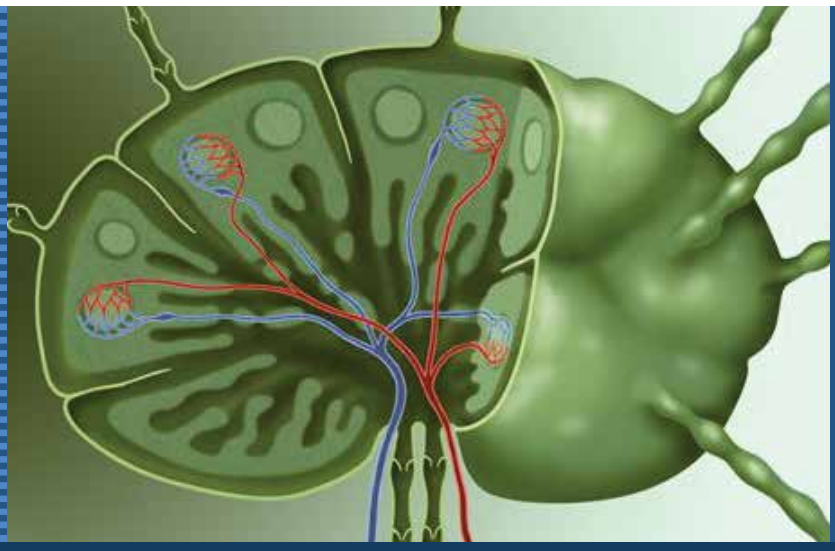
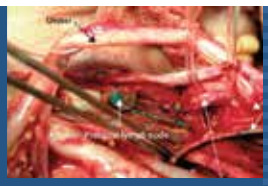
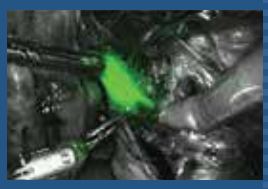
ISSN 1309-0399 • EISSN 1309-0380

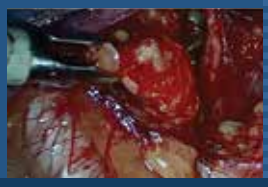
TURKISH-GERMAN GYNECOLOGICAL EDUCATION and RESEARCH FOUNDATION

Journal of the Turkish-German Gynecological Association









Volume 17
Issue 4
December
2016

Cover Picture: Sentinel nodes marked in color or by a fluorescent dye with the robot.
Alkatout et al. (Page: 224)

Original Investigations

Official Journal of the Turkish-German Gynecological Education and Research Foundation
www.tajev.org

Official Journal of the Turkish-German Gynecological Association
www.dtgg.de

www.jtgga.org

Journal of the
Turkish-German
Gynecological Association

Journal of the
Turkish-German Gynecological Association

Editorial Office

Address: Abdi İpekçi Cad. 2/7 34367 Nişantaşı, İstanbul-Turkey
Phone: +90 212 241 45 45
Fax: +90 212 241 44 08
E-mail: tajev@tajev.org

Official Journal of the Turkish-German Gynecological Education and Research Foundation
www.tajev.org

Turkish-German Gynecological Association
www.dtgg.de

AVES

Publisher
İbrahim KARA

Publication Director
Ali ŞAHİN

Deputy Publication Directors
Gökhan ÇİMEN
Dilşad GÜNEY ÖZCAN

Publication Coordinators
Esra GÖRGÜLÜ
Betül ÇİMEN
Zeynep YAKIŞIRER
Aydın Baran GÜRPINAR
Gizem KAYAN
Melike Buse ŞENAY

Project Coordinator
Hakan ERTEN

Project Assistants
Duygunur CAN
Aylin ATALAY
Şükriye YILMAZ

Graphics Department
Ünal ÖZER
Neslihan YAMAN
Deniz DURAN

Contact:
Address: Büyükdere Cad. 105/9
34394 Mecidiyeköy, Şişli, İstanbul, TURKEY
Phone: +90 212 217 17 00
Fax: +90 212 217 22 92
E-mail: info@avesyayincilik.com

Türk Alman Jinekoloji Eğitim Araştırma ve Hizmet Vakfı adına sahibi / Owned by on behalf of the Turkish German Gynecology Education, Research Foundation: M. Cihat Ünlü • Yayın türü / Publication Type: Yerel süreli / Bimonthly periodical • Basım yeri / Printed at: Matsis Matbaa Hizmetleri san. ve Tic. Ltd. Şti., İstanbul, Turkey (+90-212-6242111) • Basım tarihi / Printing Date: Aralık 2016 / December, 2016 • Türk Alman Jinekoloji Eğitim Araştırma ve Hizmet Vakfı tarafından yayınlanmaktadır / Published by Turkish German Gynecology Education Research Foundation, Abdi İpekçi Cad. 2/7 34367 Nişantaşı, İstanbul, Turkey

Journal of the
Turkish-German
Gynecological Association

Aims and Scope

Journal of the Turkish-German Gynecological Association is the official, open access publication of the Turkish-German Gynecological Education and Research Foundation and Turkish-German Gynecological Association and is published quarterly on March, June, September and December.

The target audience of Journal of the Turkish-German Gynecological Association includes gynaecologists and primary care physicians interested in gynecology practice. It publishes original work on all aspects of gynecology. The aim of Journal of the Turkish-German Gynecological Association is to publish high quality original research articles. In addition to research articles, reviews, editorials, letters to the editor and case presentations are also published.

It is an independent peer-reviewed international journal printed in English language. Manuscripts are reviewed in accordance with "double-blind peer review" process for both referees and authors.

Journal of the Turkish-German Gynecological Association is indexed in PubMed Central, Thomson Reuters – Emerging Sources Citation Index, EMBASE, Scopus, CINAHL, Gale/Cengage Learning, EBSCO, DOAJ, HINARI, ProQuest, Index Copernicus, TÜBİTAK ULAKBİM TR Index and Turkiye Citation Index.

Subscription Information
Journal of the Turkish-German Gynecological Association is distributed free of charge to all physicians, specialists in gynecology field. For subscription please contact Turkish-German Gynecological Education and Research Foundation at www.jtgga.org. The access to tables of contents, abstracts and full texts of all articles published since 2000 are free to all readers via the journal's webpage. Visit the journal's home pages for details of the aims and scope and instruction to authors.

Permission
Permission requests to reproduce copies of articles for non-commercial use may be obtained from the Editorial Office:

Editor: Cihat Ünlü, M.D.
Address: Abdi İpekçi Cad. 2/7 34367 Nişantaşı-İstanbul-Turkey
Phone: +90 212 241 45 45
Fax: +90 212 241 44 08
E-mail: tajev@tajev.org

Advertising
Enquiries concerning advertisements should be addressed to Editorial Office:

Editor: Cihat Ünlü, M.D.
Address: Abdi İpekçi Cad. 2/7 34367 Nişantaşı-İstanbul-Turkey
Phone: +90 212 241 45 45
Fax: +90 212 241 44 08
E-mail: tajev@tajev.org

Instructions for Authors
Instructions for authors page at the journal is available in the journal content and at www.jtgga.org.

Disclaimer
The statements and opinions contained in the articles of the Journal of the Turkish-German Gynecological Association are solely those of the individual authors and contributors not of the Turkish-German Gynecological Education and Research Foundation, Turkish-German Gynecological Association, Turkish Society of Reproductive Medicine, Editorial Board or AVES.

The journal is printed on acid-free paper.

Journal of the
Turkish-German
Gynecological Association

Instructions for Authors

The "Journal of the Turkish German Gynecological Association" (ISSN 1309-0399; Abbreviated as "J Turk Ger Gynecol Assoc") is the official, open access publication of the Turkish-German Gynecological Education and Research Foundation and the Turkish-German Gynecological Association. Formerly named "ARTEMIS", the journal is published quarterly (March, June, September, December) in English and publishes original peer-reviewed articles, reviews, case reports and commentaries in the fields of Gynecology, Gynecologic Oncology, Endocrinology & Reproductive Medicine and Obstetrics. Reviews will be considered for publication only if they are prepared by authors who have at least three published manuscripts in international peer reviewed journals and these studies should be cited in the review. Otherwise only invited reviews will be considered for peer review from qualified experts in the area.

The "Journal of the Turkish German Gynecological Association" is a peer reviewed journal and adheres to the highest ethical and editorial standards. The Editorial Board of the journal endorses the editorial policy statements approved by the WAME Board of Directors. The journal is in compliance with the Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals published by the International Committee of Medical Journal Editors (updated December 2015, www.icmje.org). The editors also adhere to the Committee on Publications Ethics (COPE) recommendations (http://publicationethics.org).

Submission of manuscripts
All manuscripts must be submitted via the self explanatory online submission system which is available through the journal's web page at www.jtgga.org. Manuscripts submitted via any other medium will not be evaluated. During the submission please make sure to provide all requested information to prevent any possible delays in the evaluation process.

The main document and the tables, should be prepared with "Microsoft Office Word software". Times New Roman font (size 12) should be used throughout the main document with 1.5 line spacing. The side margins of the main document should be set at 25 mm from all sides.

The figures should be submitted separately through the submission system in .JPG of .TIFF format. Please do not embed the figures in the main document. Make sure that the minimum resolution of each submitted figure is 300DPI.

A cover letter and a title page should be provided with all submissions. It should be stated in the cover letter that the manuscript was not previously published in any other publication, that it is not accepted for publication in another publication and that it is not under review for possible publication elsewhere.

Before completing your submission, please make sure to check the PDF proof of your manuscript which will be generated by the manuscript submission system and make sure that all items of the submission are displayed correctly.

Authors who have any queries regarding the submission process can contact the journal's editorial office:
Editorial Office:
Abdi İpekçi Caddesi 2/7 Nişantaşı, İstanbul / Turkey
+90 212 217 17 00
scholarone@jtgga.org

Editorial Policies
All manuscripts will be evaluated by the editorial board for their scientific contribution, originality and content. Authors are responsible for the accuracy of the data presented in their manuscript. The journal retains the right to make appropriate changes on the grammar and language of the manuscript when needed. When suitable the manuscript will be send to the corresponding author for revision. The manuscript, if accepted for publication, will become the property of the journal and copyright will be taken out in the name of the journal.

Full text of all articles can be downloaded at the web site of the journal www.jtgga.org

Preparation of Manuscripts
The "Journal of the Turkish German Gynecological Association" follows the "Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals" (International Committee of Medical Journal Editors - http://www.icmje.org/). Upon submission of the manuscript, authors are to indicate the type of trial/research and provide the checklist of the following guidelines when appropriate:

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

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

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

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

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

Human and Animal Studies
Manuscripts submitted for publication must contain a statement to the effect that all human studies have been reviewed by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards described in an appropriate version of the 1975 Declaration of Helsinki, as revised in 2000. It should also be stated clearly in the text that all persons gave their informed consent prior to their inclusion in the study. Details that might disclose the identity of the subjects under study should be omitted. Experimental animal studies should be presented with the disclosure of the appropriateness to the institutional/national/international ethical guides on care and use of laboratory animals.

Reports of animal experiments must state that the "Principles of laboratory animal care" (NIH publication No. 86-23, revised 1985) were followed, as well as specific national laws where applicable.

The editors reserve the right to reject manuscripts that do not comply with the abovementioned requirements. The author will be held responsible for false statements or for failure to fulfil the above mentioned requirements.

In a cover letter the authors should state if any of the material in the manuscript is submitted or planned for publication elsewhere in any form including electronic media. The cover letter must contain address, telephone, fax and the e-mail address of the corresponding author.

Conflict of Interest
Authors must indicate whether or not they have a financial relationship with the organization that sponsored the research. They should also state that they have had full control of all primary data and that they agree to allow the Journal to review their data if requested. Therefore manuscripts should be accompanied by the "Conflict of Interest Disclosure Form." The form can be obtained from the journal webpage (www.jtgga.org).

Copyright
The author(s) transfer(s) the copyright to his/their article to the Journal of the Turkish German Gynecological Association effective if and when the article is accepted for publication. The copyright covers the exclusive and unlimited rights to reproduce and distribute the article in any form of reproduction (printing, electronic media or any other form); it also covers translation rights for all languages and countries. For U.S. authors the copyright is transferred to the extent transferable.

Submissions must be accompanied by the "Copyright Transfer Statement". The form is available for download on the journal's manuscript submission and

evaluation site. The copyright transfer form should be signed by all contributing authors and a scanned version of the wet signed document should be submitted.

Manuscript Specifications
Submissions should have the following parts.

Title Page
A separate title page should be submitted with all submissions and should include the title of the article, name(s), affiliations and major degree(s) of the author(s) and source(s) of the work or study, a short title (running head) of no more than 50 characters. The name, address, telephone (including the mobile phone number) and fax numbers and e-mail address of the corresponding author should be listed on the title page.

Abstract
All manuscripts should be accompanied by an abstract. A structured abstract is required with original articles and it should include the following subheadings: Objective, Material and Methods, Results and Conclusion. A structured abstract is not required with review articles and case reports. The abstract should be limited to 250 words for original articles and review articles and 150 words for 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).

Original articles should have the following sections.

Introduction
State concisely the purpose and rationale for the study and cite only the most pertinent references as background.

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

Address "Institutional Review Board" issues as stated above. State the generic names of the drugs with the name and country of the manufactures. Provide information on informed consent and ethics committee approval.

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. Emphasize only your important observations; do not compare your observations with those of others. Such comparisons and comments are reserved for the discussion section.

Discussion
State the importance and significance of your findings 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 those of others. Provide information on the limitations of the study. No new data are to be presented in this section.

The main text of case reports should be structured with the following subheadings: Introduction, Case Presentation, Discussion.

References
Number references in Arabic numerals consecutively in the order in which they are mentioned in the text starting with number "1". Use the form of the "Uniform Requirements for Manuscript Submitted to Biomedical Journals" (http://www.amaassn.org/public/peer/wame/uniform.htm). If number of authors exceeds seven, list first 6 authors followed by et al.

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

Examples:
Journals;
Harrington K, Cooper D, Lees C, Hecher K, Campbell S. Doppler ultrasound of the uterine arteries: the importance of bilateral notching in the prediction of pre-eclampsia, placental abruption or delivery of a small-for-gestational-age baby. Ultrasound Obstet Gynecol 1996; 7: 182-8.

Book chapter;
Ertan AK, Tanriverdi HA, Schmidt W. Doppler Sonography in Obstetrics. In: Kurjak A, Chervenak FA, editors. Ian Donald School Textbook of Ultrasound in Obstetrics and Gynecology. New Delhi, India: Jaypee Brothers; 2003. p. 395-421.

Book;
Kohler G; Egelkraut H. In Kohler G and Egelkraut H (edts).Munchener Funktionelle Entwicklungsdiagnostik im zweitem und drittem Lebensjahr. Handanweisung. Munchen: Uni Munchen, Institut fur Soziale Paediatrie und Jugendmedizin; 1984.

Tables and Figures
Tables should be included in the main document after the reference list. Colour figures or gray-scale images must be at minimum 300 DPI resolution. 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 colour illustrations.

Units of measurement and abbreviations

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.

Revisions
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 and 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.

Accepted articles
Accepted articles are provided with a DOI number and published as ahead of print articles before they are included in their scheduled issue.

Journal and Society Web sites:
www.dtgg.de (Deutsch-Türkische Gynäkologengeselleschaft)
www.tajd.org (Türk-Alman Jinekoloji Derneği)
www.jtgga.org (Journal of the Turkish German Gynecological Association)

- Citation of published manuscripts in J Turk Ger Gynecol Assoc should be as follows: Tews G, Ebner T, Sommergruber M, Marianne M, Omar S. Ectopic Pregnancy in the Assisted Reproduction. J Turk Ger Gynecol Assoc 2004; 5: 59-62.

- The Journal name should be abbreviated as "J Turk Ger Gynecol Assoc"

Journal of the
Turkish-German
Gynecological Association

Contents

Journal of the
Turkish-German
Gynecological Association

Editorial

Dear Colleagues,

J Turk Ger Gynecol Assoc is the official, scientific, open Access publication of the Turkish-German Gynecological Education and Research Foundation that publishes original studies on all aspects of gynecology since 2000. Here you may find the last issue of 2016. With this latest issue, we published 17 manuscripts.

There are a lot to do in the future. First, we must attract good manuscripts. Then we must process them quickly and publish the accepted ones as soon as possible. These are the top eight reasons that we accept a paper as the editorial board:

1. It provides insight into an important issue – for example, by explaining the diagnosis treatment and total management of diseases effected women health.
2. The insight is useful to people who make decisions, particularly the physicians who practices in the field.
3. The insight is used to develop a framework or theory, either a new theory or advancing an existing one.
4. The insight stimulates new, important questions.
5. The methods used to explore the issue are appropriate (for example, data collection and analysis of data).
6. The methods used are applied rigorously and explain why and how the data support the conclusions.
7. Connections to prior work in the field or from other fields are made and serve to make the article's arguments clear.
8. The article tells a good story, meaning it is well written and easy to understand, the arguments are logical and not internally contradictory.

When examining improvement and development opportunities for our journal, we aim to enhance and augment the evaluation process. We are sure that the most difficult element of peer review is finding reviewers who are willing and able to evaluate a paper within a few weeks; yet this is such an integral slice of the journal's day to day running, as it helps to improve both the speed and quality of the peer review process. If you would like to contribute to our journal as a reviewer, please do not hesitate to contact us.

Dear Readers, Dear Colleagues,

We are dealing with very interesting research articles and case reports in the latest issue. We worked hard to deliver you the journal with the best manuscripts in time. In this issue, you will read several good papers from all over the world, from Taiwan and Iran to Malta and Germany. The manuscript that is searching the association of maternal serum levels of chemerin, retinol binding protein-4 (RBP-4), and visfatin with gestational diabetes mellitus (GDM) is very interesting. You will read a very informative review from Germany, that introduces the robotic surgery in Gynecology. Robotic surgery is the most dynamic development in the sector of minimally invasive operations currently. It should not be viewed as an alterna¬tive to laparoscopy, but as the next step in a process of technological evolution. Please also enjoy solving our challenging quiz.

I would like to remind you that the archive of our journal starting from September 2009 issue has been indexed in PubMed Central and available for online access. We are looking forward to receiving your valuable submissions and thank you in advance for your contributions.

Sincerely,

Prof. Cihat Ünlü, M.D.
Editor in Chief of *J Turk Ger Gynecol Assoc*
President of TAJEV

Salvage intraperitoneal chemotherapy for relapsed type II endometrial cancer: A pilot case-control study

Yi-Chen Tsai[1,2], Yen-Hou Chang[1,2], Yi-Chang[1,2], Chi-Mu Chuang[1,2]

[1]Institute of Clinical Medicine, National Yang-Ming University School of Medicine, Taipei, Taiwan

[2]Section of Gynecologic Oncology, Department of Obstetrics and Gynecology, Taipei Veterans General Hospital, Taipei, Taiwan

Abstract

Objective: Epithelial ovarian cancer and relapsed type II endometrial cancer share common characteristics. Although the role of intraperitoneal (IP) chemotherapy in the treatment of epithelial ovarian cancer has been well-established, its role in the treatment of relapsed type II endometrial cancer remains to be elucidated.

Material and Methods: From January 2000 to December 2012, patients who were diagnosed with relapsed type II endometrial cancer and underwent secondary cytoreductive surgery, patients with residual tumors less than 1 cm in diameter were initially screened for this study. Of the screened patients, consecutive patients who received salvage IP chemotherapy (IP platinum plus intravenous paclitaxel) were considered the case group. The case study group was matched to a control group that was composed of patients who received salvage systemic chemotherapy (intravenous platinum plus intravenous paclitaxel) in a 1:2 ratio. The overall survival was compared between the case group and the control group, and the IP treatment-related toxicities were reported.

Results: In total, 11 patients were assigned into the case group and 22 patients were assigned into the control group. The median overall survival (95% confidence interval) was 40.5 (25.5–56.2) months for the case group versus 28.0 (18.0–37.0) for the control group (hazard ratio=0.37 (95% confidence interval, 0.15–0.95); p=0.032, by the log-rank test). The most commonly observed toxicity was of gastrointestinal origin (81.8%). Toxicities that stemmed from hematological, cardiovascular, neurological, and catheter-related complications were similar to results published in other studies on IP chemotherapy for ovarian cancer.

Conclusion: Salvage IP chemotherapy may potentially confer a longer overall survival than conventional systemic chemotherapy in the treatment of relapsed type II endometrial cancer. (J Turk Ger Gynecol Assoc 2016; 17: 176-81)

Keywords: Intraperitoneal chemotherapy, type II endometrial cancer, overall survival

Received: 4 May, 2016 **Accepted:** 3 September, 2016

Introduction

Type II endometrial cancer with clear cell or papillary serous histology exhibits distinct biological behavior from type I endometrial cancer (1). This specific disease accounts for only 10% of all endometrial cancer cases, but analysis of historical data has shown a propensity for early extrauterine tumor spreading and a worse prognosis (2, 3). To date, randomized trials for this specific type of cancer are very rare due to the relatively low disease incidence. As such, published studies of adjuvant therapy are mainly extracted from heterogeneously treated patients (4, 5).

Currently, the standard front-line treatment for type II endometrial cancer includes surgical staging (or maximal debulking for the gross disease) and adjuvant chemotherapy, which is similar to the treatment for epithelial ovarian cancer. However, although treatment mechanisms for relapsed epithelial ovarian cancer have been well established, specific treatment for relapsed type II endometrial carcinoma has yet to be defined due to the lack of randomized trials for this relatively rare disease (6, 7).

The role of intraperitoneal (IP) chemotherapy in the treatment of epithelial ovarian cancer has been well established from published randomized trials (8-10). Nevertheless, three ongoing large-scale randomized trials are being conducted to examine the efficacy of carboplatin-based IP chemotherapy in the treatment of epithelial ovarian cancer (11, 12). In contrast, only one study of IP chemotherapy as a front-line therapy for type II endometrial cancer has been published thus far (13). Moreover, to the best of our knowledge, the role of salvage IP chemotherapy for relapsed type II endometrial cancer has yet to be elucidated.

In this work, we conducted a case-control study to evaluate the role of salvage IP chemotherapy for relapsed type II endometrial cancer. Eleven patients who were diagnosed with relapsed type II endometrial cancer were presented with peritoneal spread and received salvage IP chemotherapy after secondary cytoreductive surgery; these patients constituted

Address for Correspondence: *Chi-Mu Chuang*
e.mail: cmjuang@gmail.com

DOI: 10.5152/jtgga.2016.16087

the case group. Concurrently, twenty-two patients with the same diagnosis who received salvage systemic chemotherapy, constituted the control group. Both overall survival and instantaneous hazard function were compared between the case group and the control group. Additionally, IP treatment-related toxicities were detailed.

Material and Methods

Study population

This study entailed a retrospective analysis of prospectively collected data from an electronic health care database. Data was obtained from consecutive patients who were diagnosed with relapsed type II endometrial cancer between January 2000 and December 2012. These patients also underwent secondary cytoreductive surgery for a residual tumor less than 1 cm. Selection criteria for secondary cytoreductive surgery must meet either of the following two criteria: (1) solitary tumor recurrence and (2) diffuse intraperitoneal spreading without evidence of bowel obstruction.

We selected patients who received salvage IP chemotherapy to serve as the case group. Accordingly, each patient in the case group was matched 1:2 to the control group, which was defined as patients who received salvage intravenous chemotherapy after secondary cytoreductive surgery for a residual tumor less than 1 cm.

The institutional review board of Taipei Veterans General Hospital, Taiwan approved the current study. The procedures used in this study were conducted in accordance with the guidelines of the Declaration of Helsinki as it pertains to human subject experimentation (14).

Definition of secondary cytoreductive surgery

After a panel discussion that involved multidisciplinary care providers, once secondary cytoreductive surgery was considered appropriate for patients diagnosed with a relapsed disease; they were informed about the possibility of a secondary cytoreductive surgery. In cases when operation of the stomach, bowel, liver, diaphragm, spleen, or pancreas were seemingly necessary, consultation with an expert in the field of general surgery was issued in advance. If the patient had undergone an ostomy procedure, then a specific care team was consulted for wound care service and ostomy education.

Surgery that was performed only to relieve symptoms (e.g., relief of bowel obstruction) and performed strictly for palliative purposes (e.g., abscess drainage) or surgery within the context of primary therapy (e.g., second-look laparotomy or interval cytoreductive surgery) were excluded from the definition of secondary cytoreductive surgery.

Definition of salvage chemotherapy for the case group and the control group

The dosing schedules for the case group included IP delivery of a platinum (cisplatin (Abiplatin®; TEVA pharmaceutical, Petah Tikva, Israel) or carboplatin (Paraplatin®; Bristol-Myers Squibb Co, Princeton, NJ, USA) agent. Dosing of cisplatin (100 mg/m^2) or carboplatin (either AUC 5 or 6) occurred in 2 L of normal saline. This agent was administered via Tenckhoff catheters that were implanted during the secondary cytoreductive surgery. Concurrently, intravenous delivery of paclitaxel (Taxol®, Bristol-Myers Squibb Co.; Princeton, NJ, USA) dosing at 175 mg/m^2 was administered the same day. In summary, the case group received IP platinum plus intravenous paclitaxel.

The dosing schedule for the control group consisted of intravenous delivery of a platinum agent, dosing at cisplatin (100 mg/m^2) or carboplatin (either AUC 5 or 6), plus the intravenous delivery of paclitaxel (175 mg/m^2) on the same day. In summary, the control group received intravenous platinum plus intravenous paclitaxel.

The dosing schedules for both the case group (i.e., salvage IP chemotherapy) and the control group (i.e., salvage systemic chemotherapy) were repeated every three weeks for a total of six assigned cycles, provided that the serum creatinine concentration was less than or equal to 2.0 mg/dL, the white-cell count was higher than 3,000/mm^3, and the platelet count was higher than 80,000/mm^3.

Safety evaluation

The effects of treatment-related toxicities were evaluated according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE v4.0; http://ctep.cancer.gov/). Toxicity profiles, such as fever for more than 3 days, as well as hematological, cardiovascular, renal, and neurological profiles were recorded. In addition, IP catheter-related complications, including infection and obstruction, were also measured during the courses of treatment.

Statistical analysis

Continuous variables are presented as the mean (±standard deviations) and were compared using the Student's unpaired t-test. Categorical variables are presented as counts and percentages and were compared using the χ^2 test when appropriate (expected frequency>5). Tumor response was evaluated at 1 month after completion of chemotherapy using the RECIST criteria, version 1.1 (15). Overall survival is defined from the date of the first diagnosis to the date of the last follow-up or death. The date of last follow-up was set as December 31, 2015. An analysis of survival was conducted using the Kaplan-Meier method, and the survival of each group was compared using the log-rank test. Moreover, the hazard function, which is also called the instantaneous failure rate and force of mortality, was plotted for both the case group and the control group (16).

All analyses were performed using STATA SE version 12 (Stata Corp.; College Station, Texas, USA), and $p<0.05$ was considered statistically significant.

Results

Figure 1 depicts the process of case selection and matching. Initially, ninety-seven patients were identified. After the first round of screening, patients who met the defined criteria,

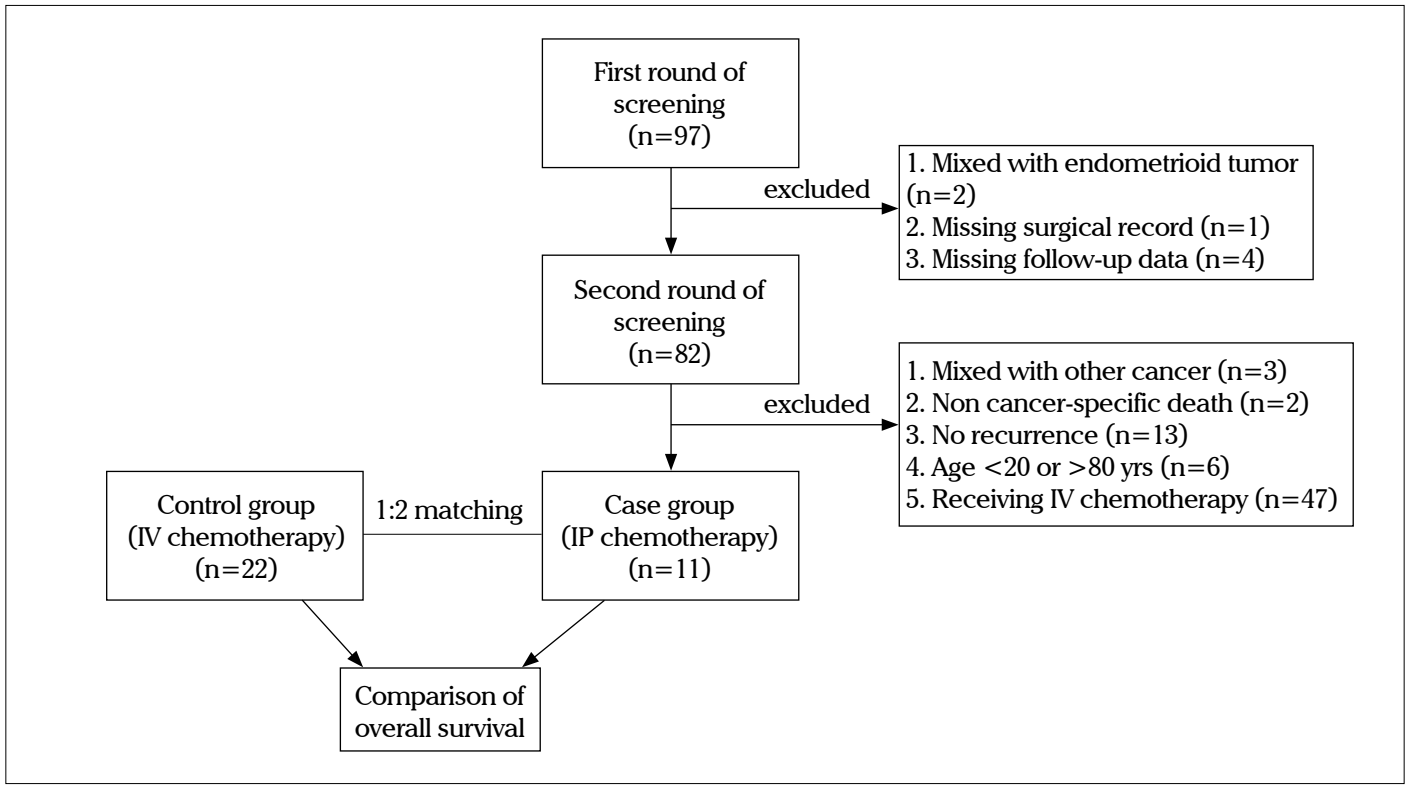


Figure 1. Flow chart describing the screening process of included patients

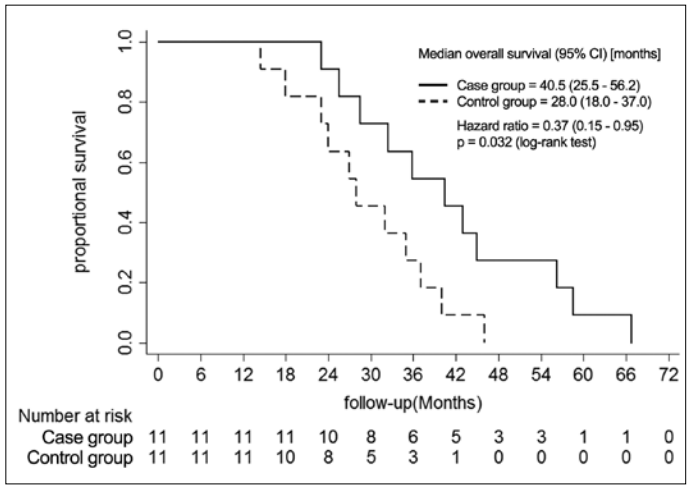


Figure 2. Kaplan-Meier overall survival for the case group (IP therapy) and the control group (systemic therapy)

including those with mixed tumors of endometrioid histology (n=12), missing surgical records (n=1), and missing follow-up data (n=4), were excluded. The remaining patients (n=82) then entered a second round of screening. Those who met the following defined criteria were also excluded: those with other cancers (n=3), non-cancer specific deaths (n=2), no recurrence (n=13), age <20 or >80 years, and treatment with systemic chemotherapy (n=47). The remaining patients formed the case group, which consisted of patients who were diagnosed with relapsed type II endometrial cancer and who had residual tumors less than 1 cm in diameter; these patients received IP chemotherapy after secondary cytoreductive surgery. The case group was 1:2 matched to the control group, which consisted of patients who were diagnosed with relapsed type II endometrial cancer and who had residual tumors less than 1 cm in diameter; these patients received systemic chemotherapy after secondary cytoreductive surgery.

Table 1 outlines the demographics and clinical characteristics of the recruited patients (n=11) who received salvage IP chemotherapy. Among these, nine patients (81.8%) were diagnosed with serous carcinoma, while the remaining two patients were diagnosed with the clear cell subtype. A total of seven patients (63.6%) completed the six assigned IP cycles. Most of the reasons for the discontinuation of IP chemotherapy were due to catheter obstruction.

Next, a Kaplan-Meier overall survival curve was constructed for the case group and the control group. The median overall survival (95% confidence interval) was 40.5 (25.5–56.2) months for the case group versus 28.0 (18.0–37.0) for the control group [hazard ratio=0.37 (0.15–0.95); p=0.032, by the log-rank test] (Figure 2).

Figure 3 presents the hazard function for both the case control groups. The hazard rate shows a significantly higher and steeper curve, which implies that patients in the control group always faced a higher instantaneous risk of death than the case group during the entire follow-up.

Lastly, grade 3 or 4 toxicities during IP chemotherapy were tabulated (Table 2). The most commonly observed toxicity was of gastrointestinal origin (81.8%). Toxicities that involved hematological, cardiovascular, neurological, and catheter-related complications were not different from those that were

Table 1. Baseline demographics and clinical characteristics of patients receiving salvage IP chemotherapy (n=11)

Case No.	Age	Histology	ECOG performance score	Tumor location	Presenting symptoms	Completed cycles	Reason for discontinuation	Tumor response
1	54	Serous	1	Peritoneal spreading	Ascites	6	NA	Partial
2	61	Serous	0	Bowel, unspecified	Fatigue	6	NA	Partial
3	53	Serous	1	Peritoneal spreading	Bloating sensation	6	NA	Complete
4	66	Clear cell	0	Peritoneal spreading	Ascites	4	Catheter obstruction	Stable
5	59	Serous	1	Spleen metastases	Poor appetite	6	NA	Partial
6	71	Serous	2	Peritoneal + liver surface spreading	Bloating sensation	3	Catheter obstruction	Stable
7	67	Clear cell	1	Peritoneal spreading	Ascites	6	NA	Progressed
8	75	Serous	1	Peritoneal spreading	Ascites	6	NA	Stable
9	64	Serous	1	Sigmoid colon + small bowel	Bloating sensation	6	NA	Partial
10	71	Serous	1	Peritoneal spreading	Bloating sensation	4	Catheter obstruction	Stable
11	62	Serous	0	Bowel, unspecified	Ascites	5	Catheter-induced infection	Complete

IP: intraperitoneal; ECOG: Eastern Cooperative Oncology Group; NA: not applicable

Table 2. Patients experiencing grade 3 and 4 toxicity during IP chemotherapy (n=11)

	Grade 3	Grade 4	Overall
Treatment-related fever	0	0	0 (0%)
Neutrophil count	3	1	4 (36.4%)
Anemia	1	0	1 (9.1%)
Platelet	2	0	2 (18.2%)
Gastrointestinal	8	1	9 (81.8%)
Cardiovascular	1	0	1 (9.1%)
Neurologic	4	1	5 (45.5%)
Renal	1	0	1 (9.1%)
Catheter-related infection	NA	NA	2 (18.2%)
Catheter obstruction	NA	NA	1 (9.1%)

IP: intraperitoneal; NA: not applicable

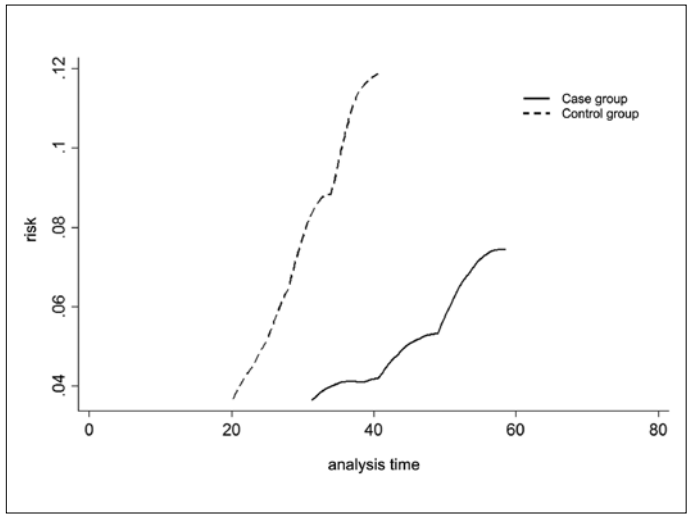


Figure 3. Hazard curve for the case group (IP therapy) and the control group (systemic therapy)

published in reports of IP chemotherapy for ovarian cancer (8-10).

Discussion

In the current matched case-control study, we aimed to analyze the efficacy of salvage IP chemotherapy for the treatment of relapsed type II endometrial cancer. Our data was in agreement with the results of published randomized trials of IP chemotherapy for ovarian cancer, and show that salvage IP chemotherapy yields a significantly longer overall survival than salvage systemic chemotherapy. Furthermore, the completion rate for the six assigned IP cycles (7/11, 63.6%) and the toxicity profiles were comparable to those in published IP randomized trials for ovarian cancer. Taken together, salvage IP chemotherapy is worthy of consideration as a potential novel therapy for patients with relapsed type II endometrial cancer.

Uterine clear cell carcinoma and uterine papillary serous carcinoma are subtypes of type II endometrial cancer that show aggressive biological behavior and a predilection for deep myometrial invasion, lympho-vascular space invasion, and a propensity for peritoneal spreading (17-19). Additionally, in the setting of relapsed type II endometrial cancer, peritoneal

spreading is the major presenting symptom in the majority of involved cases (18, 20). Thus, the peritoneal cavity provides a sanctuary site for the development of local therapies (i.e., IP chemotherapy).

Currently, no specific tailored treatment exists for relapsed type II endometrial cancer due to the rarity of this disease. Still, the current gold standard treatment for relapsed type II endometrial cancer is the same as that for relapsed type I endometrial cancer, which is based on a series of published phase III randomized trials that focused on the efficacy of chemotherapy (7, 21-25). However, given the apparent differences in clinical behavior between type I and type II endometrial cancers, it has been suggested that treatments should be tailored according to the histologic type. To date, potential chemotherapy regimens for relapsed type II endometrial cancer include cisplatin, cyclophosphamide, topotecan, doxorubicin, and pegylated liposomal doxorubicin (26). Furthermore, patients with relapsed type II endometrial cancer are unlikely to be successfully treated with surgery or radiation as salvage treatments, and demonstrate a less favorable response rate to chemotherapy than patients with endometrioid cancer (27). As such, the development of novel treatment modalities for relapsed type II endometrial cancer is imperative.

In addition to the established role of IP chemotherapy in the treatment of epithelial ovarian cancer, according to published randomized trials, IP chemotherapy even shows long-term survival benefits that extend beyond 10 years. The long-term survival benefits may encourage more clinicians to adopt IP chemotherapy in their communities (28). Nonetheless, according to a recent report, the adoption of IP chemotherapy is an underused strategy for eligible patients in general, and the integration of IP chemotherapy into clinical practice varies significantly among institutions (29). Thus, the implementation of IP chemotherapy merits further education and encouragement for gynecologic oncologists.

The current study has two primary advantages. First, the current study adopted IP delivery of platinum plus intravenous delivery of paclitaxel, whereas a previous study adopted IP delivery of platinum plus intravenous delivery of doxorubicin and cyclophosphamide (13). Apparently, the regimen used in the current study is favorable with respect to modern practices for endometrial cancer treatment. Second, aside from epithelial ovarian cancer, the current study found that IP chemotherapy is potentially feasible and useful in the treatment of relapsed type II endometrial cancer.

Some limitations of our study should be emphasized. First, we acknowledge the lack of some important patient-level information in the current work. For example, socioeconomic factor has been linked to cancer mortality, and the lack of this information may lead to biased results (30). Second, follow-up protocols after secondary cytoreductive surgery were not strictly defined (e.g., frequency of image study), which means that the exact timing of the relapse was in doubt. Third, because the current study is essentially a retrospective study, many factors relevant in the front-line treatment setting were not as strictly controlled as for those in a randomized trial. As such, the results of overall survival may have been prone to major bias. Lastly, the sample size in the platinum-refractory/resistant group is relatively small, which may have affected the validity of the conclusion.

In conclusion, this matched case-control study reveals that salvage IP chemotherapy may potentially confer longer overall survival than conventional systemic chemotherapy in the treatment of relapsed type II endometrial cancer. Moreover, salvage IP chemotherapy is associated with comparable IP-related toxicities compared with those of previously published randomized trials for epithelial ovarian cancer. This study may widen the application of IP chemotherapy, but a prospective study with an adequate sample size is still needed to validate its application.

Ethics Committee Approval: *Ethics committee approval was received for this study from the ethics committee of Taipei Veterans General Hospital. / IRB No. 2016-05-004CC.*

Informed Consent: *N/A.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Concept - Y.C.T., C.M.C.; Design - Y.C.T., Y.H.C., C.M.C.; Supervision - C.M.C.; Resources - Y.C.T., Y.H.C., Y.C., C.M.C.; Data Collection and/or Processing - Y.C.T., Y.H.C., C.M.C.; Analysis and/or Interpretation - Y.C.T., Y.H.C., C.M.C.; Literature Search - Y.C.T.; Writing Manuscript - Y.C.T., Y.H.C., Y.C., C.M.C.; Critical Review - Y.C.T., Y.H.C., Y.C., C.M.C.*

Acknowledgements: *The authors would like to thank the Task Force on Excellence on Intraperitoneal Chemotherapy Research group, Lilly Wen, MD (Catholic Cardinal Tien Hospital, Taipei), and Kuo-Chang Wen, MD, Chih-Yu Chen, MD, Peng-Hui Wang, MD, Nae-Fong Twu, MD, and Hsiang-Tai Chao, MD (Taipei Veterans General Hospital).*

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

Financial Disclosure: *The authors declared that this study has partially supported by the National Science Council, Taiwan (No. NSC 104-NU-E-010-004-NU & MOST 103-2410-H-264-004).*

Yi-Chen Tsai and Yen-Hou Chang contributed equally to this study.

References

1. Mendivil A, Schuler KM, Gehrig PA. Non-endometrioid adenocarcinoma of the uterine corpus: a review of selected histological subtypes. Cancer Control 2009; 16: 46-52.
2. Boruta DM 2nd, Gehrig PA, Fader AN, Olawaiye AB. Management of women with uterine papillary serous cancer: a Society of Gynecologic Oncology (SGO) review. Gynecol Oncol 2009; 115: 142-53. [CrossRef]
3. Greggi S, Mangili G, Scaffa C, Scala F, Losito S, Iodice F, et al. Uterine papillary serous, clear cell, and poorly differentiated endometrioid carcinomas: a comparative study. Int J Gynecol Cancer 2011; 21: 661-7. [CrossRef]
4. Fields AL, Einstein MH, Novetsky AP, Gebb J, Goldberg GL. Pilot phase II trial of radiation "sandwiched" between combination paclitaxel/platinum chemotherapy in patients with uterine papillary serous carcinoma (UPSC). Gynecol Oncol 2008; 108: 201-6. [CrossRef]
5. Johnson N, Bryant A, Miles T, Hogberg T, Cornes P. Adjuvant chemotherapy for endometrial cancer after hysterectomy. Cochrane Database Syst Rev 2011: CD003175. [CrossRef]
6. Network NCC. NCCN Clinical Practice Guidelines in Oncology: Ovarian Cancer Version 2 2015 [cited 2015 December 1]. Available from: http://www.nccn.org/professionals/physician_gls/f_guidelines.asp.
7. Network NCC. NCCN Clinical Practice Guidelines in Oncology: Uterine Cancers Version 1 2015 [cited 2015 December 1]. Available

from: http://www.nccn.org/professionals/physician_gls/f_guidelines.asp.
8. Alberts DS, Liu PY, Hannigan EV, O'Toole R, Williams SD, Young JA, et al. Intraperitoneal cisplatin plus intravenous cyclophosphamide versus intravenous cisplatin plus intravenous cyclophosphamide for stage III ovarian cancer. N Engl J Med 1996; 335: 1950-5. [CrossRef]
9. Markman M, Bundy BN, Alberts DS, Fowler JM, Clark-Pearson DL, Carson LF, et al. Phase III trial of standard-dose intravenous cisplatin plus paclitaxel versus moderately high-dose carboplatin followed by intravenous paclitaxel and intraperitoneal cisplatin in small-volume stage III ovarian carcinoma: an intergroup study of the Gynecologic Oncology Group, Southwestern Oncology Group, and Eastern Cooperative Oncology Group. J Clin Oncol 2001; 19: 1001-7.
10. Armstrong DK, Bundy B, Wenzel L, Huang HQ, Baergen R, Lele S, et al; Gynecologic Oncology Group. Intraperitoneal cisplatin and paclitaxel in ovarian cancer. N Engl J Med 2006; 354: 34-43. [CrossRef]
11. Gadducci A, Conte PF. Intraperitoneal chemotherapy in the management of patients with advanced epithelial ovarian cancer: a critical review of the literature. Int J Gynecol Cancer 2008; 18: 943-53. [CrossRef]
12. Fujiwara K. Three ongoing intraperitoneal chemotherapy trials in ovarian cancer. J Gynecol Oncol 2012; 23: 75-7. [CrossRef]
13. Chambers JT, Chambers SK, Kohorn EI, Carcangiu ML, Schwartz PE. Uterine papillary serous carcinoma treated with intraperitoneal cisplatin and intravenous doxorubicin and cyclophosphamide. Gynecol Oncol 1996; 60: 438-42. [CrossRef]
14. Association WM. Declaration of Helsinki [cited 2003 November 5]. Available from: http://www.wma.net/e/ethicsunit.
15. Nishino M, Jagannathan JP, Ramaiya NH, Van den Abbeele. Revised recist guideline version 1.1: What oncologists want to know and what radiologists need to know. AJR Am J Roentgenol 2010; 195: 281-9. [CrossRef]
16. Bradburn MJ, Clark TG, Love SB, Altman DG. Survival analysis part II: multivariate data analysis--an introduction to concepts and methods. Br J Cancer 2003; 89: 431-6. [CrossRef]
17. Lindahl B, Persson J, Ranstam J, Willén R. Long-term survival in uterine clear cell carcinoma and uterine papillary serous carcinoma. Anticancer Res 2010; 30: 3727-30.
18. Murphy KT, Rotmensch J, Yamada SD, Mundt AJ. Outcome and patterns of failure in pathologic stages I-IV clear-cell carcinoma of the endometrium: implications for adjuvant radiation therapy. Int J Radiat Oncol Biol Phys 2003; 55: 1272-6. [CrossRef]
19. Tay EH, Ward BG. The treatment of uterine papillary serous carcinoma (UPSC): are we doing the right thing? Int J Gynecol Cancer 1999; 9: 463-9. [CrossRef]
20. Sood BM, Jones J, Gupta S, Khabele D, Guha C, Runowicz C, et al. Patterns of failure after the multimodality treatment of uterine papillary serous carcinoma. Int J Radiat Oncol Biol Phys 2003; 57: 208-16. [CrossRef]
21. Fleming GF, Brunetto VL, Cella D, Look KY, Reid GC, Munkarah AR, et al. Phase III trial of doxorubicin plus cisplatin with or without paclitaxel plus filgrastim in advanced endometrial carcinoma: a Gynecologic Oncology Group Study. J Clin Oncol 2004; 22: 2159-66. [CrossRef]
22. Fleming GF, Filiaci VL, Bentley RC, Herzog T, Sorosky J, Vaccarello L, et al. Phase III randomized trial of doxorubicin + cisplatin versus doxorubicin + 24-h paclitaxel + filgrastim in endometrial carcinoma: a Gynecologic Oncology Group study. Ann Oncol 2004; 15: 1173-8. [CrossRef]
23. Gallion HH, Brunetto VL, Cibull M, Lentz SS, Reid G, Soper JT, et al. Randomized phase III trial of standard timed doxorubicin plus cisplatin versus circadian timed doxorubicin plus cisplatin in stage III and IV or recurrent endometrial carcinoma: a Gynecologic Oncology Group Study. J Clin Oncol 2003; 21: 3808-13. [CrossRef]
24. Thigpen JT, Brady MF, Homesley HD, Malfetano J, DuBeshter B, Burger RA, et al. Phase III trial of doxorubicin with or without cisplatin in advanced endometrial carcinoma: a gynecologic oncology group study. J Clin Oncol 2004; 22: 3902-8. [CrossRef]
25. Homesley HD, Filiaci V, Gibbons SK, Long HJ, Cella D, Spirtos NM, et al. A randomized phase III trial in advanced endometrial carcinoma of surgery and volume directed radiation followed by cisplatin and doxorubicin with or without paclitaxel: A Gynecologic Oncology Group study. Gynecol Oncol 2009; 112: 543-52. [CrossRef]
26. Muggia FM, Blessing JA, Sorosky J, Reid GC. Phase II trial of the pegylated liposomal doxorubicin in previously treated metastatic endometrial cancer: a Gynecologic Oncology Group study. J Clin Oncol 2002; 20: 2360-4. [CrossRef]
27. McMeekin DS, Filiaci VL, Thigpen JT, Gallion HH, Fleming GF, Rodgers WH, et al. The relationship between histology and outcome in advanced and recurrent endometrial cancer patients participating in first-line chemotherapy trials: a Gynecologic Oncology Group study. Gynecol Oncol 2007; 106: 16-22. [CrossRef]
28. Tewari D, Java JJ, Salani R, Armstrong DK, Markman M, Herzog T, et al. Long-term survival advantage and prognostic factors associated with intraperitoneal chemotherapy treatment in advanced ovarian cancer: a gynecologic oncology group study. J Clin Oncol 2015; 33: 1460-6. [CrossRef]
29. Wright AA, Cronin A, Milne DE, Bookman MA, Burger RA, Cohn DE, et al. Use and Effectiveness of Intraperitoneal Chemotherapy for Treatment of Ovarian Cancer. J Clin Oncol 2015; 33: 2841-7. [CrossRef]
30. Albano JD, Ward E, Jemal A, Anderson R, Cokkinides VE, Murray T, et al. Cancer mortality in the United States by education level and race. J Natl Cancer Inst 2007; 99: 1384-94. [CrossRef]

The association between level of maternal serum leptin in the third trimester and the occurrence of moderate preterm labor

Fereshteh Fakor[1], Seyedeh Hajar Sharami[1], Forozan Milani[1], Fariba Mirblouk[1], Sodabeh Kazemi[1], Davoud Pourmarzi[2], Hannan Ebrahimi[1], Seyedeh Fatemeh Dalil Heirati[1]

[1]Reproductive Health Research Center, Department of Obstetrics and Gynecology, Guilan University of Medical Sciences, Rasht, Iran

[2]Reproductive Health Research Center, Department of Epidemiology, Guilan University of Medical Sciences, Rasht, Iran

Abstract

Objective: We aimed to investigate the relationship between the level of maternal serum leptin and the occurrence of moderate preterm labor.

Material and Methods: This was a case control study conducted on pregnant women referred to Al- Zahra Hospital in Rasht, north of Iran in 2013. Cases included 30 moderate preterm delivering women and 30 control pregnant women with the same gestational age. The maternal serum leptin was measured for each mother at the time of entering the study.

Results: The mean serum leptin in the control group (56.66±34.18) was significantly higher than the preterm (33.65±16.70) group. There were no significant differences between the groups in terms of body mass index and age. Logistic regression revealed that age and body mass index did not have a significant relationship to moderate preterm birth. However, an increased leptin level as low as 1 microgram per liter was associated with the risk of moderate preterm birth incidence (OR: 0.973, CI: 0.948–0.997).

Conclusion: Higher levels of leptin in pregnant women are associated with a decreased risk of moderate preterm birth. Further investigations are recommended with a larger sample size. (J Turk Ger Gynecol Assoc 2016; 17: 182-5)

Keywords: Preterm birth, leptin, delivery, pregnancy

Received: 3 July, 2016 **Accepted:** 16 October, 2016

Introduction

Preterm birth is a serious health problem associated with neonatal morbidity and mortality (1). Preterm birth is the leading cause of neonatal mortality and the second prevalent cause of mortality in children aged less than 5 years (2). Additionally, the rate of death by other causes, such as neonatal infections, in preterm neonates are higher than term neonates (3).

The World Health Organization defined preterm labor as live birth before 37 weeks of gestation. Preterm birth is classified to three subgroups as defined by gestational age: extremely preterm (<28 weeks), very preterm (28–<32 weeks), and moderate preterm (32–<37 completed weeks of gestation) (4). Moderate preterm birth includes late preterm birth (34–<37 completed weeks of gestation). The occurrence of preterm birth was reported as 5–18%, worldwide. The prevalence of preterm labor in Iran has been estimated to be 10–15% of deliveries (4, 5). Recent strategies to avert preterm labor are used to prevent mechanisms that increase the frequency and severity of uterine contractions (6). Although, various tocolytic drugs are commonly used in this regard, there is no consensus on which is best (7).

Leptin is a peptide and a tocolytic factor hormone. It is the product of the ob gene with a 16 kda molecular weight, 167 amino acids long, and a half-life of 25 (8-10). During pregnancy, leptin is secreted from placental trophoblasts and enters from the placenta to the fetal and maternal body. Leptin can have a significant role on fetal growth and development, angiogenesis, and hematopoiesis (8, 11). Leptin levels increase during pregnancy, in which the lowest level can be assessed in the first trimester, and the highest during the second trimester. A plateau is noted in the third trimester of pregnancy (8, 12).

Results have shown that leptin can affect physiological processes such as glucose metabolism and immune system responses (9, 13, 14). Dotsch et al. (15) mentioned that the mean leptin level in term neonates was 6-fold higher than preterm neonates.

According to previous investigations, various factors, such as maternal body mass index (BMI), maternal age, and smoking cigarettes could influence the umbilical leptin levels (16). Additionally, there was a linear relationship between leptin

Address for Correspondence: *Seyedeh Hajar Sharami*
e.mail: sharami@gums.ac.ir

DOI: 10.5152/jtgga.2016.16121

level and the body lipid content (17). Generally, obese pregnant women had a lower risk of preterm birth and had a higher rate of post term birth compared to normal weight mothers (18). Laboratory studies indicated that the myometrium in obese women had a lower ability to contract and this issue might be caused by a higher level of leptin (10, 19).

As there are limited investigations on the level of leptin as a predictor for preterm birth, most studies were cross-sectional and they assessed leptin levels during birth. In this study, we aimed to investigate the relationship between levels of maternal serum leptin and the incidence of moderate preterm labor.

Material and Methods

Setting and patients

A case control study was conducted on pregnant women at Al Zahra Hospital in Rasht, north of Iran in 2013. The study was approved by the Ethical Committee of the Guilan University of Medical Sciences (Number: 1920251601, Date: 7.13.2013), and all participants provided written informed consent. The study was a pilot study to understand the relationship of maternal serum leptin levels and the incidence of moderate preterm labor. The cases included 30 pregnant women at 30–34 weeks of gestation without rupture of the membrane. They were hospitalized with a diagnosis of moderate preterm labor (uterine contractions with at least three centimeter dilation and 80% effacement). The gestational age was estimated by ultrasound reports (<20 weeks) and the date of the last menstrual period. Women were excluded if they had known associating factors such as multiple pregnancy, rupture of the membrane, known vaginal infection, cervical incontinence (cervix length less than 30 millimeter as measured by ultrasound), and conditions affecting leptin levels such as diabetes, polycystic ovarian syndrome (PCOS, based on the Rotterdam criteria including menstrual disorders, PCOS morphology, and hyperandrogenism), preeclampsia (blood pressure≥140/90 and proteinuria after 20 weeks of gestation), corticosteroid therapy, and cytotoxic drug use.

For each case, a gestational age-matched control was included in the study. The control group included pregnant women that attended the hospital prenatal clinic for routine prenatal care at 30–34 weeks of gestation. They did not mention the symptoms and signs of preterm delivery. If women in the control group delivered before 34 weeks of gestational age, we would consider them as a case and would find a control who matched, in terms of gestational age, at the time of entering into the study. In this study, we did not observe this type of participant in the control group. Data including leptin level, age, weight, height, BMI, neonatal weight, and gestational age at delivery were collected.

Height was measured in standing position and weight was measured with bare feet using a standard scale. The BMI was calculated by dividing the weight (kg) by height (m^2). Normal, overweight, and obese mothers were identified based on BMI values of <25, 25–30, and ≥30, respectively.

Leptin measurement

Five cc blood samples were drawn from each participant and were sent to the specific laboratory at 30–34 weeks of gestation. A specific human leptin LBN kit (LDN, Germany) using the enzyme-linked immunosorbent assay (ELISA) method was used to measure serum leptin levels. The measurement was performed based on the manufacturers' instruction. All blood samples were drawn and analyzed in one pre-specified laboratory.

Statistical analysis

Statistical analysis was done using SPSS software version 21.0 (IBM corp.; Armonk, NY, USA). Data was reported as mean and standard deviation. The independent t test was used for comparing means between the two groups. Multiple logistic regression analysis was applied to investigate the predicting effect of leptin level on incidence of preterm delivery. P values less than 0.05 were considered statistically significant and a 95% confidence interval was noted.

Results

The mean age in the case group (27.83±5.89) was higher than the control group (25.33±4.66), but the difference was not statistically significant (p=0.074). In the control group, BMI (p=0.017), gestational age at delivery (p=0.0001), and neonatal weight (p=0.028) were significantly higher than the case group. None of the women were smokers. The mean level of leptin in the control group (56.66±34.18) was significantly higher than the case group (33.65±16.70) (p=0.002), as shown in Table 1.

Logistic regression revealed that age and BMI did not have a significant relationship to preterm birth incidence. However, increasing the leptin level to 1 microgram per liter could decrease the risk of moderate preterm birth by 2.7% (Table 2).

Discussion

According to the results, a higher level of leptin in pregnant women could decrease the risk of birth before 34 weeks of

Table 1. Comparing demographic/reproductive characteristics between the case and control groups

Variables	Case group (n=30)	Control group (n=30)	p (t test)
Age (year)	27.83±5.89	25.33±4.66	0.074
Body Mass Index (kg/m^2)	27.59±5.78	31.10±5.28	0.017
Gestational age at delivery (week)	32.19±1.13	36.24±2.21	0.0001
Neonatal weight (gr)	2293.47±813.51	2724.83±664.86	0.028
Leptin level (μg/L)	33.65±16.70	56.66±34.18	0.002

Table 2. Logistic regression analysis to reveal the effects of maternal age, BMI, and leptin level on moderate preterm birth incidence

Variables	OR (95% Confidence Interval)	p
Age (year)	1.093 (0.975–1.225)	0.127
Body Mass Index (kg/m^2)	0.919 (0.820–1.030)	0.146
Leptin level (μg/L)	0.973 (0.948–0.997)	0.031
OR: odds ratio		

gestation. In this study, maternal age and BMI did not have significant effects on the risk of moderate preterm delivery. Leptin might change the pregnancy outcome by affecting the cytokines balance in the feto-placental unit. Furthermore, leptin could affect the activity of cytotrophoblast (20) and, by angiogenic activity, might affect placental growth (21). Some of the previous studies have shown the effect of leptin on myometrial contractions. Wuntakal et al. (10) assessed myometrial biopsies in obese women. They mentioned that access to the leptin had a protecting effect on induced myometrial contraction. They found leptin as a cause for dysfunctional labor in obese women, which led to a higher rate of cesarean section. They recommended it as a tocolytic factor that could prevent preterm birth (10). Moynihan et al. (19) assessed 18 pregnant women who underwent cesarean section after oxytocin induction. They exposed mothers to leptin and observed a decreased frequency and severity of contractions. They also reported the protecting effect of leptin on myometrial contractions (19). Laird et al. (22) assessed 53 women with a history of repeated spontaneous abortion at 5–6 and 7–8 weeks of gestation. Among them, 23 abortions, 23 term delivery, and 7 preterm births were noted. Results showed that, in both periods of assessing leptin, women with term delivery had significantly higher levels of leptin compared to women with spontaneous abortion. While slightly higher leptin was noted in term deliveries, no significant difference was noted between preterm and term groups, which might be because of the limited sample size of the preterm group. They mentioned that age and BMI had no significant effect on preterm birth, but the level of leptin could be a predictor for preterm birth (22). Shroff et al. (23) studied 1304 pregnant women in 16–27 weeks of gestation. They mentioned that maternal leptin level in term appropriate-for-gestational age infants was significantly higher than in preterm appropriate-for-gestational age infants and small for gestational age infants. This difference was noted even after controlling pre-pregnancy BMI, blood pressure disorders, and diabetes (23).

Furthermore, Palchevska et al. (24) indicated that leptin levels in 110 term neonates were higher than preterm neonates (24). Laivuori et al. (25) study on women with pre-eclampsia showed that leptin levels in pre-eclamptic women with term delivery was higher than pre-eclamptic women with preterm delivery and normal healthy women with term and preterm deliveries. Also, higher leptin levels were noted in term deliveries compared to preterm deliveries (25).

Limitations

In this study, we only assessed maternal leptin levels. It seems that leptin is a biomarker affecting preterm labor. The leptin levels in the case group were measured at a time near the delivery, which can affect our findings. Also, many socio-economic factors that can affect preterm delivery were not assessed.

In conclusion, a higher level of leptin can decrease the risk of moderate preterm birth. As leptin is secreted from lipid cells, it seems that short-term use (24 h) of leptin for postponing delivery does not have severe complications on the mothers or the fetus. Distinguishing the mechanism of tocolytic agents is recommended before they are applied and further investigations are recommended to assess leptin safety and efficacy on the prevention of preterm birth.

Ethics Committee Approval: *Ethics committee approval was received for this study from the ethics committee of Guilan University of Medical Sciences (Number: 1920251601, Date: 7.13.2013).*

Informed Consent: *Written informed consent was obtained from patients who participated in this study.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Concept - F.F., S.H.S., F.Milani; Design - F.F., S.H.S., F.Mirblouk, S.K., D.P.; Supervision - F.F., S.H.S.; Resources - F.F., S.H.S., F.Milani, F.Mirblouk, S.K.; Materials - F.F., S.H.S., F.Milani, F.Mirblouk, S.K.; Data Collection and/or Processing - D.P., H.E., S.F.D.H.; Analysis and/or Interpretation - S.H.S., D.P, H.E.; Literature Search - F.F., S.K., H.E., S.F.D.H.; Writing Manuscript - F.F., S.H.S., F.Milani, F.Mirblouk, S.K., D.P., H.E., S.F.D.H.; Critical Review - F.F., S.H.S., F.Milani, F.Mirblouk, S.K., D.P.*

Acknowledgements: *The authors would like to thank Dr. Hadi Hajizadeh, Ms. Afagh Hasanzadeh and Dr. Hadiseh Eghtedari for their cooperation. We gratefully thank the Vice Chancellor for research and technology of Guilan University of Medical Sciences for its collaboration.*

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

Financial Disclosure: *The authors declared that this study has received financial support from Vice- Chancellorship of Research and technology Gulian University of Medical Sciences.*

References

1. Beck S, Wojdyla D, Say L, Betran AP, Merialdi M, Requejo JH, et al. The worldwide incidence of preterm birth: a systematic review of maternal mortality and morbidity. Bull World Health Organ 2010; 88: 31-8. [CrossRef]
2. Liu L, Johnson HL, Cousens S, Perin J, Scott S, Lawn JE, et al. Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. Lancet 2012; 379: 2151-61. [CrossRef]
3. Lawn JE, Cousens S, Zupan J; Lancet Neonatal Survival Steering Team. 4 million neonatal deaths: when? Where? Why? Lancet 2005; 365: 891-900. [CrossRef]
4. Organisation Wh. Preterm birth, Fact sheet N°363 Genova: World health organisation; 2015. Available from: http://www.who.int/mediacentre/factsheets/fs363/en/.
5. Blencowe H, Cousens S, Chou D, Oestergaard M, Say L, Moller A, et al. Chapter 2: 15 million preterm births: Priorities for action based on national, regional and global estimates. Born Too Soon: The Global Action Report on Preterm Birth New York: March of Dimes, PMNCH, Save the Children, World Health Organization. 2012.
6. Olson DM, Christiaens I, Gracie S, Yamamoto Y, Mitchell BF. Emerging tocolytics: challenges in designing and testing drugs to delay preterm delivery and prolong pregnancy. Expert Opin Emerg Drugs 2008; 13: 695-707. [CrossRef]
7. James DK, Steer PJ, Weiner CP, Gonik B. High risk pregnancy: management options-expert consult: Elsevier Health Sciences; 2010.
8. Hauguel-de Mouzon S, Lepercq J, Catalano P. The known and unknown of leptin in pregnancy. Am J Obstet Gynecol 2006; 194: 1537-45. [CrossRef]
9. Bouloumié A, Drexler HC, Lafontan M, Busse R. Leptin, the product of Ob gene, promotes angiogenesis. Circ Res 1998; 83: 1059-66. [CrossRef]
10. Wuntakal R, Hollingworth T. Leptin–A tocolytic agent for the future? Med Hypotheses 2010; 74: 81-2. [CrossRef]
11. Lea R, Howe D, Hannah L, Bonneau O, Hunter L, Hoggard N. Placental leptin in normal, diabetic and fetal growth-retarded pregnancies. Mol Hum Reprod 2000; 6: 763-9. [CrossRef]

12. Helland IB, Reseland JE, Saugstad OD, Drevon CA. Leptin levels in pregnant women and newborn infants: gender differences and reduction during the neonatal period. Pediatrics 1998; 101: e12-e.
13. Mise H, Yura S, Itoh H, Nuamah MA, Takemura M, Sagawa N, et al. The relationship between maternal plasma leptin levels and fetal growth restriction. Endocr J 2007; 54: 945-51. [CrossRef]
14. Mantzoros CS, Moschos SJ. Leptin: in search of role (s) in human physiology and pathophysiology. Clin Endocrinol 1998; 49: 551-67. [CrossRef]
15. Dötsch J, Nüsken KD, Knerr I, Kirschbaum M, Repp R, Rascher W. Leptin and neuropeptide Y gene expression in human placenta: ontogeny and evidence for similarities to hypothalamic regulation. J Clin Endocrinol Metab 1999; 84: 2755-8. [CrossRef]
16. Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, et al. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N Engl J Med 1996; 334: 292-5. [CrossRef]
17. Nakao K, Nakata K, Ohtsubo N, Maeda M, Moriuchi T, Ichikawa T, et al. Association between nonalcoholic fatty liver, markers of obesity, and serum leptin level in young adults. Am J Gastroenterol 2002; 97: 1796-801. [CrossRef]
18. Vahratian A, Siega-Riz AM, Savitz DA, Zhang J. Maternal pre-pregnancy overweight and obesity and the risk of cesarean delivery in nulliparous women. Ann Epidemiol 2005; 15: 467-74. [CrossRef]
19. Moynihan AT, Hehir MP, Glavey SV, Smith TJ, Morrison JJ. Inhibitory effect of leptin on human uterine contractility in vitro. Am J Obstet Gynecol 2006; 195: 504-9. [CrossRef]
20. González RR, Simón C, Caballero-Campo P, Norman R, Chardonnens D, Devoto L, et al. Leptin and reproduction. Hum Reprod Update 2000; 6: 290-300. [CrossRef]
21. Sierra-Honigmann MR, Nath AK, Murakami C, García-Cardeña G, Papapetropoulos A, Sessa WC, et al. Biological action of leptin as an angiogenic factor. Science 1998; 281: 1683-6. [CrossRef]
22. Laird S, Quinton N, Anstie B, Li T, Blakemore A. Leptin and leptin-binding activity in women with recurrent miscarriage: correlation with pregnancy outcome. Hum Reprod 2001; 16: 2008-13. [CrossRef]
23. Shroff MR, Holzman C, Tian Y, Evans RW, Sikorskii A. Mid-pregnancy maternal leptin levels, birthweight for gestational age and preterm delivery. Clin Endocrinol (Oxf) 2013; 78: 607-13. [CrossRef]
24. Palchevska SKM, Shukarova E, Aluloska N, Jakimoska M, Kocevski D, et al. Comparing Preterm and Term Newborns Serum Adiponectin and Leptin Concentrations and their Correlations with Anthropometric Parameters. Macedonian J Med Sci 2012; 5: 317-23.
25. Laivuori H GM, Collura L, Crombleholme W, Markovic N, Rajakumar A, et al. Relationships between maternal plasma leptin, placental leptin mRNA and protein in normal pregnancy, pre-eclampsia and intrauterine growth restriction without pre-eclampsia. Mol Hum Reprod 2006; 12: 551-6. [CrossRef]

Are adipokines associated with gestational diabetes mellitus?

Ümit Görkem[1], Ferit Kerim Küçükler[2], Cihan Toğrul[1], Tayfun Güngör[1]
[1]Department of Obstetrics and Gynecology, Hitit University School of Medicine, Çorum, Turkey
[2]Department of Endocrinology, Hitit University School of Medicine, Çorum, Turkey

Abstract

Objective: To investigate the association of maternal serum levels of chemerin, retinol binding protein-4 (RBP-4), and visfatin with gestational diabetes mellitus (GDM).

Material and Methods: 158 pregnant women were screened between 24 and 28 weeks of gestation. They were divided into two groups: GDM group (n=76) and control group (n=82). Maternal serum concentrations of chemerin, RBP-4, visfatin, insulin, and homeostasis model assessment-insulin resistance (HOMA-IR) were assessed.

Results: There were no differences in age and gestational age between the GDM group and the control group (p=0.058 and p=0.820, respectively). Body mass index (BMI) at 24 to 28 weeks of gestation was higher in the GDM group (p<0.001). The serum concentrations of RBP-4, chemerin, and visfatin did not demonstrate significant differences between the GDM and control groups (p=0.871, p=0.100, and p=0.886, respectively). Significant differences in serum level of insulin and HOMA-IR were found between the GDM and control groups (14.94 vs 9.87, p<0.001 and 3.73 vs 1.77, p<0.001, respectively). Correlation analyses of chemerin, RBP-4, visfatin, insulin, and HOMA-IR in both groups revealed a weak degree of positive correlation between RBP-4 and chemerin (Spearman r=0.251, p=0.026) and a strong positive correlation between maternal insulin and HOMA (Spearman r=0.868, p<0.001).

Conclusion: No differences were found in serum chemerin, RBP-4, and visfatin between pregnant women with GDM and healthy pregnant women. Further prospective studies will be essential to elucidate the contribution of adipokines to GDM and the positive correlation between maternal RBP-4 and chemerin. (J Turk Ger Gynecol Assoc 2016; 17: 186-90)

Keywords: Chemerin, retinol binding protein-4, visfatin, gestational diabetes

Received: 13 July, 2016 **Accepted:** 15 October, 2016

Introduction

Important alterations in maternal metabolism and increased insulin resistance coincide with the progressive accumulation of adiposity during the course of normal pregnancy. Insulin resistance shows an increase in the late second trimester to levels that are observed in type 2 diabetes mellitus (T2DM) (1). Most pregnant women remain normoglycemic due to adequate beta-cell compensation for this higher insulin secretion. When the beta-cell compensation for insulin resistance and hepatic glucose production is inadequate, gestational diabetes mellitus (GDM) ultimately develops (1). Moreover, 10% to 50% of GDM cases are reported to develop T2DM in the postpartum period (2).

Gestational diabetes mellitus is defined as a condition of carbohydrate intolerance with onset or first recognition during pregnancy (3). The American Diabetes Association states that the incidence of GDM is between 1% and 14%, and it complicates almost 7% of pregnancies (4). GDM carries numerous risks for mothers, fetuses, and even offspring. GDM causes vascular and obstetric complications, including diabetic nephropathy, retinopathy, macrosomia, increased operative deliveries, and unexplained fetal demise. Neonatal complications, such as hypoglycemia, hypocalcemia, jaundice, respiratory distress syndrome, and cardiomyopathy, are also more prevalent. Offspring born to women with diabetes have a 1% to 3% risk of cardiovascular and metabolic disorders (2, 5).

Maternal adiposity is an important, modifiable risk factor for the development of GDM (6). Adipose tissue is not only involved in energy storage, but also functions as an active endocrine organ (7). Recent evidence supports the crucial roles of specific hormones and adipokines (i.e., cytokines) secreted by adipose tissue. Among those identified to date are adiponectin, leptin, resistin, tumor necrosis factor alpha (TNF-alpha), progranulin, omentin, chemerin, retinol binding protein-4 (RBP-4), and visfatin.

Although adipokine chemerin has conventionally been associated with regulation of adaptive and innate immunity, it also induces insulin resistance and impairs glucose tolerance. Chemerin is a novel adipokine that is secreted by various tissues, especially white adipose tissue; it regulates insulin sensitivity in adipocytes and skeletal muscle (8, 9). Increased

Address for Correspondence: *Ümit Görkem*
e.mail: drumitgorkem@hotmail.com

DOI: 10.5152/jtgga.2016.16112

levels of chemerin are detected in obesity and are associated with multiple components of metabolic syndromes, including body mass index (BMI), triglycerides, high density cholesterol, hypertension, inflammation, and markers of liver pathology (10). Chemerin is also expressed in human placenta (11). Serum levels of chemerin correlate significantly with systemic markers of inflammation, such as TNF-alpha, interleukin 6, and C-reactive protein (12). Overall, these findings suggest that chemerin levels are related to adiposity and glucose metabolism. They also represent a possible link between obesity and the development of GDM.

Retinol binding protein-4 is a blood carrier protein for retinol that is synthesized in hepatocytes and adipocytes (13). Increased levels of RBP-4 have been demonstrated in several metabolic conditions, including obesity, insulin resistance, polycystic ovary syndrome, and cardiovascular disease (14). Also, RBP-4 is believed to induce expression of enzymes involved in gluconeogenesis in hepatocytes and to impair insulin signaling pathways in skeletal muscle (15).

Visfatin is predominantly secreted by visceral tissue; however, it is also found in skeletal muscle, liver, bone marrow, lymphocytes, and placenta (16). Visfatin promotes adipogenesis and exerts insulin-mimetic effects (17). It also upregulates production of proinflammatory cytokines by monocytes (18). Circulating levels of visfatin are increased in patients with type 1 and 2 DM and obesity (13, 19). However, the association of visfatin with GDM is still unclear (20).

Only a few adipokines have been investigated with respect to their involvement in GDM. Evidence in the existing literature does not support clear roles of chemerin, RBP-4, and visfatin in the prediction of GDM. In this study, we aimed to investigate the association of maternal serum levels of chemerin, RBP-4, visfatin, and insulin with GDM. Therefore, we performed a prospective cross-sectional study in pregnant women with GDM and with normal glucose tolerance.

Material and Methods

Study population

This cross-sectional study was conducted in the Obstetrics and Gynecology Department of Hitit University between March 2015 and September 2015. The ethics committee of Ankara Numune Hospital approved the study, which was in accordance with the Declaration of Helsinki, 2013 Brazil version (20796219-724.131). All participants gave written informed consent for the study. The patients were 18 to 35 years of age and had singleton pregnancies. Pregnant women who presented themselves to our obstetrics department were screened between 24 and 28 weeks of gestation for GDM according to the recommendations of the American College of Obstetricians and Gynecologists (ACOG) (24). Briefly, all pregnant women in the low risk group were evaluated with a 50-g glucose challenge test (GCT). Women with serum glucose ≥140 mg/dL at 1 h after GCT were subjected to a 100-g oral glucose tolerance test (OGTT). Serum glucose concentrations were measured at 0, 1, 2, and 3 h after glucose ingestion. The diagnosis of GDM was based on the criteria of Carpenter and Couston, in which, after a 100-g oral glucose load, 2 or more of the following plasma values must be obtained: fasting ≥95 mg/dL, 1h ≥180 mg/dL, 2h ≥155 mg/d L, and 3h ≥140 mg/dL (21). The estimation of pregnancy duration was based on routine ultrasonographic examination performed in the first trimester. BMI was calculated using pregnancy weight and height, which were recorded at the time of blood sampling. The exclusion criteria were as follows: (1) smoking, (2) a history of diabetes mellitus and/or GDM, (3) a history of chronic disease, (4) a history of congenital malformation, (5) a family history of DM. A total of 158 pregnant women met the inclusion criteria and were divided into two groups: 76 were in the GDM group, and 82 were in the control group. The demographic characteristics and biochemical parameters of the study population, including age, BMI, and gestational age, were recorded in the second trimester.

Assays

Blood samples for adipokines and insulin were obtained from the antecubital vein after overnight fasting between 8:00 A.M. and 10:00 A.M. The samples for adipokines and insulin were centrifuged (1500 g for 25 min), and the serum was immediately stored at -80 °C until analysis. Serum glucose was determined daily using the glucose hexokinase method (Siemens Healthcare Diagnostic Limited; Camberley, UK). The serum chemerin concentration was measured by the enzyme-linked immunosorbent assay (ELISA) method (Biovendor, Biovendor-Laboratomi Medicina; Brno, Czech Republic). Serum RBP-4 concentration was determined by ELISA (Immundiagnostik, Immundiagnostik AG; Bensheim, Germany). Serum visfatin level was also determined by ELISA (Cusabio, Cusabio Biotech Co. Ltd.; Hubei, China). Serum insulin concentration was measured by chemiluminescence assay (Advia Centaur, Siemens Medical Solutions Diagnostics; Tarrytown, USA). Homeostasis model assessment-insulin resistance (HOMA-IR) was calculated using the following formula: Plasma glucose (mg/dL)×fasting plasma insulin (IUmg/L) in the fasting state divided by 405 (22).

Statistical analysis

Data were analyzed using SPSS (Statistical Package for the Social Sciences) software version 21 (IBM Corp.; Armonk, NY, USA). Continuous variables were first evaluated for normality of statistical distribution by the Shapiro-Wilk test. As the continuous variables were not normally distributed, a non-parametric method (Mann-Whitney U test) was used to perform the statistical analysis. Descriptive statistics were expressed as the median (minimum-maximum) and number (percentage %). Spearman correlation tests were used to determine the correlations of continuous variables. P values <0.05 were considered to be significant in all comparisons.

Results

The maternal demographic characteristics and biochemical parameters of the study participants are summarized in Table 1; all continuous variables are given as the median (minimum-maximum). There were no differences in age and gestational

Table 1. Comparison of maternal demographic characteristics and biochemical parameters in the gestational diabetes mellitus (GDM) and control groups

	GDM group	Control group	p
Number	76 (48.1%)	82 (51.9%)	
Maternal age (years)	29 (19-35)	26 (18-35)	0.058
Gestational age (weeks)	26.5 (24-28)	25 (24-28)	0.820
BMI (kg/m^2)	33.25 (22.80-52.20)	26.43 (19.10-47.00)	0.000*
Chemerin (ng/mL)	3.64 (2.37-7.43)	3.47 (2.19-4.63)	0.100
RBP-4 (mg/mL)	15.29 (10.08-19.63)	14.91 (10.64-31.16)	0.871
Visfatin (ng/mL)	0.07 (0.03-0.44)	0.07 (0.03-0.47)	0.886
Insulin (mIU/mL)	14.94 (1.39-32.26)	9.87 (3.53-23.98)	0.000*
HOMA-IR	3.73 (0.33-8.40)	1.77 (0.60-4.59)	0.000*

BMI: body mass index; RBP-4: retinol binding protein-4; HOMA-IR: homeostasis model assessment of insulin resistance
*p values indicate statistical significance (p<0.05).
Values are shown as median (minimum-maximum).

Table 2. Correlation analyses of data

		Chemerin	Visfatin	Insulin	HOMA-IR
RBP-4	r	0.251	-0.071	-0.018	0.021
	p	0.026*	0.534	0.873	0.854
Chemerin	r		0.192	0.196	0.161
	p		0.090	0.083	0.155
Visfatin	r			0.143	0.071
	p			0.207	0.535
Insulin	r				0.868
	p				0.000*

RBP-4: retinol binding protein-4; HOMA-IR: homeostasis model assessment of insulin resistance
*p values indicate statistical significance (p<0.05).

age at the time of the study between the GDM group and the control group (p=0.058 and p=0.820, respectively). However, the BMI of the GDM group was higher than that of the control group (p<0.001). The serum concentrations of RBP-4, chemerin, and visfatin did not demonstrate significant differences between the groups (p=0.871, p=0.100, and p=0.886, respectively). As expected, there were significant differences in serum levels of insulin and HOMA between the GDM and control groups [14.94 (1.39–32.26) vs 9.87 (3.53–23.98, p<0.001 and 3.73 (0.33–8.43) vs 1.77 (0.6–4.59), p<0.001, respectively]. The insulin levels and HOMA of the GDM group were significantly higher than those of the control group. Correlation analyses of chemerin, RBP-4, visfatin, insulin, and HOMA-IR in both groups revealed a weak degree of positive correlation between maternal RBP-4 and chemerin (Spearman r=0.251, p=0.026) and a strong positive correlation between maternal insulin and HOMA (Spearman r=0.868, p<0.001), as shown in Table 2.

Discussion

Various adipokines contribute to diabetogenic resistance to insulin, especially during the last half of pregnancy. This study mainly focused on potential alterations of specific adipokine concentrations (chemerin, RBP-4, and visfatin) in pregnant women with GDM. Here, we demonstrate that there were no significant differences in these adipokines between pregnant women with GDM and healthy pregnant women in the second trimester. However, the women with GDM were more likely to be overweight compared to matched healthy controls.

Chemerin has been proposed to be an insulin-sensitizing adipokine; its secretion has been demonstrated to increase, presumably as a compensatory mechanism, in insulin-resistant subjects (11). However, our results demonstrate the exact opposite. Various studies have evaluated the correlation of chemerin with clinical parameters in pregnant women. To date, no study has precisely determined the predictive value of chemerin concentration for risk of GDM. A recent study from Germany reported that circulating levels of chemerin did not show a significant difference between pregnant women with GDM and healthy pregnant controls (1). Garces et al. (23) found no significant association of insulin and HOMA levels with chemerin, as did other independent studies (11, 24, 25). We found similar results for chemerin in pregnant women with GDM; however, other studies presented an association of chemerin with insulin resistance in pregnancy (26, 27). Overall, it appears that chemerin may not be useful in predicting GDM.

As previously outlined, inconsistent data has been published concerning the association of RBP-4 with GDM. Some, but not all, studies have proposed that RBP-4 is positively and independently correlated with insulin resistance (28). Many studies demonstrated increased RBP-4 concentrations in GDM (14, 29), while others did not show a relationship of chemerin with GDM or normal glucose tolerance, as in our study (30, 31). One study even reported that RBP-4 concentration was decreased in GDM (26). A study was conducted by Wójcik et al. (13) concerning the relationship between adipose tissue hormones and GDM. This findings of this study conflicted with those in previous reports.

In the present study, we also found that the visfatin levels of pregnant women with and without GDM were not significantly different. Current evidence regarding the association of visfatin concentration with GDM is contradictory. Some studies demonstrated increased serum levels of visfatin in women with GDM (11, 32, 33), while others reported that visfatin concentrations were significantly lower in women with GDM (34, 35). Contrary to our study, Lewandowski et al. (32) and Akturk et al. (35) reported a significant correlation between visfatin and HOMA. It is presumed that this discrepancy may be related to differences in gestational duration at the time of sampling, the variety of diagnostic criteria for GDM, or even racial differences (36). Furthermore, Morgan et al. (37) suggested that visfatin may act locally as a paracrine/autocrine agent and not as a hormone. However, the exact mechanism remains unclear. In a study by Rezvan, no correlation of visfatin was observed with insulin or HOMA (38).

Despite the high number of studies evaluating the role of adipokines in GDM in the existing literature, interpretation of the results is somewhat laborious for several reasons. First, the diagnostic criteria for GDM vary greatly. Second, the gestational age at the study times ranges from early first trimester to late third trimester. Third, diverse assay methods may also cause heterogenous results. Obesity is accompanied by altered secretion of adipokines from adipose tissue (39, 40). Adipokine levels are usually higher in obese women. Although we demonstrated that pregnant women with GDM had higher BMI values than healthy pregnant women, there were no differences in the levels of the studied adipokines between the two groups. This may be due to the regulation of various adipokines by pregnancy or to insufficient matching of control and GDM patients for BMI.

Limitations in the present study that should be mentioned are the relatively small number of participants in each group and the assessment of maternal adipokines only in the second trimester. Furthermore, we did not analyze adipokines during the pre-pregnancy period, during the course of pregnancy, and in the postpartum period. Finally, power analysis was not performed due to the limited financial resources of this study, which was supported by the Scientific Research Unit of Hitit University.

In conclusion, despite these limitations, our study supports that serum chemerin, RBP-4, and visfatin levels in pregnant women with GDM do not differ from those of healthy pregnant women. We suggest that long-term observations of adipokines during pre-pregnancy, pregnancy, and postpartum periods would increase our understanding of the pathogenesis of GDM. Therefore, further prospective studies are essential to elucidate the contribution of adipokines to GDM and the positive correlation between maternal RBP-4 and chemerin.

Ethics Committee Approval: *Ethics committee approval was received for this study from the ethics committee of Ankara Numune Hospital (20796219-724.131).*

Informed Consent: *Written informed consent was obtained from patients who participated in this study.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Concept - Ü.G.; Design - Ü.G.; Supervision - T.G.; Data Collection and/or Processing - Ü.G., F.K.K.; Analysis and/or Interpretation - Ü.G., C.T.; Literature Search - Ü.G., C.T.; Writing Manuscript - Ü.G.; Other - T.G.*

Acknowledgements: *The authors would like to thank the staff at Hitit University for collecting the data and their assistance.*

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

Financial Disclosure: *The authors declared that this study has received financial support from the Scientific Research Unit of Hitit University, Çorum, Turkey.*

References

1. Fasshauer M, Blüher M, Stumvoll M. Adipokines in gestational diabetes. Lancet Diabetes Endocrinol 2014; 2: 488-99. [CrossRef]
2. Uzun G, Oktem S. Visfatin and its effects. Turk Klinik Biyokimya Derg 2013; 11: 119-30.
3. Teede HJ, Harrison CL, Teh WT, Paul E, Allan CA. Gestational diabetes: Development of an early risk prediction tool to facilitate opportunities for prevention. Aust N Zeal J Obstet Gynaecol 2011; 51: 499-504. [CrossRef]
4. American Diabetes Association. Gestational diabetes mellitus (Position Statement). Am J Obstet Gynecol 1987; 156: 488-9.
5. American Diabetes Association. Gestational diabetes mellitus. Diabetes Care 2004; 27(Suppl 1): S88-90. [CrossRef]
6. Ben-Haroush A, Yogev Y, Hod M. Epidemiology of gestational diabetes mellitus and its association with type 2 diabetes. Diabet Med 2004; 21: 103-13. [CrossRef]
7. Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. Nat Rev Immunol 2011; 11: 85-97. [CrossRef]
8. Roman AA, Parlee SD, Sinal CJ. Chemerin: a potential endocrine link between obesity and type 2 diabetes. Endocrine 2012; 42: 243-51. [CrossRef]
9. Sell H, Laurencikiene J, Taube A, Eckardt K, Cramer A, Horrighs A, et al. Chemerin is a novel adipocyte-derived factor inducing insulin resistance in primary human skeletal muscle cells. Diabetes 2009; 58: 2731-40. [CrossRef]
10. Catalán V, Gómez-Ambrosi J, Rodríguez A, Ramírez B, Rotellar F, Valentí V, et al. Increased levels of chemerin and its receptor, chemokine-like receptor-1, in obesity are related to inflammation: tumor necrosis factor-α stimulates mRNA levels of chemerin in visceral adipocytes from obese patients. Surg Obes Relat Dis 2013; 9: 306-14. [CrossRef]
11. van Poppel MN, Zeck W, Ulrich D, Schest EC, Hirschmugl B, Lang U, et al. Cord blood chemerin: differential effects of gestational diabetes mellitus and maternal obesity. Clin Endocrinol 2014; 80: 65-72. [CrossRef]
12. Wittamer V, Bondue B, Guillabert A, Vassart G, Parmentier M, Communi D. Neutrophil-mediated maturation of chemerin: a link between innate andadaptive immunity. J Immunol 2005; 175: 487-93. [CrossRef]
13. Wójcik M, Chmielewska-Kassassir M, Grzywnowicz K, Woźniak L, Cypryk K. The relationship between adipose tissue-derived hormones and gestational diabetes mellitus (GDM). Endokryno Polska 2014; 65: 134-42. [CrossRef]
14. Miehle K, Stepan H, Fasshauer M. Leptin, adiponectin and other adipokines in gestational diabetes mellitus and pre-eclampsia. Clin Endocrinol 2012; 76: 2-11. [CrossRef]
15. Bergmann K, Sypniewska G. Diabetes as a complication of adipose tissue dysfunction. Is there a role for potential new biomarkers? Clin Chem Lab Med 2013; 51: 177-85. [CrossRef]
16. Fukuhara A, Matsuda M, Nishizawa M, Segawa K, Tanaka M, Kishimoto K, et al. Visfatin: A protein secreted by visceral fat that mimics the effects of insulin. Science 2005; 307: 426-30. [CrossRef]
17. Briana DD, Malamitsi-Puchner A. Reviews: Adipocytokines in normal and complicated pregnancies. Reprod Sci 2009; 16: 921-37. [CrossRef]
18. Moschen AR, Kaser A, Enrich B, Mosheimer B, Theurl M, Niederegger H, et al. Visfatin, an adipocytokine with proinflammatory and immunomodulating properties. J Immunol 2007; 178: 1748-58. [CrossRef]
19. Lopez-Bermejo A, Chico-Julia B, Fernandez-Balsells M, Recasens M, Esteve E, Casamitjana R, et al. Serum visfatin increases with progressive β-cell deterioration. Diabetes 2006; 55: 2871-5. [CrossRef]
20. Haider DG, Handisurya A, Storka A, Vojtassakova E, Luger A, Pacini G, et al. Visfatin response to glucose is reduced in women with gestational diabetes mellitus. Diabetes Care 2007; 30: 1889-91. [CrossRef]
21. ACOG Practice Bulletin. Clinical management guidelines for obstetrician-gynecologists Number 30, September 2001 (replaces Technical Bulletin Number 200, December 1994). Gestational diabetes. Obstet Gynecol 2001; 98: 525-38.

22. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985; 28: 412-9. [CrossRef]
23. Garces MF, Sanchez E, Ruíz-Parra AI, Rubio-Romero JA, Angel-Müller E, Suarez MA, et al. Serum chemerin levels during normal human pregnancy. Peptides 2013; 42: 138-43. [CrossRef]
24. Barker G, Lim R, Rice GE, Lappas M. Increased chemerin concentrations in fetuses of obese mothers and correlation with maternal insulin sensitivity. J Matern Fetal Neonatal Med 2012; 25: 2274-80. [CrossRef]
25. Pfau D, Stepan H, Kratzsch J, Verlohren M, Verlohren HJ, Drynda K, et al. Circulating levels of the adipokine chemerin in gestational diabetes mellitus. Horm Res Paediatr 2010; 74: 56-61. [CrossRef]
26. Hare KJ, Bonde L, Svare JA, Randeva HS, Asmar M, Larsen S, et al. Short Report: Pathophysiology Decreased plasma chemerin levels in women with gestational diabetes mellitus. Diabet Med 2014; 31: 936-40. [CrossRef]
27. Li XM, Ji H, Li CJ, Wang PH, Yu P, Yu DM. Chemerin expression in Chinese pregnant women with and withoutgestational diabetes mellitus. Ann Endocrinol (Paris) 2015; 76: 19-24. [CrossRef]
28. Kotnik P, Fischer-Posovszky P, Wabitsch M. RBP4: a controversial adipokine. Eur J Endocrinol 2011; 165: 703-11. [CrossRef]
29. Huang QT, Huang Q, Luo W, Li F, Hang LL, Yu YH, et al. Circulating retinol-binding protein 4 levels in gestational diabetes mellitus: A meta-analysis of observational studies. Gynecol Endocrinol 2015; 31: 337-44. [CrossRef]
30. Nanda S, Nikoletakis G, Markova D, Poon LC, Nicolaides KH. Maternal serum retinol-binding protein-4 at 11–13 weeks' gestation in normal and pathological pregnancies. Metabolism 2013; 62: 814-9. [CrossRef]
31. Abetew DF, Qiu C, Fida NG, Dishi M, Hevner K, Williams MA, et al. Association of retinol binding protein 4 with risk of gestational diabetes. Diabetes Res Clin Pract 2013; 99: 48-53. [CrossRef]
32. Lewandowski KC, Stojanovic N, Press M, Tuck SM, Szosland K, Bienkiewicz M, et al. Elevated serum levels of visfatin in gestational diabetes: a comparative study across various degrees of glucose tolerance. Diabetologia 2007; 50: 1033-7. [CrossRef]
33. Coskun A, Ozkaya M, Kiran G, Kilinc M, Arikan DC. Plasma visfatin levels in pregnant women with normal glucose tolerance, gestational diabetes and pre-gestational diabetes mellitus. J Matern Fetal Neonatal Med 2010; 23: 1014-8. [CrossRef]
34. Chan TF, Chen YL, Lee CH, Chou FH, Wu LC, Jong SB, et al. Decreased plasma visfatin concentrations in women with gestational diabetes mellitus. J Soc Gynecol Investig 2006; 13: 364-7. [CrossRef]
35. Akturk M, Altinova AE, Mert I, Buyukkagnici U, Sargin A, Arslan M, et al. Visfatin concentration is decreased in women with gestational diabetes mellitus in the third trimester. J Endocrinol Invest 2006; 31: 610-3. [CrossRef]
36. Krzyzanowska K, Krugluger W, Mittermayer F, Rahman R, Haider D, Shnawa N, et al. Increased visfatin concentrations in women with gestational diabetes mellitus. Clin Sci (Lond) 2006; 110: 605-9. [CrossRef]
37. Morgan SA, Bringolf JB, Seidel ER. Visfatin expression is elevated in normal human pregnancy. Peptides 2008; 29: 1382-9. [CrossRef]
38. Rezvan N, Hosseinzadeh-Attar MJ, Masoudkabir F, Moini A, Janani L, Mazaherioun M. Serum visfatin concentrations in gestational diabetes mellitus and normal pregnancy. Arch Gynecol Obstet 2012; 285: 1257-62. [CrossRef]
39. Rasouli N, Kern PA. Adipocytokines and the metabolic complications of obesity. J Clin Endocrinol Metab 2008; 93: S64-73. [CrossRef]
40. Galic S, Oakhill JS, Steinberg GR. Adipose tissue as an endocrine organ. Mol Cell Endocrinol 2010; 316: 129-39. [CrossRef]

Furan toxicity on testes and protective role of lycopene in diabetic rats

Özlem Kara, Hatice Baş, Dilek Pandır

Department of Biology, Bozok University School of Arts and Science, Yozgat, Turkey

Abstract

Objective: Furan (C_4H_4O) is a heat-induced food contaminant that is utilized as an industrial chemical agent. Lycopene is a natural substance that is produced by plants and tomatoes. We aimed to evaluate the toxicity of furan on testes and the protective effect of lycopene in diabetic rats.

Material and Methods: Male Wistar albino rats were divided into five groups: Group 1 (control group) received 1 mL/kg corn oil. Group 2 (diabetic control group) received 55 mg/kg STZ and 1 mL/kg corn oil. Group 3 (diabetic lycopene group) received 55 mg/kg STZ and 4 mg/kg lycopene. Group 4 (diabetic furan group) received 55 mg/kg STZ and 40 mg/kg furan. Group 5 (diabetic furan + lycopene group) received 55 mg/kg STZ, 40 mg/kg furan, and 4 mg/kg lycopene. After 28 days, the testes were extirpated in all groups. In the testicular tissue samples, the level of malondialdehyde (MDA) and the activities of catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD), and reducted glutathione (GST) were studied. Serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone levels were measured. Histopathologic examination was performed by light microscope.

Results: The MDA level and the activities of CAT, GPx, SOD, and GST were found to be higher in the furan group than in the control and diabetic control groups ($p<0.05$). The MDA level and the activities of CAT, GPx, SOD, and GST were significantly lower in the furan + lycopene group than in the furan group ($p<0.05$).

Conclusion: The low blood testosterone level in the rats who received furan suggested the presence of endocrinological defects and cellular degenerative changes. Lycopene may be effective to reverse furan toxicity in diabetic rat testes. (J Turk Ger Gynecol Assoc 2016; 17: 191-6)

Keywords: Furan, rat, testis, toxicity, lycopene, diabetes mellitus

Received: 23 August, 2016 **Accepted:** 15 October, 2016

Introduction

Furan is a chemical agent that is used in industry for the production of organic compounds, herbicides, plastics, and pharmaceuticals (1). Coffee, canned meats, sodium caseinate, nuts, hydrolyzed soy protein, and rape seed protein are natural products that contain furan (2). However, furan should be used with caution due to potential harmful effects on adipose tissue, liver, and kidneys (3). Polychlorinated dibenzofurans accumulate in human fat tissue; if the critical range is exceeded, water enters the cells. This event leads to an increase in the production of reactive oxygen species (ROS). ROS cause mutations in protein and DNA, lipid peroxidation in membranes, apoptosis, and damage to cellular structures (4, 5).

The amount of oxygen is very low in the testes due to weak vascularity. Therefore, oxidative stress can be detrimental to spermatogenesis and steroidogenesis in testicular tissue. Although the testes contain low levels of oxygen, they are sensitive to oxidative stress because they contain large amounts of unsaturated fatty acids and ROS-generating systems (6). Cells produce antioxidants as protection from the harmful effects of ROS. The actions of catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD), and reducted glutathione (GST) increase in testicular tissue due to oxidative stress (7).

Lycopene is a natural substance that is found in fruits and vegetables such as tomatoes, carrots, strawberries, and cherries (8). Structurally, it is a carotenoid derivative, and it acts as a free-radical scavenger. Lycopene exhibits antioxidant activity due to its long chain and conjugated double bonds (9). Coyne et al. (10) reported increased lycopene levels in adults with type 2 diabetes mellitus; this effect was found to be associated with decreased ROS levels. Li et al. (11) investigated the effects of lycopene in diabetic patients. They found that lycopene could be used in the prevention and treatment of diabetic retinopathy. This is the first study demonstrating the effects of furan toxicity on the testes. Therefore, we aimed to assess the toxicity of furan and the protective effects of lycopene in the testes of diabetic rats.

Material and Methods

35 male adult Wistar albino rats weighing 300 to 320 g were obtained from the Experimental Animal Laboratory of Çukurova University. The rats were inserted into special plas-

Address for Correspondence: *Özlem Kara*
e.mail: ozlemozturk34@hotmail.com

DOI: 10.5152/jtgga.2016.16144

tic cages and were fed with free access to both water and a standard pellet diet. The study was approved by the Institutional Ethics Committee of Çukurova University.

Chemicals

In this experimental study, three substances, including streptozotosin (STZ), furan, and lycopene, were administered; all chemicals used were obtained from Sigma. Furan and lycopene were dissolved in corn oil before being administered to the animals.

Implementation plan for animals

Chemicals were administered to the rats between 9:00 A.M. and 11:00 A.M. 55 mg/kg STZ was given to each rat by intraperitoneal (ip) injection as a single dose. Two days later, the blood glycose levels of the rats were measured. Rats with a glucose range of 300 mg/dL were accepted as diabetic. Furan was administered one hour after lycopene administration. These two substances were given to the rats by gavage once daily for 28 days.

Randomization

The animals were randomly allocated into groups. The randomization was performed using www.randomization.com. The study crew did not know which animal was in which group. The animals were divided into five groups.

Study design

Thirty-five male rats were divided into five groups: group 1 (control group) received 1 mL/kg corn oil. Group 2 (diabetic control group) received 55 mg/kg STZ and 1 mL/kg corn oil. Group 3 (diabetic lycopene group) received 55 mg/kg STZ and 4 mg/kg lycopene. Group 4 (diabetic furan group) received 55 mg/kg STZ and 40 mg/kg furan. Group 5 (diabetic furan + lycopene group) received 55 mg/kg STZ, 40 mg/kg furan, and 4 mg/kg lycopene. After 28 days, the testes of the rats in all groups were resected. In the testicular tissue samples, the level of MDA and the activities of CAT, GPx, SOD, and GST were studied. Serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone levels were measured. Histopathologic examination was performed by light microscope.

Ketamine hydrochloride (100 mg/kg, Alfamine, Egevet; İzmir, Turkey) and xylazin hydrochloride (10 mg/kg, Xylazinbio, Intermed; Ankara, Turkey) were administered intraperitoneally for anesthesia. All the rats were allowed to breathe throughout the intervention. Ultimately, the testes were extirpated.

The testicular samples were treated with sodium phosphate solution, and the tissues were maintained at –80°C. The Aebi method was used to measure CAT enzyme activity (12). The Paglia and Valentine method was used to measure GPx activity (13). The Marklund and Marklund method (14) was utilized to measure SOD activity. The MDA level was assessed utilizing the test described by Ohkawa et al. (15) GST activity was determined by the Habig test (16). Protein content was evaluated by the Lowry method (17).

Histopathological examination

The testicular samples were maintained in 10% formalin solution for 1 day. Then, the tissues were dehydrated and fixed in paraffin. The thickness of the tissues was 5 to 6 μm. The sections were stained with hematoxylin and eosin dye. The sections were analyzed and photographed with an Olympus BX 51 light microscope (Olympus Corp.; Tokyo, Japan).

The histopathological assessment was performed as follows: grade 0, normal; grade I, no hemorrhage and no follicular degeneration, no leukocyte infiltration, mild edema and congestion; grade II, no hemorrhage and no follicular degeneration, no leukocyte infiltration, moderate edema and congestion; grade III, severe edema and congestion, hemorrhage, follicular degeneration, and leukocyte infiltration.

Statistical analysis

The Statistical Package for the Social Sciences version 17.00 (SPSS Inc.; Chicago, IL, USA) was utilized for the statistical analyses. The homogenity of the data was evaluated with the Kolmogorov-Smirnov test. The Kruskal–Wallis test was used to evaluate the tissue damage scores and biochemical markers. $p<0.05$ was accepted as significant.

Results

Evaluation of biochemical parameters

There were significant differences between the control and diabetic control groups in terms of MDA level and enzymatic activities (Figure 1).

MDA level

The MDA level was higher in the diabetic control group than in the control group ($p<0.05$). The MDA level was lower in group 3 than in group 2 ($p<0.05$). The MDA level was lower in group 5 than in group 4 ($p<0.05$) (Figure 1a).

SOD activity

SOD activity was higher in the diabetic furan group than in the diabetic control group ($p<0.05$). SOD activity was lower in group 5 than in group 4 ($p<0.05$) (Figure 1b).

CAT activity

CAT activity was greatly diminished in the diabetic furan + lycopene group compared to the diabetic furan group ($p<0.05$) (Figure 1c).

GPx activity

GPx activity was higher in group 4 than in group 2 ($p<0.05$). Group 5 had higher GPx activity than group 4 ($p<0.05$) (Figure 1d).

GST activity

GST activity was found to be lower in group 5 than in group 4 ($p<0.05$) (Figure 1e).

Effects of furan and lycopene treatment on plasma hormone levels

Plasma FSH level

When plasma FSH levels were compared, a statistically significant decrease was found in group 4 compared to group 5 ($p<0.05$).

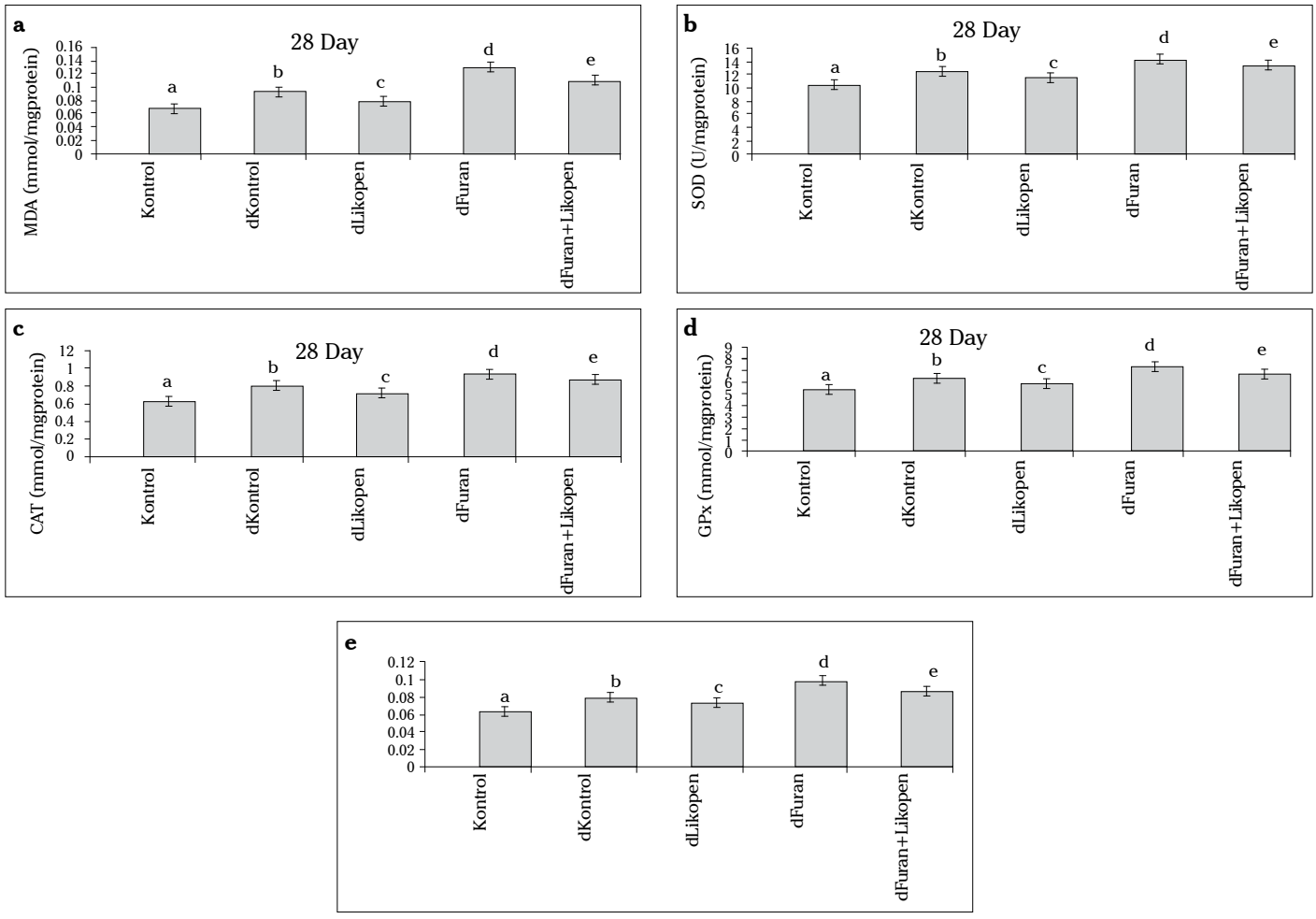


Figure 1. a-e. Comparison of the MDA level (a) and SOD (b), CAT (c), GPx (d), and GST activities in the testes of the control and experimental groups.

Table 1. Mean hormone levels between control and experimental groups

Group	FSH (mIU/mL)	LH (mIU/mL)	Testosterone (ng/mL)	p
Control	3.32±0.03	1.42±0.021	4.04±0.06[a]	0.89
Diabetic control	3.21±0.02	1.29±0.035	3.93±0.02	0.57
Diabetic lycopene	3.26±0.02	1.36±0.024	3.85±0.04	0.13
Diabetic furan	2.67±0.05*	0.88±0.047*	2.81±0.03*	0.01[a]
Diabetic furan + lycopene	2.81±0.07	0.97±0.033	2.96±0.08	0.94

FSH: follicle-stimulating hormone; LH: luteinizing hormone
*Statistically significant.
[a]Kruskal-Wallis test was used.

Plasma LH level

The lowest plasma LH level was in group 4; the addition of lycopene minimally increased the plasma LH level, and this difference was found to be significant ($p<0.05$).

Plasma testosterone level

The plasma testosterone range was lower in group 4 than in group 5 ($p<0.05$) (Table 1).

Evaluation of histopathological damage

The morphology of the testicular tissue was normal in the control group (Figure 2a). After STZ administration, mild edema and vascular congestion were detected in the diabetic control group (Figure 2b). The tissue of the diabetic lycopene group showed mild edema and detachments of the basal cells of the seminiferous tubules (Figure 2c). In group 4, leukocyte infiltration and edema were detected in the testicular stroma. Degeneration was observed in the seminiferous tubules and germ cells (Figure 2d). Treatment with lycopene reduced testicular tissue damage. No leukocyte infiltration, edema, hemorrhage, or follicular degeneration was observed in the diabetic furan + lycopene group (Figure 2e).

The histopathological assessment was performed as follows: grade 0, normal; grade I, no hemorrhage and no follicular degeneration, no leukocyte infiltration, mild edema and congestion; grade II, no hemorrhage and no follicular degeneration, no leukocyte infiltration, moderate edema and congestion; grade III, severe edema and congestion, hemorrhage, follicular degeneration, and leukocyte infiltration. The testicular tissue damage scores in group 5 were lower than in group 4, and this difference was found to be significant ($p<0.05$). The control and diabetic furan + lycopene groups were the least-influenced groups; the damage scores and biochemical values were superior in these groups (Table 2).

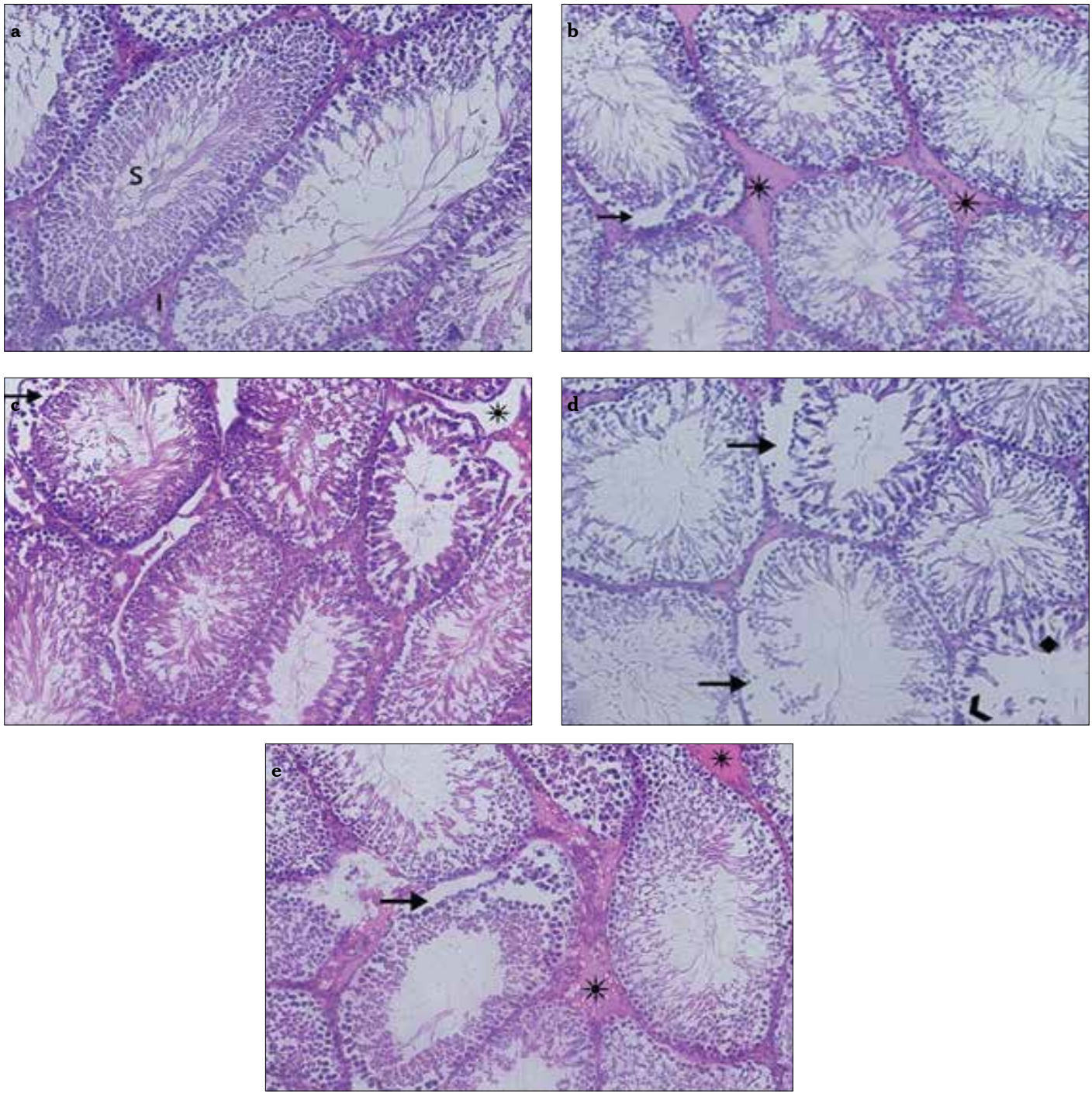


Figure 2. a-e. Appearance of testes by light microscope. Control group (a), S: seminar tubul, I: interstitial space, x200. Diabetic control group (b), (*) edema, (black arrow) separation in the basal cells of the seminiferous tubules. Diabetic lycopene group (c), (black arrow) separation in the basal cells of the seminiferous tubules, (*) edema. Diabetic furan group (d), (black arrow) separation in the basal cells of the seminiferous tubules, (♦) degeneration, (>) basal cell loss in the seminiferous tubules. Diabetic furan + lycopene group (e), (*) edema, (black arrow) separation in the basal cells of the seminiferous tubules

Discussion

In this study, the toxicity of furan and the protective effect of lycopene on the testes of diabetic rats were evaluated. The results of this study demonstrate that tissue MDA levels and the enzymatic activities of SOD, CAT, GPx, and GST appear to be elevated with furan treatment. A decrease was detected in the MDA level and enzymatic activities after supplementation with lycopene. Also, plasma FSH, LH, and testosterone levels were found to decrease when furan was administered. Lycopene

Table 2. Distribution of histopathologic findings according to the number of animals

Group	Histopathological score			
	0	I	II	III
Control	6	-	-	-
Diabetic control	-	4	2	-
Diabetic lycopene	2	3	1	-
Diabetic furan	-	-	1	5
Diabetic furan + lycopene	-	-	5	1

addition appears to reverse the negative effects of furan on testicular tissue. This is the first study describing the effects of lycopene on diabetic rats treated with furan.

Furan is an important environmental contaminant with a heterocyclic structure. The toxic effects of furan are well known from animal studies (1-3). Gill et al. (18) studied the toxic effects of furan on the hepatocytes of rats. They found that furan showed no adverse effects or hepatic toxicity at a dose of 0.03 mg/kg. However, we administered a furan dose of 40 mg/kg in our study. Furan is mutagenic, and some tumors have been demonstrated to be associated with furan usage (19, 20). Moser et al. (21) reported that oxidative stress induced by furan in cells causes an increase in ROS levels.

Studies have reported improvements in cellular and seminiferous structures by elimination of obstructions due to testicular artery ligation (22, 23). Oxidative stress can be detrimental to spermatogenesis and steroidogenesis in Leydig cells (7). Therefore, we believed that utilization of lycopene might protect testes from furan-induced toxicity.

Lycopene is an antioxidant that has been investigated for the prevention and treatment of oxidative stress. It neutralizes free radicals and has antioxidant properties (24). Lycopene has been demonstrated to have protective effects against breast, endometrium, and liver cancers (25). Oxidative stress due to diabetes mellitus can lead to endotelial dysfunction. Li et al. (11) showed that lycopene could repair vascular endothelial damage in diabetic patients. Coyne et al. (10) reported that lycopene and serum carotenoids could reverse the harmful effects of type 2 diabetes mellitus. In our study, control and diabetic control groups were compared in terms of antioxidant enzyme activities. The enzyme activities were significantly higher in the diabetic control group than in the control group. In the diabetic lycopene group, antioxidant enzyme activities were lower than in the diabetic control group. The light microscopic findings and hormone levels, such as FSH, LH, and testosterone, indicated cellular degenerative changes.

Testicular tissue uses different antioxidant enzymes to protect itself from damage by free radicals. These enzymes include CAT, GPx, SOD, and GST (6). Therefore, we studied the tissue MDA levels and the enzyme activities of CAT, GPx, SOD, and GST to assess the oxidative damage due to furan and the possible protective effects of lycopene. According to our results, the MDA level and CAT, GPx, SOD, and GST activities were found to be higher in group 4 than group 1 and 2 ($p<0.05$). When plasma FSH, LH, and testosterone levels were compared, they were lower in group 4 than in the other groups ($p<0.05$).

Although these findings are short term, the results could ultimately be useful to protect the testes of diabetic rats from furan toxicity. However, large-scale randomized studies are needed to evaluate the protective effects of lycopene on furan damage in the testis.

Ethics Committee Approval: *Ethics committee approval was received for this study from the ethics committee of Çukurova University (09/12/2013/11).*

Informed Consent: *N/A.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Concept - Ö.K., D.P.; Design - Ö.K., D.P.; Supervision - Ö.K., H.B., D.P.; Resources - Ö.K., H.B.; Materials - Ö.K., H.B.; Data Collection and/or Processing - Ö.K., H.B.; Analysis and/or Interpretation - Ö.K., H.B.; Literature Search - Ö.K., H.B.; Writing Manuscript - Ö.K., H.B., D.P.; Critical Review - Ö.K., H.B., D.P.*

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

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

References

1. Lynne WE, Alphonse ES. Phenotypic caracterization of metaplastic intestinal glands and ductular hepatocytes in cholangiofibrotic lesions rapidly induced in the caudate liver lobe of rats treated with furan. Cancer Res 1991; 51: 5752-9.
2. Maga JA. Furans in foods. CRC Crit Rev Food Sci Nutr 1979; 11: 355-400. [CrossRef]
3. Kedderis GL, Ploch SA. The biochemical toxicology of furan. Chemical Industry Institute of Toxicol 1999; 19:1-8.
4. Parlakpinar H, Sahna E, Acet A, Mizrak B, Polat A. Protective effect of caffeic acid phenethyl ester (CAPE) on myocardial ischemia-reperfusion induced apoptotic cell death. Toxicol 2005; 209:1-14. [CrossRef]
5. Peterson LA, Cummings ME, Chan JY, Vu CC, Mateer BA. Identification of a cis- 2- butene-1,4 –dial-derived glutathione conjugate in the urine of furan- treated rats, Chemical Research in Toxicol 2006; 19: 1138-41. [CrossRef]
6. Atiken RJ, Roman SD. Antioxidant systems and oxidative stress in the testes. Oxid Med Cell Longev 2008; 1: 15-24. [CrossRef]
7. Peltola V, Huhtaniemi I, Ahotupa M. Antioxidant enzyme activity in the maturing rat testis. J Androl 1992; 13: 450-5.
8. Hadley CW, Clinton SK, Schwartz SJ. The consumption of processed tomato products enhances plasma lycopene concentretions in association with a reduced lipoprotein sensitivity to oxidative damage. J Nutr 2003; 133: 727-32.
9. Bast A, Haenen GR, Van Den Berg R, Van Den Berg H. Antioxidant effects of carotenoids. Int J Vitam Nutr Res 1998; 68: 399-403.
10. Coyne T, Ibiebele TI, Baade PD, Dobson A, Mc Clintock C, Dunn S, et al. Diabetes mellitus and serum carotenoids: findings of a population-based study in Queensland, Australia. Am J Clin Nutr 2005; 82: 685-93.
11. Li ZZ, Lu XZ, Ma CC, Chen L. Serum lycopene levels in patients with diabetic retinopathy. Eur J Ophthalmol 2010; 20: 719-23.
12. Aebi H. Catalase in vitro. Methods Enzymol 1984; 105: 121-6. [CrossRef]
13. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of glutathione peroxidase. J Lab Med 1987; 70: 158-65.
14. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem 1974; 47: 469-74. [CrossRef]

15. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid rection. Anal Biochem 1979; 95: 351-8. [CrossRef]
16. Habig WH, Pabst MJ, Jakoby WB. Glutathione-S-transferases: the first enzymatic step in mercapturic acid formation. J Biol Chem 1974; 249: 7130-9.
17. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193: 265-75.
18. Gill S, Bondy G, Lefebvre DE, Becalski A, Kavanagh M, Hou Y, et al. Subchronic oral toxicity study of furan in Fischer-344 rats. Toxicol Pathol 2010; 38: 619-30. [CrossRef]
19. Perez Locas C, Yaylayan VA. Origin and mechanistic pathways of formation of the parent furan, a food toxicant. J Agric Food Chem 2004; 52: 6830-6. [CrossRef]
20. Mariotti MS, Toledo C, Hevia K, Gomez JP, Fromberg A, Granby K, et al. Are Chileans exposed to dietary furan? Food Addit Contam 2013; 30: 1715-21. [CrossRef]
21. Moser GJ, Foley J, Burnett M, Goldsworthy TL, Maronpot R. Furan induced dose-response relationships for liver cytotoxicity, cell proliferation, and tumorigenicity (furan-induced tumorigenicity). Exp Toxicol Pathol 2009; 61: 101-11. [CrossRef]
22. Ozgur H, Kaya M, Doran SS. Ultrastructure of the seminiferous tubules in human testes before and after varicocelectomy. Anat Embryol 2003; 207: 343-53. [CrossRef]
23. Kaya M. Sertoli cells and various types of multinucleates in the rat seminiferous tubules following temporary ligation of the testicular artery. J Anat 1986; 144: 15-29.
24. Hadley CW, Clinton SK, Schwartz SJ. The consumption of processed tomato products enhances plasma lycopene concentretions in association with a reduced lipoprotein sensitivity to oxidative damage. J Nutr 2003; 133: 727-32.
25. Erhardt JG, Meisner C, Bode JC. Lycopene, beta-carotene and colorectal adenomas. Am J Clin Nutr 2003; 78: 1219-24.

Impact of office hysteroscopy in repeated implantation failure: Experience of a single center

Emre Göksan Pabuçcu[1], İbrahim Yalçın[2], Taylan Bodur[3], Gamze Sinem Çağlar[1], Recai Pabuçcu[1,4]

[1]Department of Obstetrics and Gynecology, Ufuk University School of Medicine, Ankara, Turkey

[2]Department of Obstetrics and Gynecology, Zekai Tahir Burak Women's Health and Research Hospital, Ankara, Turkey

[3]Department of Obstetrics and Gynecology, Yücelen Muğla Hospital, Muğla, Turkey

[4]Centrum Clinic Women's Health and ART Centre, Ankara, Turkey

Abstract

Objective: Repeated implantation failure (RIF) is a clinical entity affecting many couples undergoing assisted reproductive technology (ART). Various intrauterine pathologies contribute to RIF. Nevertheless, vaginal sonography and hysterosalpingography, which are the common diagnostic tools for the initial follow-up, have limited sensitivities. In this context, we aimed to evaluate the impact of office hysteroscopy (oHS) on live birth rates (LBRs) when performed prior to subsequent ART cycles in women with a history of RIF.

Material and Methods: The database of an assisted reproduction center was retrospectively reviewed to detect eligible cases. A total of 363 women out of 2875 admissions were consecutively included in the analysis, of which 119 formed the oHS group and 244 formed the non-oHS group prior to a new ART cycle. Women in the oHS arm were examined during their early follicular phase via a vaginoscopic approach 1–6 months before the beginning of a new cycle. The standard *in-vitro* fertilization-intracytoplasmic sperm injection (IVF/ICSI) cycle was applied to all the women.

Results: In the oHS group (n=119), 61 patients had intrauterine abnormalities, with an overall abnormality rate of 51.2%. Implantation, pregnancy, and LBRs of the groups were statistically similar. LBRs of the women with abnormal oHS findings (15/61, 24.5%), with normal oHS findings (14/58, 24.1%), and without oHS (39/244, 16%) were statistically similar (p=0.41).

Conclusion: Unrecognized intrauterine pathologies can be easily detected and concurrently treated during oHS with high success rate. However, a beneficial impact depends on the extent of the pathology and thus, routine application to enhance reproductive outcomes is still not warranted. (J Turk Ger Gynecol Assoc 2016; 17: 197-200)

Keywords: Endometrium, *in-vitro* fertilization, office hysteroscopy, pregnancy

Received: 23 September, 2016 **Accepted:** 17 October, 2016

Introduction

Repeated implantation failure (RIF) is a distressing clinical entity and refers to a situation when transferred embryo(s) repeatedly fail to implant despite numerous attempts via assisted reproductive technology (ART). Several uterine pathologies, such as polyps, adhesions, septum, or myomas, have been linked with poor reproductive outcomes when detected prior to ART (1, 2). Such pathologies have been reported in up to 50% of women with RIF, leading to suggestions that corrections could improve reproductive outcomes (3). Office hysteroscopy (oHS) is considered as the ''gold standard'' for assessment of the uterine cavity, which also provides a chance for concurrently treating uterine pathologies. Despite several advantages, the European Society of Human Reproduction and Endocrinology does not recommend routine oHS in women undergoing ART, unless there is an apparent uterine pathology (4). Moreover, two recent randomized controlled trials have failed to show any clear benefit of this procedure either before the first IVF cycle or in repeated failed cycles (5, 6).

The present study aimed to evaluate the incidence of unrecognized uterine pathologies with oHS in women with RIF as well as the impact of oHS on live birth rates (LBRs) when performed prior to a new ART cycle.

Material and Methods

This is a retrospective cohort study of infertile patients who were treated at a private fertility center between 2007 and 2014. As this study was retrospectively designed, institutional review board approval was not obtained. RIF was defined as two or more unsuccessful ART/embryo transfer cycles despite the availability of good quality embryos (7). According to the ART center's policy, all women with a history of RIF were suggested to undergo an oHS procedure preceding a new ovarian stimulation cycle despite normal vaginal sonography (TVS) or hysterosalpingography (HSG).

Address for Correspondence: *Emre Göksan Pabuçcu*
e.mail: pabuccu@hotmail.com

DOI: 10.5152/jtgga.2016.16166

Subjects who underwent the procedure formed the oHS group, whereas the remaining subjects formed the non-oHS group. All included patients were between 18 and 40 years of age and had follicle-stimulating hormone (FSH) levels of <15 IU/mL. The exclusion critertia were 1) poor ovarian response according to the Bologna criteria (8) or women with premature ovarian failure; 2) male subjects with severe oligozoospermia, oligoasthenozoospermia, or azoospermia; 3) preimplantation genetic screening and cryopreserved/thawed embryo transfer cycles; 4) women with confirmed endometriosis; 5) women with hypothalamic amenorrhea; and 6) women who underwent oHS more than 6 months prior to a new cycle.

Office hysteroscopy procedure

All patients were examined during their early follicular phase, 1–6 months before the start of a new ART cycle, via the vaginoscopic approach as previously described (9). No routine preoperative analgesia, antibiotics, sedation, or cervical preparation was used. Briefly, a rigid hysteroscope (continuous flow; 30° forward oblique view) with an outer diameter of 4 mm using 0.9% normal saline was used. Following adequate distension of the uterine cavity, systematic inspection was performed. Standard gynecologic surgical procedures were used to treat the recognized pathologies, such as removing all polyps and adhesions. A senior physician (R.P.) performed all the procedures.

Ovarian stimulation protocol

All the couples were subjected to ICSI and all sperm injections were performed with fresh specimens. One ART cycle of each patient was included in the study. All the OS cycles were conducted using the short antagonist protocol either with recombinant (Gonal-F, Merck Serono; İstanbul, Turkey) or with human menopausal gonadotropins (Menogon, Ferring; İstanbul, Turkey) (150–300 IU/day s.c.). Ovarian stimulation, oocyte retrieval, and embryo transfer procedures were performed as described elsewhere (10). Top quality embryos were defined as those with ≥7 evenly sized cells and ≤10% fragmentation on day 3 and with a ≥3 AA quality of blastocyst morphology on day 5. During the study period, one embryo was transferred to patients aged <35 years, while two embryos were transferred for those ≥35 years, in accordance with the local legislation. Embryo implantation was defined as the proportion of women with an intrauterine gestational sac on an ultrasound scan ≥4 weeks after embryo transfer, with the rate calculated as the number of gestational sacs divided by the number of embryos transferred. The miscarriage rate was defined as the proportion of women with pregnancy loss before 24 weeks of gestation. The pregnancy rate was defined as the proportion of women with a positive quantitative serum human chorionic gonadotropin test 12 days after embryo transfer, while LBR was defined as the delivery of a live fetus beyond 24 weeks of gestation after one ART cycle.

Statistical analysis

SPSS 22.0 statistical software (IBM Corp.; Armonk, NY, USA) was used for data analysis. The values of the measurement data are expressed herein as the mean±SD when applicable. Between-group differences were compared using the independent samples t-test. Classified information was statistically analyzed using Pearson's χ^2 test, one-way analysis of variance, and Fisher's exact test. Statistical significance was defined as $p<0.05$.

Results

A total of 440 RIF cases were detected out of 2875 admissions during the study period, of which 366 met the inclusion criteria. Among those, three women were excluded from the analysis due to operative HS work out, and finally, 363 women were consecutively selected for the analysis, of which 119 formed the oHS group and 244 formed the non-oHS group. The time interval between oHS and a new ART cycle was 1–6 months and during this period, the procedure was the first and only one performed for those in the oHS arm. There were no statistically significant differences between the two groups with regard to demographic properties, basal patient characteristics, and general cycle outcomes, as shown in Table 1, 2, respectively.

In the oHS group (n=119), 61 patients had intrauterine abnormalities, with an overall abnormality rate of 51.2%. The types and incidence of abnormalities were mild intrauterine and cervical adhesions (25/61, 40.9%), endometrial polyps (23/61, 37.7%), polypoid endometrium (11/61, 18.1%), and arcuate uterus (2/61, 3.2%).

According to the reproductive outcomes, implantation, pregnancy, and LBRs of the groups were statistically similar (Table 3). LBRs of the women with abnormal oHS findings (15/61, 24.5%), normal oHS (14/58, 24.1%), and women without oHS (39/244, 16%)

Table 1. Basal patient characteristics of the groups

Variable	oHS group	Non-HS group	p
Age (years)	30.7±5.3	31.93±4.4	0.52
Duration of infertility (years)	6.94±3.72	7.83±3.47	0.39
AFC (in both ovaries)	8.3±3.0	8.66±2.43	0.47
Number of previous ART attempts	4.04±1.5	4.06±1.21	0.89

AFC: antral follicle count; ART: assisted reproductive technology; oHS: office hysteroscopy; non-HS: non-hysteroscopy
Results are given in terms of the mean (±SD).

Table 2. Cycle characteristics of the groups

Variable	oHS group	Non-HS group	p
Total gonadotropin dosage (IU)	2318±800	2556±941	0.01*
Total stimulation days, n	10.92±1.92	10.76±2.48	0.56
Peak endometrial echo (mm)	11.4±1.8	10.6±1.4	0.46
No of retrieved oocytes, n	9.04±4.36	8.21±5.03	0.12
No of transferred embryos, n	1.63±0.58	1.61±0.62	0.70
Fertilization rate (%)	67.3±20.7	63.37±25.02	0.38

oHS: office hysteroscopy; non-HS: non-hysteroscopy
Results are given in terms of the mean (±SD).
*p<0.05.

Table 3. Reproductive outcomes of the groups

	oHS group (119)	Non-HS group (244)	p
Implantation rate (%)	22.39±39.86	18.70±36.57	0.38
Positive hCG, n (%)	41 (34.4)	63 (25.8)	0.08
LBR/ET, n (%)	29 (24.3)	39 (15.9)	0.06
Miscarriages, n (%)	10 (8.4)	18 (7.3)	0.26

hCG: human chorionic gonadotropin; LBR: live birth rate; oHS: office hysteroscopy; non-HS: non-hysteroscopy; ET: embryo transfer
Results are given in terms of the mean (±SD).

were statistically similar (p=0.41). There were no complications encountered during or after the procedure in women who underwent oHS.

Discussion

According to our results, oHS prior to a new OS cycle does not improve LBRs in women with RIF. A slightly higher LBR was noted in the oHS group without reaching statistical significance. In routine practice, TVS and HSG are the main tools to document the uterine texture prior to ART; however, the diagnostic accuracies of these are quite low with limited sensitivities and specificities (11-14). Diagnostic limitation is likely to be more prominent in those with a history of RIF, as the frequency of unrecognized pathologies may be up to 50% (2, 15). In the study by Gao et al. (16), nearly 80% of intrauterine abnormalities were found to be undiagnosed with HSG or TVS in those with RIF. This rate is approximately 50% in our data. As all the women in the oHS arm underwent their first oHS procedure in our study, the prevalence of abnormalities was considered to be relatively high. It seems that a considerable number of RIF cases are somehow misdiagnosed as having "normal" uterine texture for a period of time, unless oHS is performed prior to a new cycle. All the given data justify the need for oHS despite normal findings on TVS or HSG in RIF cases.

One of the most beneficial impacts of oHS is the correction of specific uterine cavity abnormalities when detected. Endometrial polyps and different degrees of adhesions are the most common findings in women with RIF in the literature and in our data as well (2, 17). Adhesions are likely to be unrecognized with TVS during initial follow-up, and they should be better removed when detected in order to maintain successful implantation (18-20). Endometrial polyps are quite common and have been shown to compromise pregnancies, depending on the size, by interfering with embryo implantation (21). It has been shown that polypectomy prior to IVF, even for small polyps (<2 cm), might improve the take-home baby rate in patients undergoing IVF (22). Thus, the routine removal of polyps prior to a new ART attempt is also suggested (21). Despite previous data underlining the beneficial impact of correcting unsuspected uterine cavity abnormalities, a very recent TROPHY trial failed to demonstrate such an impact (5). This might be explained with the fact that they identified cervical or uterine cavity abnormalities only in 26% of women and two-thirds of those were not treated. In our data, apparent polyps and adhesions were the most common findings, and 40% of the women in the oHS group were treated.

It has been shown that the fertility-enhancing effect of oHS could also be independent from the correction of intrauterine abnormalities. Hysteroscopy has been proposed to improve ART outcomes through an endometrial injury process leading to embryo implantation (23, 24). The outcomes of the women in the oHS arm without any pathologic findings being generally more favorable compared to those without oHS in our data is speculating this impact. In their meta-analysis, El-Toukhy et al. (1) showed a significant improvement in the outcome of the normal hysteroscopy subgroup compared to in controls (RR=1.63, 95% CI 1.35–1.98, p<0.001). The time interval between mechanical injury and ET is also speculative to influence pregnancy outcomes. In a recent meta-analysis, endometrial scratching (four studies) or oHS (three studies) was shown to increase the CPRs of women with a history of RIF when induced in the preceding cycle of OS (25). The same favorable results have been reported when oHS was performed within 50 days (13) or even within 6 months prior to a new ART (16). On the other hand, this impact is somehow questionable in light of the recent conflicting evidence (5, 26). Further research is still needed to optimize instrumentation and timing.

The limitations of this study are its retrospective design and lack of power analysis. On the other hand, the complete data set was from a single center and the same senior physician performed all the oHS procedures, as this should better overcome inter-observer discrepancies. In conclusion, unrecognized intrauterine pathologies can be easily detected and concurrently treated during the oHS procedure with high success rates. However, the overall beneficial impact in terms of reproductive outcomes seems depending on the extent of the pathology.

Ethics Committee Approval: *N/A.*

Informed Consent: *N/A.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Concept - E.G.P.; Design - E.G.P., G.S.Ç.; Supervision - R.P.; Resources - R.P.; Materials - R.P., G.S.Ç.; Data Collection and/or Processing - E.G.P., İ.Y., T.B.; Analysis and/or Interpretation - E.G.P., G.S.Ç., İ.Y.; Literature Search - E.G.P., İ.Y., T.B., G.S.Ç.; Writing Manuscript - E.G.P., G.S.Ç.; Critical Review - R.P.*

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

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

References

1. El-Toukhy T, Sunkara S, Coumarasamy A, Grace J, Khalaf Y. Outpatient hysteroscopy and subsequent IVF cycle outcome: a systematic review and meta-analysis. Reprod Biomed Online 2008; 16: 712-9. [CrossRef]
2. Makrakis E, Hassiakos D, Stathis D, Vaxevanoglou T, Orfanoudaki E, Pantos K. Hysteroscopy in women with implantation failures after invitro fertilization: findings and effects on subsequent pregnancy rates. J Minim Invasive Gynecol 2009; 16: 181-7. [CrossRef]
3. Moini A, Kiani K, Ghaff ari F, Hosseini F. Hysteroscopic findings in patients with a history of two implantation failures following in vitro fertilization. Int J Fertil Steril 2012; 6: 27-30.

4. Crosignani PG, Rubin BL. Optimal use of infertility diagnostic tests and treatments. The ESHRE Capri Workshop Group. Hum Reprod 2000; 15: 723-32. [CrossRef]
5. El-Toukhy T, Campo R, Khalaf Y, Tabanelli C, Gianaroli L, Gordts SS, et al. Hysteroscopy in recurrent in-vitro fertilisation failure (TROPHY): a multicentre, randomised controlled trial. Lancet 2016; 387: 2614-21. [CrossRef]
6. Smit JG, Kasius JC, Eijkemans MJ, Koks CA, van Golde R, Nap AW, et al. Hysteroscopy before in-vitro fertilisation (inSIGHT): a multicentre, randomised controlled trial. Lancet 2016; 387: 2622-9. [CrossRef]
7. Polanski LT, Baumgarten MN, Quenby S, Brosens J, Campbell BK, Raine-Fenning NJ. What exactly do we mean by 'recurrent implantation failure'? A systematic review and opinion. Reprod Biomed Online 2014; 28: 409-23. [CrossRef]
8. Ferraretti P, La Marca A, Fauser B, Tarlatzis B, Nargund G, Gianaroli L; ESHRE working group on Poor Ovarian Response Definition. ESHRE consensus on the definition of 'poor response' to ovarian stimulation for in vitro fertilization: the Bologna criteria. Hum Reprod 2011; 26; 1616-24. [CrossRef]
9. El-Mazny A, Abou-Salem N, El-Sherbiny W, Saber W. Outpatient hysteroscopy: a routine investigation before assisted reproductive techniques? Fertil Steril 2011; 95: 272-6. [CrossRef]
10. Pabuccu R, Pabuccu EG, Gursoy AY, Caglar GS, Yilmaz MB, Ozdegirmenci O. Improved cycle outcomes after laparoscopic ovarian diathermy in hyper-responder patients with previous ART failure. Gynecol Endocrinol 2014; 30: 881-4. [CrossRef]
11. Cunha-Filho J, de Souza C, Salazar C, Facin A, Freitas F, Passos E. Accuracy of hysterosalpingography and hysteroscopy for intrauterine lesions in infertile patients in an assisted-fertilization programme. Gynecologic Endoscopy 2001; 10: 45-8. [CrossRef]
12. Grimbizis G, Tsolakidis D, Mikos T, Anagnostou E, Asimakopoulos E, Stamatopoulos P, et al. A prospective comparison of transvaginal ultrasound, saline infusion sonohysterography, and diagnostic hysteroscopy in the evaluation of endometrial pathology. Fertil Steril 2010; 94: 2720-5. [CrossRef]
13. Karayalçin R, Ozyer S, Ozcan S, Uzunlar O, Gürlek B, Moraloğlu O, et al. Office hysteroscopy improves pregnancy rates following IVF. Reprod Biomed Online 2012; 25: 261-6. [CrossRef]
14. Taskin E, Berker B, Ozmen B, Sönmezer M, Atabekoglu C. Comparison of hysterosalpingography and hysteroscopy in the evaluation of the uterine cavity in patients undergoing assisted reproductive technologies. Fertil Steril 2011; 96: 349-52. [CrossRef]
15. Bozdag G, Aksan G, Esinler I, Yarali H. What is the role of office hysteroscopy in women with failed IVF cycles? Reprod Biomed Online 2008; 17: 410-5. [CrossRef]
16. Gao M, Sun Y, Xie H, Fang S, Zhao X. Hysteroscopy prior to repeat embryo transfer may improve pregnancy outcomes for asymptomatic women with repeated implantation failure. J Obstet Gynaecol Res 2015; 41: 1569-76. [CrossRef]
17. Dicker D, Ashkenazi J, Feldberg D, Farhi J, Shalev J, Ben-Rafael Z. The value of repeat hysteroscopic evaluation in patients with failed in-vitro fertilization cycles. Fertil Steril 1992; 58: 833-5. [CrossRef]
18. Soares SR, Barbosa dos Reis MM, Camargos AF. Diagnostic accuracy of sonohysterography, transvaginal sonography, and hysterosalpingography in patients with uterine cavity diseases. Fertil Steril 2000; 73: 406-11. [CrossRef]
19. Margalioth EJ, Ben Chetrit A, Gal M, Eldar-Geva T. Investigation and treatment of repeated implantation failure following IVF-ET. Human Reprod 2006; 21: 3036-43. [CrossRef]
20. Oliveira F, Abdelmassih V, Diamond M, Dozortsev D, Nagy ZP, Abdelmassih R. Uterine cavity findings and hysteroscopic interventions in patients undergoing in vitro fertilization- embryo transfer who repeatedly cannot conceive. Fertil Steril 2003; 80: 1371-5. [CrossRef]
21. Coughlan C, Ledger W, Wang Q, Liu F, Demirol A, Gurgan T, et al. Recurrent implantation failure: definition and management. Reprod Biomed Online 2014; 28: 14-38. [CrossRef]
22. Lass A, Williams G, Abusheikha N, Brinsden P. The effect of endometrial polyps on outcomes of in-vitro fertilization (IVF) cycles. J Assist Reprod Genet 1999; 16: 410-5. [CrossRef]
23. Demirol A, Gürgan T. Effect of treatment of intrauterine pathologies with office hysteroscopy in patients with recurrent IVF failure. Reprod Biomed Online 2004; 8: 590-4. [CrossRef]
24. Kilic Y, Bastu E, Ergun B. Validity and efficacy of office hysteroscopy before in vitro fertilization treatment. Arch Gynecol Obstet 2013; 287: 577-81. [CrossRef]
25. Potdar N, Gelbaya T, Nardo LG. Endometrial injury to overcome recurrent embryo implantation failure: a systematic review and meta-analysis. Reprod Biomed Online 2012; 25: 561-71. [CrossRef]
26. Panagiotopoulou N, Karavolos S, Choudhary M. Endometrial injury prior to assisted reproductive techniques for recurrent implantation failure: a systematic literature review. Eur J Obstet Gynecol Reprod Biol 2015; 193: 27-33. [CrossRef]

Metabolic and carbohydrate characteristics of different phenotypes of polycystic ovary syndrome

Ebru Çelik[1], Ilgın Türkçüoğlu[1], Barış Ata[2], Abdullah Karaer[1], Pınar Kırıcı[1], Sevil Eraslan[1], Çağatay Taşkapan[3], Bülent Berker[4]

[1]Department of Obstetrics and Gynecology, İnönü University School of Medicine, Malatya, Turkey

[2]Department of Obstetrics and Gynecology, Koç University School of Medicine, İstanbul, Turkey

[3]Department of Clinical Biochemistry, İnönü University School of Medicine, Malatya, Turkey

[4]Department of Obstetrics and Gynecology, Ankara University School of Medicine, Ankara, Turkey

Abstract

Objective: To compare the prevalence of various metabolic and cardiovascular risk factors and insulin resistance between polycystic ovary syndrome (PCOS) patients with or without hyperandrogenism.

Material and Methods: This is a retrospective cross-sectional study involving women with PCOS as diagnosed according to the Androgen Excess (AE) Society definition (n=504) and women with normoandrogenemic PCOS (n=183). Anthropometrics, lipid profile, glucose, insulin, oral glucose tolerance test (OGTT), and reproductive hormone levels were evaluated.

Results: Women with PCOS diagnosed according to the AE Society had a significantly higher prevalence of metabolic syndrome compared with the normoandrogenemic PCOS phenotype: odds ratio (OR) 2.95 [95% confidence interval (CI) 1.21–7.21]. There was no significant difference in the prevalence glucose intolerance test between the groups [OR: 2.15, 95% CI 0.71–6.56]. The prevalence of low high density lipoprotein (HDL)-cholesterol in the group under the AE-PCOS Society criteria was higher than that of the normoandrogenemic PCOS group [OR: 2.82, 95%CI 1.29–3.36].

Conclusion: The risks of metabolic syndrome and cardiovascular disease may vary among the phenotypes of PCOS based on the Rotterdam criteria. This new data may be of reference in informing women with PCOS, although further prospective studies are needed to validate this proposition. (J Turk Ger Gynecol Assoc 2016; 17: 201-8)

Keywords: Polycystic ovary syndrome, diagnostic categories, metabolic syndrome, hyperanderogenism, insulin resistance

Received: 1 August, 2016 **Accepted:** 27 October, 2016

Introduction

Polycystic ovary syndrome (PCOS) is a frequent endocrine abnormality of women in the reproductive period, and is related with infertility and menstrual irregularities (1, 2). PCOS is a heterogeneous condition, presenting with hyperandrogenism, polycystic ovarian morphology (POM), and oligomenorrhea (3-5).

The etiology of PCOS is elusive, but its long-term implications, including the risk of cardiovascular disease, type-2 diabetes mellitus, and endometrial carcinoma, are well known (6-8). However, PCOS is regarded as the most common endocrine abnormality of women in the reproductive period; its predicted frequencies vary in the literature, ranging from 2.2% to up to 26% (2, 9-12). A major reason for this variation is the absence of a consensus on the diagnostic criteria. Opinions differ on whether the presence of hyperanderogenism is an absolute requirement for a diagnosis of PCOS. The same holds true for the requirement of POM (13).

Chronologically, the National Institutes of Health (NIH) proposed the first diagnostic criteria. The NIH criteria required the combination of chronic oligo/anovulation and clinical or biochemical evidence of hyperandrogenism, with the exclusion of related disease (14). Subsequently, the Rotterdam European Society of Human Reproduction and the Embryology/American Society for Reproduction Medicine (ESHRE/ASRM) Sponsored PCOS (Rott-PCOS) Consensus Workshop group proposed the addition of POM to the NIH criteria, with a statement that PCOS could be diagnosed when any two of these three criteria were present (13). More recently, the Androgen Excess Society (AES) proposed new diagnostic criteria and stated that androgen excess is the *sine qua non* of PCOS and the syndrome must only be diagnosed in the presence of hyperanderogenism in combination with oligo/anovulation and/or POM (15). Compared to the recent AES criteria, the Rotterdam criteria include an additional PCOS phenotype, comprising women with POM and oligo/anovulation in the absence of androgen excess. It is still unknown whether this new phenotype is also related to long-term health risks, such as metabolic syndrome, insulin resistance, and obesity (13, 16). Diagnosing normoandrogenemic women with PCOS has attracted

Address for Correspondence: *Ebru Çelik*
e.mail: ecelik05@googlemail.com

DOI: 10.5152/jtgga.2016.16133

criticism (15). Although the normoandrogenemic PCOS phenotype appears to be weakly associated with adverse reproductive outcomes and metabolic syndrome compared with hyperandrogenemic PCOS, some evidence suggests that women with normoandrogenemic PCOS present with more severe insulin resistance (IR) and dyslipidemia (17-19).

Whether women with only POM and oligo/amenorrhea bear similar metabolic and cardiovascular risks as compared with hyperandrogenemic women diagnosed with PCOS as per the AES criteria remains to be determined. This study aims to compare the prevalence of various metabolic and cardiovascular risk factors and insulin resistance between women diagnosed as PCOS with or without hyperandrogenism.

Material and Methods

This is a retrospective cohort study involving women who presented to our Gynecology Clinic between April 2011 and August 2012. The study protocol was approved by the Institutional Ethic Committee.

Demographic features and medical information regarding menstrual cycles, obstetric and gynecological history, and previous medication and/or disease were collected from the medical records. Body mass index (BMI) was calculated as body weight (in kilograms) divided by height (in meters squared). The waist to hip ratio (W/H) was calculated from dividing the waist circumference by the hip circumference.

A total of 1048 consecutive women diagnosed with PCOS were analyzed. PCOS was diagnosed with regard to the Rotterdam criteria, i.e., according to the existence of at least two of the following three features: 1) oligo- or amenorrhea; 2) clinical or biochemical hyperandrogenism; or 3) POM on pelvic ultrasound, after exclusion of other causes of hyperandrogenism, thyroid disorders, hyperprolactinemia, Cushing syndrome, and late onset congenital adrenal hyperplasia. Women with type-1 diabetes mellitus, hepatic or renal pathology, or receiving medication, which could affect carbohydrate metabolism or lipid profile, were also excluded. The follow-chart of subjects is presented in Figure 1.

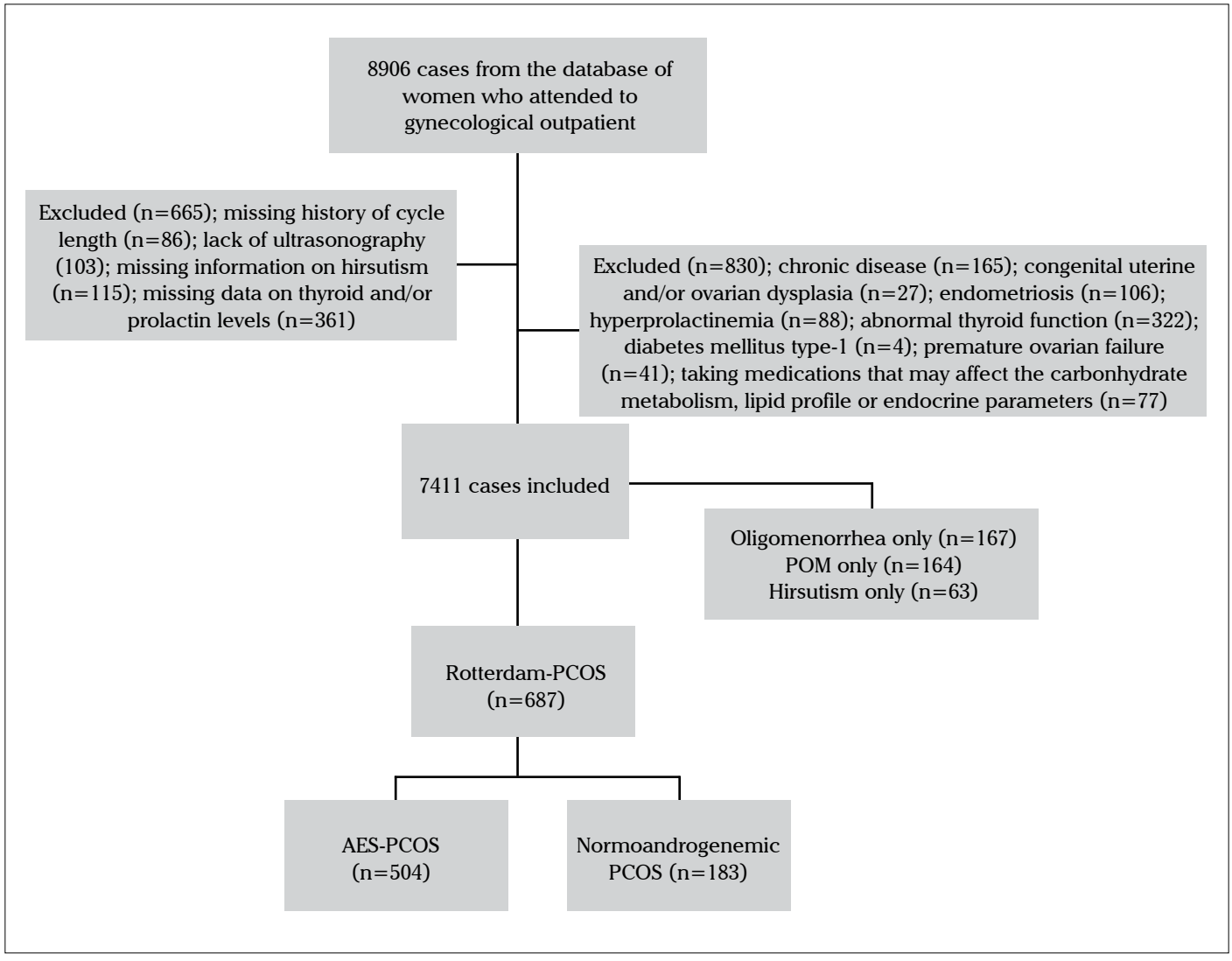


Figure 1. The flowchart of subjects who attended the clinic

PCOS subtypes

The participants were divided into two groups: i) those fulfilling the AES criteria (n=504) (15) and ii) women with normoandrogenemic PCOS (oligomernorrhea with POM; n=183).

The criteria used to determine the components of the PCOS phenotypes were:

- Clinical hyperandrogenism was determined with a modified Ferriman-Gallewey score>8 (20).
- Biochemical hyperandrogenism was defined with the existence of at least one of the following findings: serum total testosterone level (tT)>65.82 ng/dL, serum dehydroepiandrosterone sulfate (DHEAS) level>374.9 μg/dL and free androgen index (FAI)>4.94 (calculated on the basis of the 95th percentile of basal serum androgen normality in the control group of 70 healthy, non-hirsute, eumenorrheic women from the same area (unpublished data).
- Oligomenorrhea was defined as having <8 menstrual cycles/year or menstrual cycles for more than 35 days.
- POM was defined with an antral follicle count (2–9 mm) of≥12 in at least one ovary.

Definition of insulin resistance

Insulin resistance was predicted by using the homeostatic model assessment of insulin resistance (HOMA-IR) (21). The following formula was used to calculate HOMA-IR: (fasting insulin in μU/mL x fasting glucose in mg/dL)/405. A HOMA-IR value ≥3.8 was considered to represent insulin resistance (22). A 120-minute 75-g oral glucose tolerance test (OGTT) was done on a random day of the cycle. Impaired glucose tolerance test (IGTT) was defined by an abnormal glucose value following the 75-g OGTT, with cut-off values between 140 and 199 for 120 minute. A value of 200 mg/dl or higher subsequent to 75-g OGTT were considered as diabetes mellitus type-2 (23).

Definition of metabolic syndrome (MetS) and dyslipidemia

The diagnosis of MetS was made in accordance to the definitions proposed by the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III). Based on the NCEP ATP III, the diagnosis of MetS in women was defined by the presence of at least three of the following features: (i) waist circumference≥88 cm, (ii) serum triglyceride (TG) level≥150 mg/dL, (iii) serum high density lipoprotein (HDL-cholesterol) level<50 mg/dL or the previous consumption of lipid lowering medication, (iv) blood pressure≥130/85 mmHg or the use of anti-hypertensive medication, (v) fasting blood glucose≥100 mg/dL (24).

Dyslipidemia was diagnosed according to the definitions proposed by the Framingham/Adult Treatment Panel (ATP) III criteria (25). Regarding Framingham/ATP III, the diagnosis of dyslipidemia in women was defined by the following features: (i) low density lipoprotein (LDL-cholesterol)≥130 mg/dL, (ii) HDL-cholesterol<50 mg/dL, (iii) triglyceride≥150 mg/dL, (iv) total cholesterol (TC)≥200 mg/dL, (v) TC/HDL≥5.6.

Laboratory analyses

Comprehensive endocrine screening, including early follicular phase follicle stimulating hormone (FSH), luteinizing hormone (LH), total testosterone (tT), dehydroepiandrosterone sulfate (DHEAS), sex hormone-binding globulin (SHBG), thyroid stimulating hormone (TSH), and prolactin, was carried out. Plasma insulin, FSH, LH, SHBG, DHEAS, and TSH levels were analyzed by a chemiluminescence method (Immulite 2000, Siemens Medical Solutions Diagnostics; Los Angeles, CA, USA). FAI was determined as follows: FAI=tTx100/SHBG.

Fasting glucose and insulin, lipids, and carbohydrates levels were also determined. The plasma glucose, total cholesterol, HDL-cholesterol, and TG levels were tested by the spectrophotometric method (Abbott Trade C16000, Abbott Laboratories; Abbott Park, IL, USA). LDL-cholesterol and VLDL-cholesterol were calculated using Friedewald formulation. Highly sensitive C-reactive protein (hs-CRP) was analyzed by the nephelometric method (Siemens Dade-Behring BNII, Siemens Healthcare Diagnostics Inc.; Newark, BE, USA).

Statistical analyses

Continuous variables are defined herein with the mean±standard deviation (SD), and categorical variables with numbers and percentages as appropriate. Baseline features of the different PCOS phenotypes were compared by independent samples t test. Proportions were compared using the chi-square test. Statistical significance was set at a two sided p value≤0.05. The Statistical Package for Social Sciences (SPSS) was used for statistical analysis (version 19.0, SPSS Inc.; Chicago, IL, USA).

Results

Study population

The medical records of 8906 women were reviewed; 665 women were excluded due to inadequate data, including for unavailable data on the history of the cycle length (n=86), ultrasonographic examination (n=103), hirsutism (n=115), thyroid and/or prolactin laboratory assay (n=361). An additional 830 women were excluded due to the presence of concomitant pathology, including other relevant chronic diseases (n=165), congenital uterine anomalies and/or ovarian dysplasia (n=27), endometriosis (n=106), hyperprolactinemia (n=88), abnormal thyroid function (n=322), diabetes mellitus type-1 (n=4), premature ovarian failure (n=41), and the previous consumption of medications that may affect the carbohydrate pathway, lipid profile, or endocrine parameters (n=77) (Figure 1). The final study population included 687 women diagnosed with PCOS based on Rott-PCOS criteria. While 504 of these women met the AES -PCOS criteria, 183 women were classified as normoandrogenemic PCOS. The prevalence of different PCOS phenotypes is presented in Table 1.

The prevalence of women with clinical and/or biochemical hyperandrogenism, oligomenorhea (OM), and POM was 7.6, 8.5, and 7.1%, respectively.

Demographic characteristics, and the biochemical and anthropometric features of the participants are presented in Table 2. There were no differences among the PCOS subgroups regarding age (p=0.62; Table 2). The mean concentration of C-reactive protein (CRP) in women diagnosed as PCOS according to the AES criteria was significantly higher than that of women classified as normoandrogenemic PCOS (5.96±6.92 vs. 4.21±3.12 mg/L, p=0.003).

Table 1. Prevalence of PCOS phenotypes among the same population

Phenotypes	n	%
Screened population	7411	
Rotterdam Criteria - PCOS	687	9.3
AES Criteria - PCOS	504	6.8
PCOS with all 3 criteria (HA+POM+OM)	277	3.7
Normoandrogenemic PCOS (OM+POM)	183	2.5
Ovulatory PCOS (HA+POM)	144	1.9
HA+OM	83	1.1

PCOS: polycystic ovary syndrome; AES: Androgen Excess Society; HA: hyperandrogenism; POM: polycystic ovary morphology; OM: oligemenorrhea

Prevalence of insulin resistance

A comparison of the data between AES-PCOS and normoandrogenemic PCOS revealed a significant difference for the carbohydrate traits assessed, including HOMA-IR (p=0.004), fasting insulin (0.007), and fasting glucose (p=0.001).

The overall prevalence of elevated fasting blood glucose and insulin resistance among the PCOS patients was 17.8% and 31.7%, respectively. While the rates of elevated blood glucose and insulin resistance were 19.9% and 32.8% in the AES-PCOS group, within the women classified as normoandrogenemic PCOS, these rates were 10.0% and 26.3%, respectively (p=0.01 and 0.34, respectively). The respective odds ratios for insulin resistance in patients diagnosed as PCOS according to the AES criteria was 1.36 [95% confidence interval (CI), 0.77–2.58] when compared with the subjects classified as normoandrogenemic PCOS (Table 3).

Table 2. Demographic, anthropometric, and metabolic parameters of various PCOS phenotypes

Variables	AES-PCOS (n_{avail})	Normoandrogenemic PCOS (n_{avail})	p
Age	24.73±5.86 (504)	24.48±5.54 (183)	0.62
Body Mass Index (kg/m^2)	26.12±6.26 (168)	23.96±4.51 (24)	0.10
Waist circumference (mm)	78.66±13.53 (158)	77.92±17.20 (19)	0.82
Hip circumference (mm)	103.25±17.09 (158)	102.46±21.03 (19)	0.85
Waist to hip ratio	0.79±0.09 (158)	0.76±0.10 (19)	0.17
Systolic blood pressure (mmHg)	110 (110-120) (99)	115 (102-120) (19)	0.53
Diastolic blood pressure (mmHg)	70 (62-80) (99)	70 (60-80) (19)	0.20
Follicule stimulating hormone (mIU/mL)	5.67±2.09 (502)	5.77±2.03 (183)	0.55
Luteinizing hormone (mIU/mL)	7.23±5.24 (503)	8.13±5.51 (183)	0.05
Thyroid stimulating hormone (mIU/mL)	1.58±0.84 (504)	1.58±0.83 (183)	0.99
Prolactin (ng/mL)	13.16±7.81 (504)	13.58±7.88 (183)	0.54
Total testosterone (ng/dL)	48.03±89.04 (346)	31.84±12.46 (72)	0.12
Free testosterone (ng/dL)	6.40±9.35 (183)	2.88±2.11 (32)	<0.01*
Sex hormone binding globulin (nmoL/mL)	40.73±31.95 (346)	64.64±43.84 (72)	<0.01
Free androgen index	6.11±9.43 (346)	2.32±1.39 (72)	<0.01*
Dehydroepiandrosterone sulfate (μg/dL)	243.75±117.05 (368)	181.89±79.67 (80)	<0.001*
Ferriman-Gallewey score, median (IQR)	13 (10-18) (404)	6 (4-7) (175)	<0.001*
Fasting Insulin (μIU/mL)	15.69±20.35 (298)	10.84±10.16 (57)	0.007*
Fasting glucose (mg/dL)	91.81±12.60 (442)	87.83±9.83 (120)	0.001*
§HOMA-IR	4.53±6.65 (296)	2.94±2.96 (57)	0.004*
Total Cholesterol (mg/dL)	170.09±35.59 (382)	168.60±38.22 (87)	0.73
Triglycerides (mg/dL)	113.14±64.62 (382)	98.98±52.41 (87)	0.03*
†HDL-cholesterol (mg/dL)	48.01±11.76 (383)	52.28±13.15 (85)	0.004*
‡LDL-cholesterol (mg/dL)	101.15±29.14 (374)	96.95±31.98 (86)	0.23
¥VLDL-cholesterol (mg/dL)	23.29±14.88 (373)	19.84±11.26 (83) (Matthews, Hosker et al.)	0.02*
Triglycerides/†HDL-cholesterol ratio	2.63±1.92 (376)	2.06±1.41 (85)	0.002*
C-reactive protein (mg/L)	5.96±6.92 (253)	4.21±3.12 (62)	0.003*

AES: Androgen Excess Society; n_{avail}.: the number of women available for each characteristic measured; HOMA-IR: homeostatic model assessment of insulin resistance; HDL-cholesterol: high density lipoprotein; LDL-cholesterol: low density lipoprotein; VLDL-cholesterol: very low density lipoprotein
Data are expressed as the mean±SD unless otherwise was stated.
*Statistically significant (p<0.05).

Table 3. The prevalence of obesity, insulin resistance, and the components of metabolic syndrome according to the International Diabetes Federation criteria and the compositions of dyslipidemia according to the Adult Treatment Model III National Cholesterol Education Program criteria among all the PCOS phenotypes

Variables	AES-PCOS (n)	Normoandrogenemic PCOS (n)	p*	Odds Ratio (95% Confidence interval)
BMI≥25 (kg/m²)	49.4% (83/168)	41.7% (10/24)	0.48	0.73 [0.31-1.74]
BMI≥27 (kg/m²)	39.9% (67/168)	37.5% (9/24)	0.82	0.90 [0.37-2.18]
BMI≥30 (kg/m²)	25.6% (43/168)	12.5% (3/24)	0.16	1.59 [0.63-3.97]
Insulin resistance (HOMA-IR≥3.8)	32.8% (97/296)	26.3% (15/57)	0.34	1.36 [0.77-2.58]
Oral Glucose Test Intolerance	21.2% (31/146)	11.1% (4/36)	0.23	2.15 [0.71-6.56]
Diabetes Mellitus type-2	1.4% (2/146)	-	-	-
Fasting glucose ≥100 mg/dL	19.9% (88/442)	10.0% (12/120)	0.01*	2.23 [1.17-4.24]
Waist circumference ≥88 cm	50.6% (80/158)	42.1% (8/19)	0.48	1.41 [0.54-3.69]
Blood pressure ≥130/85 mmHg	5.1% (5/99)	5.3% (1/19)	0.96	0.96 [0.15-6.06]
HDL-cholesterol <50 mg/dL	59.4% (228/384)	41.2% (35/85)	0.002*	2.82 [1.29-3.36]
Triglycerides ≥150 mg/dL	23.0% (88/322)	14.9% (13/87)	0.09	1.70 [0.90-3.21]
Total cholesterol ≥200 mg/dL	18.6% (71/382)	17.2% (15/87)	0.77	1.09 [0.59-2.02]
LDL-cholesterol ≥130 mg/dL	15.2% (57/374)	11.6% (10/86)	0.39	1.21 [0.59-2.48]
Triglycerides/HDL-cholesterol ≥5.6	8.5% (32/376)	3.5% (3/85)	0.12	2.54 [0.76-8.51]
Metabolic syndrome (any more than two components)	25.4% (62/244)	10.3% (6/58)	0.01*	2.95 [1.21-7.21]

PCOS: polycystic ovary syndrome; BMI: body mass index; AES-PCOS: Androgen Excess Society-Polycystic Ovary Syndrome; HOMA-IR: homeostatic model assessment of insulin resistance; HDL: high density lipoprotein; LDL: low density lipoprotein
Data are expressed as the number and percentage unless otherwise stated.
*Statistically significant (p<0.05).

The prevalence of IGTT was 19.9% in the whole PCOS population. In the AES-PCOS group, 31 of 146 patients (21.2%) had impaired glucose tolerance during OGTT and of these, two patients had DM type 2 (1.4%), while the prevalence rate for the abnormal glucose tolerance test in the normoandrogenic PCOS group was 11.1% (p=0.23). The respective odds ratio for the glucose intolerance test in patients diagnosed as PCOS according to the AES criteria was 2.15 [95% CI, 0.71–6.56] when compared with subjects classified as normoandrogenemic PCOS.

Prevalence of MetS and dyslipidemia

While the overall rate of metabolic syndrome in women with PCOS was 22.5%, within women diagnosed as PCOS according to the AES-PCOS criteria and normoandrogenemic PCOS, these rates were 25.4 and 10.3%, respectively (p=0.01; Table 3). The respective odds ratio for MetS in women with AES-PCOS was 2.95 [95% CI, 1.21–7.21] when compared with subjects classified as normoandrogenemic PCOS. The distribution of the components of metabolic syndrome is also presented in Table 3.

Regarding the lipid profile, the concentrations of HDL-cholesterol, TG, TG/HDL-cholesterol ratio, and VLDL-C were significantly different in women with AES-PCOS from the subjects with normoandrogenemic PCOS (p=0.004, p=0.03, p=0.002, and p=0.02, respectively; Table 2). The prevalence of low HDL-cholesterol in the group under the AE-PCOS Society criteria was higher than that of normoandrogenemic PCOS (59.4 vs 41.2%, respectively; p=0.002; Table 3), while the prevalence of low HDL-cholesterol was 56.1% in the whole study group. While the prevalence of elevated total cholesterol (≥200 mg/dL) and LDL-C (≥130 mg/dL) parameters in the whole population were 18.3 and 14.6%, respectively, there were no statistically significant differences between women diagnosed as PCOS according to the AE-PCOS Society criteria and women with normoandrogenemic PCOS (18.6% vs. 17.2% and 15.2% vs. 11.6%; p=0.77 and p=0.39, respectively). While the prevalence of elevated TG (≥150 mg/dL) was 21.5% in the whole study group, within the patients diagnosed as PCOS according to the AE-PCOS Society criteria and normoandrogenemic PCOS, these rates were 23.0 and 14.9%, respectively (p=0.09). While the prevalence of elevated TC to HDL-cholesterol ratio (≥5.6) in the whole population was 7.6% within women diagnosed as PCOS according to AE-PCOS, this rate did not differ from subjects with normoandrogenemic PCOS (8.5 and 3.5%, respectively; p=0.12).

Discussion

In the current study, the prevalence of PCOS according to the Rotterdam and AE-PCOS Society criteria in a Caucasian population were 9.3 and 6.8%, respectively. Although the results of the current study are in concurrence with the previously reported frequency's ranging from 11.2 to 20.9% according to the Rotterdam criteria (18, 26-28), we found lower figures than a prior Turkish cohort study using the AE-PCOS Society criteria (18).

Further, data from this study illustrated that the prevalence of insulin resistance and impaired glucose tolerance barely rise in the overall PCOS population (31.7 and 17.8%, respectively) when compared to that reported from another study of Turkish women with PCOS (29). This study has also shown that women with PCOS have a higher prevalence of metabolic syndrome (22.5%) than that consistently reported from the literature, albeit with a varying rate (17, 18, 28).

Although there is limited data on the association of the new phenotype (normoandrogenemic PCOS) and its implications on the metabolic profile, it has been reported that the normoandrogenemic PCOS phenotype has a milder metabolic syndrome with a rather similar rate of insulin resistance (30). The evidence currently suggests that patients with PCOS diagnosed according to AE-PCOS Society criteria have the most severe metabolic features (15, 17). Women in PCOS without hyperandrogenism are still the subject of debate (2, 15, 16). The Androgen Excess and PCOS Society suggested that PCOS initially should be accepted as a condition of hyperandrogenism (15). The current study demonstrated that in women diagnosed according to AE-PCOS Society criteria, the proportion of increased serum glucose level was double in comparison with that in women identified as normoandrogenemic PCOS, while the rate of women with an abnormal glucose tolerance test and insulin resistance rose by almost 10%. These findings appear to support the previous observations that women with normoandrogenemic PCOS exhibit a small proportion of hyperglycemia and insulin resistance (31, 32).

An impaired lipid profile is a prevalent finding in women with PCOS (33, 34), and also it was recently noted that the TG, LDL-cholesterol, and TC levels are significantly higher in obese PCOS women than in lean/normo-weight PCOS women, irrespective of the PCOS phenotypes (35). Women with hyperanderogenemic PCOS showed the most atherogenic lipid profiles, with higher apolipoprotein B compared to the other PCOS phenotypes (36). However, conflicting data are present in the literature. Moreover, despite the similar rate of IR in BMI-matched PCOS phenotypes, free testosterone and FAI were positively correlated with triglyceride as well as being inversely correlated with HDL-cholesterol (37). Conversely, other researchers speculate that lipid abnormalities are not different in women with hyperandrogenemic PCOS (38, 39). In accordance with the previous study, we also observed that women with normoandrogenemic PCOS have a lower triglyceride/HDL-cholesterol ratio, triglyceride, and VLDL-cholesterol concentrations as well as higher HDL-cholesterol levels when compared with those of women diagnosed as PCOS according to the AES-PCOS criteria, although BMI was not significantly different between the PCOS phenotypes (37). These findings suggest that hyperandrogenemia may adversely affect the lipid metabolism regardless of the presence of MetS or IR. However, the higher prevalence of decreased HDL-cholesterol with a similar rate of elevated TG, total cholesterol, and LDL-cholesterol observed in this study may indicate the effects of insulin resistance on HDL-cholesterol. Further, this finding would be an indirect reflection of increased ovarian androgen secretion in women with PCOS, as proposed in previous reports (40, 41).

According to the new diagnostic criteria (2, 13), PCOS may *de facto* involve a large-scale number of abnormalities, consisting of women with mild clinical and hormonal abnormalities (42, 43). As regards metabolic syndrome (evaluated with the International Diabetes Federation criteria), our cohort study showed a proportion of 22.1% had it in the overall PCOS population according to the Rotterdam criteria consistent with a previous cohort study in a Mediterranean population (44). Furthermore, women diagnosed with PCOS according to the AE-PCOS Society criteria had a higher rate of MetS than those with normoandrogenemic PCOS (20.4 vs 9.2%). This significant difference is in line with previous studies (45-48). The explanation for this quite pronounced difference may be that the key features of MetS comprising abdominal obesity, insulin resistance, and impaired glucose tolerance are present less frequently in normoandrogenemic PCOS (24). Indeed, a current meta-analysis showed that elevated serum androgen levels have a positive correlation with the prevalence of MetS (49). Overall, women with normal androgen levels appear to be part of the milder PCOS spectrum, but this might be due to the less frequently elevated blood glucose level, which is related with lower health-risk-related factors.

It has also been found that subclinical atherosclerosis expressed as low-grade inflammation (increased hs-CRP levels) could be a factor for an increased risk of cardiovascular disease and DM type 2 in women with PCOS (50-52). Moreover, an elevation in circulating hs-CRP irrespective of obesity is evidence that chronic low-grade inflammation may have an effect on the pathogenesis of PCOS, particularly in hyperanderogenemic phenotypes (4, 53). In accordance with previous reports, we also observed a significant elevation of hs-CRP in women with hyperanderogenemia compared to in women with normal androgen levels, despite there being no difference in BMI between the phenotypes (4, 53). This assumes that a high androgen level independently may be a factor that affects the establishment of cardiovascular disease in women diagnosed as PCOS.

Limitations for the study include that the methodology of study was a retrospective cohort, and also the numbers in the normoandrogenemic PCOS group were too small to assess the statistical significance for the oral glucose test intolerance.

In conclusion, women with hyperandrogenemic PCOS usually present an impaired lipid profile, insulin resistance, and abnormal glucose tolerance, which may be related to the subsequent development of MetS. PCOS patients with androgen excess appear to be exposed to a higher risk of cardiovascular disease than normoanderogenemic PCOS patients (54, 55). The observations of the present study suggest that the androgen excess per se is related with a long-term-health-risk of PCOS. This new data may be of reference in informing women diagnosed as PCOS, even though further prospective studies are needed to validate this proposition.

Ethics Committee Approval: *Ethics committee approval was received for this study from the ethics committee of İnönü University.*

Informed Consent: *N/A.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Concept - E.Ç., I.T.; Design - E.Ç., I.T.; Supervision - B.B.; Materials - E.Ç., Ç.T.; Data Collection and/or Processing - S.E., P.K.; Analysis and/or Interpretation - E.Ç., B.A.; Literature Search - E.Ç., A.K.; Writing Manuscript - E.Ç., B.A.; Critical Review - B.B.*

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

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

References

1. Koivunen R, Pouta A., Franks S, Martikainen H, Sovio U, Hartikainen AL, et al; Northern Finland Birth Cohort 1966 Study. Fecundability and spontaneous abortions in women with self-reported oligoamenorrhea and/or hirsutism: Northern Finland Birth Cohort 1966 Study. Hum Reprod 2008; 23: 2134-9. [CrossRef]
2. Azziz R, Woods KS, Reyna R, Key TJ, Knochenhauer ES, Yildiz BO. The prevalence and features of the polycystic ovary syndrome in an unselected population. The Journal of clinical endocrinology and metabolism. 2004; 89: 2745-9. [CrossRef]
3. Hull MG. Epidemiology of infertility and polycystic ovarian disease: endocrinological and demographic studies. Gynecological endocrinology: the official journal of the International Society of Gynecological Endocrinology. 1987; 1: 235-45. [CrossRef]
4. Escobar-Morreale HF, Luque-Ramirez M, San Millan JL. The molecular-genetic basis of functional hyperandrogenism and the polycystic ovary syndrome. Endocr Rev 2005; 26: 251-82. [CrossRef]
5. Rebar R, Judd HL, Yen SS, Rakoff J, Vandenberg G, Naftolin F. Characterization of the inappropriate gonadotropin secretion in polycystic ovary syndrome. J Clin Invest 1976; 57: 1320-9. [CrossRef]
6. Amowitz LL, Sobel BE. Cardiovascular consequences of polycystic ovary syndrome. Endocrinology and metabolism clinics of North America. 1999; 28(2): 439-58, viii. Epub 1999/06/03.
7. Dokras A. Cardiovascular disease risk factors in polycystic ovary syndrome. Semin Reprod Med 2008; 26: 39-44. [CrossRef]
8. Dahlgren E, Friberg LG, Johansson S, Lindstrom B, Oden A, Samsioe G, et al. Endometrial carcinoma; ovarian dysfunction--a risk factor in young women. Eur J Obstet Gynecol Reprod Biol 1991; 41: 143-50. [CrossRef]
9. Knochenhauer ES, Key TJ, Kahsar-Miller M, Waggoner W, Boots LR, Azziz R. Prevalence of the polycystic ovary syndrome in unselected black and white women of the southeastern United States: a prospective study. J Clin Endocrinol Metab 1998; 83: 3078-82. [CrossRef]
10. Asuncion M, Calvo RM, San Millan JL, Sancho J, Avila S, Escobar-Morreale HF. A prospective study of the prevalence of the polycystic ovary syndrome in unselected Caucasian women from Spain. J Clin Endocrinol Metab 2000; 85: 2434-8. [CrossRef]
11. Diamanti-Kandarakis E, Kouli CR, Bergiele AT, Filandra FA, Tsianateli TC, Spina GG, et al. A survey of the polycystic ovary syndrome in the Greek island of Lesbos: hormonal and metabolic profile. J Clin Endocrinol Metab 1999; 84: 4006-11. [CrossRef]
12. Michelmore KF, Balen AH, Dunger DB, Vessey MP. Polycystic ovaries and associated clinical and biochemical features in young women. Clinical endocrinology. 1999; 51: 779-86. [CrossRef]
13. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod 2004; 19: 41-7. [CrossRef]
14. Zawadzki JK DA. Diagnostic criteria for polycystic ovary syndrome: towards a rational approach. Polycystic Ovary Syndromes. In: Dunaif AGJ, Haseltine F (eds): Boston: Blackwell Scientific; 1992. p. 377-84.
15. Azziz R, Carmina E, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Futterweit W, et al. Task Force on the Phenotype of the Polycystic Ovary Syndrome of The Androgen Excess and PCOS Society. The Androgen Excess and PCOS Society criteria for the polycystic ovary syndrome: the complete task force report. Fertil Steril 2009; 91: 456-88. [CrossRef]
16. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril 2004; 81: 19-25. [CrossRef]
17. Moran L, Teede H. Metabolic features of the reproductive phenotypes of polycystic ovary syndrome. Hum Reprod Update 2009; 15: 477-88. [CrossRef]
18. Yildiz BO, Bozdag G, Yapici Z, Esinler I, Yarali H. Prevalence, phenotype and cardiometabolic risk of polycystic ovary syndrome under different diagnostic criteria. Hum Reprod 2012; 27: 3067-73. [CrossRef]
19. Moran LJ, Misso ML, Wild RA, Norman RJ. Impaired glucose tolerance, type 2 diabetes and metabolic syndrome in polycystic ovary syndrome: a systematic review and meta-analysis. Hum Reprod Update 2010; 16: 347-63. [CrossRef]
20. Ferriman D, Gallwy JD. Clinical assessment of body hair growth in women. J Clin Endocrinol Metab 1961; 21: 1440-7. [CrossRef]
21. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis Model Assessment - Insulin Resistance and Beta-Cell Function from Fasting Plasma-Glucose and Insulin Concentrations in Man. Diabetologia 1985; 28: 412-9. [CrossRef]
22. Legro RS, Castracane VD, Kauffman RP. Detecting insulin resistance in polycystic ovary syndrome: purposes and pitfalls. Obstet Gynecol Surv 2004; 59: 141-54. [CrossRef]
23. American Diabetes Association. Standards of Medical Care in Diabetes--2010. Diabetes Care 2010; 33: S11-61. [CrossRef]
24. A worldwide consensus definition for the metabolic syndrome. Rev Panam Salud Publica 2005; 18: 451-4.
25. Talbert RL. Role of the National Cholesterol Education Program Adult Treatment Panel III guidelines in managing dyslipidemia. Am J Health-Syst Ph 2003; 60: S3-8.
26. Wiltgen D, Spritzer PM. Variation in metabolic and cardiovascular risk in women with different polycystic ovary syndrome phenotypes. Fertil Steril 2010; 94: 2493-6. [CrossRef]
27. Boyle JA, Cunningham J, O'Dea K, Dunbar T, Norman RJ. Prevalence of polycystic ovary syndrome in a sample of Indigenous women in Darwin, Australia. Med J Aust 2012; 196: 62-6. [CrossRef]
28. March WA, Moore VM, Willson KJ, Phillips DIW, Norman RJ, Davies MJ. Prevalence of the Polycystic Ovary Syndrome in a Community Sample Assessed under Contrasting Diagnostic Criteria. Aust Nz J Obstet Gyn 2010; 50: 16-7. [CrossRef]
29. Moran C, Tena G, Moran S, Ruiz P, Reyna R, Duque X. Prevalence of Polycystic Ovary Syndrome and Related Disorders in Mexican Women. Gynecol Obstet Inves 2010; 69: 274-80. [CrossRef]
30. Celik C, Abali R, Bastu E, Tasdemir N, Tasdemir UG, Gul A. Assessment of impaired glucose tolerance prevalence with hemoglobin A1c and oral glucose tolerance test in 252 Turkish women with polycystic ovary syndrome: a prospective, controlled study. Hum Reprod 2013; 28: 1062-8. [CrossRef]
31. Panidis D, Tziomalos K, Misichronis G, Papadakis E, Betsas G, Katsikis I, et al. Insulin resistance and endocrine characteristics of the different phenotypes of polycystic ovary syndrome: a prospective study. Hum Reprod 2012; 27: 541-9. [CrossRef]
32. Broekmans FJ, Knauff EA, Valkenburg O, Laven JS, Eijkemans MJ, Fauser BC. PCOS according to the Rotterdam consensus criteria: Change in prevalence among WHO-II anovulation and association with metabolic factors. BJOG 2006; 113: 1210-7. [CrossRef]
33. Guastella E, Longo RA, Carmina E. Clinical and endocrine characteristics of the main polycystic ovary syndrome phenotypes. Fertil Steril 2010; 94: 2197-201. [CrossRef]

34. Legro RS, Kunselman AR, Dunaif A. Prevalence and predictors of dyslipidemia in women with polycystic ovary syndrome. Am J Med 2001; 111: 607-13. [CrossRef]
35. Cussons AJ, Stuckey BG, Watts GF. Cardiovascular disease in the polycystic ovary syndrome: new insights and perspectives. Atherosclerosis 2006; 185: 227-39. [CrossRef]
36. Rizzo M, Berneis K, Hersberger M, Pepe I, Di Fede G, Rini GB, et al. Milder forms of atherogenic dyslipidemia in ovulatory versus anovulatory polycystic ovary syndrome phenotype. Hum Reprod 2009; 24: 2286-92. [CrossRef]
37. Castelo-Branco C, Steinvarcel F, Osorio A, Ros C, Balasch J. Atherogenic metabolic profile in PCOS patients: role of obesity and hyperandrogenism. Gynecological endocrinology: the official journal of the International Society of Gynecol Endocrinol 2010; 26: 736-42. [CrossRef]
38. Maffei L, Murata Y, Rochira V, Tubert G, Aranda C, Vazquez M, et al. Dysmetabolic syndrome in a man with a novel mutation of the aromatase gene: effects of testosterone, alendronate, and estradiol treatment. J Clin Endocrinol Metab 2004; 89: 61-70. [CrossRef]
39. Carmina E, Chu MC, Longo RA, Rini GB, Lobo RA. Phenotypic variation in hyperandrogenic women influences the findings of abnormal metabolic and cardiovascular risk parameters. J Clin Endocrinol Metab 2005; 90: 2545-9. [CrossRef]
40. Carmina E, Lobo RA. Prevalence and metabolic characteristics of adrenal androgen excess in hyperandrogenic women with different phenotypes. J Endocrinol Invest 2007; 30: 111-6. [CrossRef]
41. Rizzo M, Longo RA, Guastella E, Rini GB, Carmina E. Assessing cardiovascular risk in Mediterranean women with polycystic ovary syndrome. J Endocrinol Invest 2011; 34: 422-6. [CrossRef]
42. Karkanaki A, Piouka A, Katsikis I, Farmakiotis D, Macut D, Panidis D. Adiponectin levels reflect the different phenotypes of polycystic ovary syndrome: study in normal weight, normoinsulinemic patients. Fertil Steril 2009; 92: 2078-81. [CrossRef]
43. Carmina E, Orio F, Palomba S, Longo RA, Cascella T, Colao A, et al. Endothelial dysfunction in PCOS: role of obesity and adipose hormones. Am J Med 2006; 119: 356.e1-6. [CrossRef]
44. Barber TM, Wass JA, McCarthy MI, Franks S. Metabolic characteristics of women with polycystic ovaries and oligo-amenorrhoea but normal androgen levels: implications for the management of polycystic ovary syndrome. Clin Endocrinol 2007; 66: 513-7. [CrossRef]
45. Amato MC, Galluzzo A, Finocchiaro S, Criscimanna A, Giordano C. The evaluation of metabolic parameters and insulin sensitivity for a more robust diagnosis of the polycystic ovary syndrome. Clin Endocrinol 2008; 69: 52-60. [CrossRef]
46. Apridonidze T, Essah PA, Iuorno MJ, Nestler JE. Prevalence and characteristics of the metabolic syndrome in women with polycystic ovary syndrome. J Clin Endocrinol Metab 2005; 90: 1929-35. [CrossRef]
47. Cussons AJ, Watts GF, Burke V, Shaw JE, Zimmet PZ, Stuckey BG. Cardiometabolic risk in polycystic ovary syndrome: a comparison of different approaches to defining the metabolic syndrome. Hum Reprod 2008; 23: 2352-8. [CrossRef]
48. Cheang KI, Huszar JM, Best AM, Sharma S, Essah PA, Nestler JE. Long-term effect of metformin on metabolic parameters in the polycystic ovary syndrome. Diab Vasc Dis Res 2009; 6: 110-9. [CrossRef]
49. Tziomalos K, Katsikis I, Papadakis E, Kandaraki EA, Macut D, Panidis D. Comparison of markers of insulin resistance and circulating androgens between women with polycystic ovary syndrome and women with metabolic syndrome. Hum Reprod 2013; 28: 785-93. [CrossRef]
50. Brand JS, van der Tweel I, Grobbee DE, Emmelot-Vonk MH, van der Schouw YT. Testosterone, sex hormone-binding globulin and the metabolic syndrome: a systematic review and meta-analysis of observational studies. Int J Epidemiol 2011; 40: 189-207. [CrossRef]
51. Türkçüoğlu I, Kafkasli A, Meydanli MM, Ozyalin F, Taşkapan C. Independent predictors of cardiovascular risk in polycystic ovarian syndrome. Gynecol Endocrinol 2011; 27: 915-9. [CrossRef]
52. Karaer A, Cavkaytar S, Mert I, Buyukkagnici U, Batioglu S. Cardiovascular risk factors in polycystic ovary syndrome. J Obstet Gynaecol 2010; 30: 387-92. [CrossRef]
53. Athyros VG, Kakafika AI, Karagiannis A, Mikhailidis DP. Do we need to consider inflammatory markers when we treat atherosclerotic disease? Atherosclerosis 2008; 200: 1-12. [CrossRef]
54. Daskalopoulos GN, Karkanaki A, Karagiannis A, Mikhailidis DP, Athyros VG. Is the risk for cardiovascular disease increased in all phenotypes of the polycystic ovary syndrome? Angiology 2011; 62: 285-90. [CrossRef]
55. Wild RA, Carmina E, Diamanti-Kandarakis E, Dokras A, Escobar-Morreale HF, Futterweit W, et al. Assessment of cardiovascular risk and prevention of cardiovascular disease in women with the polycystic ovary syndrome: a consensus statement by the Androgen Excess and Polycystic Ovary Syndrome (AE-PCOS) Society. J Clin Endocrinol Metab 2010; 95: 2038-49. [CrossRef]
56. Makedos A, Goulis DG, Papanikolaou A, Panidis D. Serum high-sensitivity C-reactive protein and homocysteine changes during hormonal therapy in women with polycystic ovary syndrome: a prospective, matched study. Angiology 2010; 61: 595-601. [CrossRef]

Why do multiparous women with a history of vaginal delivery give birth by cesarean section?

Hale Göksever Çelik[1], Ayşegül Bestel[1], Engin Çelik[2], Alev Atış Aydın[1]

[1]Department of Obstetrics and Gynecology, Kanuni Sultan Süleyman Training and Research Hospital, İstanbul, Turkey

[2]Department of Obstetrics and Gynecology, İstanbul University İstanbul School of Medicine Hospital, İstanbul, Turkey

Abstract

Objective: A cesarean section (C-section) is performed to deliver a baby through the mother's abdomen. In recent years, the rate of incidences requiring a C-section is steadily increasing all over the world. Advanced maternal age, chronic health problems, multiple pregnancies as a result of the development of assisted reproductive technologies, and an insufficient supplementary health network can be considered as the reasons why mothers and obstetricians prefer a C-section. Our study aimed to identify the risk factors for the need of C-section in women with a history of vaginal delivery.

Material and Methods: 238 multiparous women with a history of vaginal birth at 37-42 gestational weeks were enrolled in our study. 110 women had underwent C-section. Control group was chosen randomly from women giving birth by vaginal route.

Results: Overall, 238 multiparous women with a history of vaginal delivery at 37–42 gestational weeks were enrolled in our study. The history of operative delivery, that of labor induction and presence of meconium and the indication of admission to the delivery room were different between groups. A lower Bishop score and biophysical profile, smaller gestational period, and lower birth weight were associated with the group requiring a C-section, whereas older age and a long time interval from the previous birth were associated with the group not requiring a C-section.

Conclusion: A strategy involving either labor induction or not could be individualized for each patient to eliminate the risk factors for adverse outcomes. To identify criteria for the standardization of labor management, further studies are needed. (J Turk Ger Gynecol Assoc 2016; 17: 209-13)

Keywords: Cesarean section, vaginal birth, multiparity

Received: 14 June, 2016 **Accepted:** 29 October, 2016

Introduction

A cesarean section (C-section) is performed to deliver a baby through the mother's abdomen. Unexpected conditions related to the baby such as the position of the baby, signs of fetal distress, and number of babies or related to the mother such as health problems and previous operations or abnormalities of placentation result in a requirement of C-section. Some mothers demand this delivery route because they think that it is relatively safe for the baby and herself.

In recent years, the rate of incidences of C-section is steadily increasing all over the world. This procedure was used for 37% of the deliveries in 2008 and more than 48.1% of the deliveries in 2013 in Turkey (1). Advanced maternal age, chronic health problems such as hypertension and diabetes mellitus, multiple pregnancies as a result of the development of assisted reproductive technologies, and an insufficient supplementary health network can be considered as the reasons why mothers and obstetricians prefer a C-section (2). Our study aimed to identify the risk factors for C-section in women with a history of vaginal delivery. The primary outcome for the study was a requirement of a C-section in these women.

Material and Methods

A hospital-based retrospective cohort study comprising 238 multiparous women was performed at the Department of Obstetrics and Gynecology at Kanuni Sultan Süleyman Training and Research Hospital. Women with a history of vaginal delivery were included. In total, 110 women had undergone a C-section. An age-matched control group was randomly chosen from women giving birth through the vaginal route. Operative deliveries with vacuum or forceps and patients with fetal demise and a prior C-section were excluded from the study. All the demographic and clinical characteristics of the patients were recorded from our own hospital's database, including age, number of gravida and parity, body mass index (BMI), weight gain, time interval from previous delivery, birth weight of previous and present babies, and gestational week. We analyzed the presence of maternal health problems (diabetes mellitus, goiter, hypertension, asthma, epilepsy, psychotic diseases, and cardiac problems), indication for admission to the delivery room and that of C-section, placental location, presence of meconium, history of operative intervention, and induction in previous birth(s). Records were also reviewed to identify induction and route of delivery.

Address for Correspondence: *Hale Göksever Çelik*
e.mail: hgoksever@yahoo.com

DOI: 10.5152/jtgga.2016.16119

All the gestational weeks were confirmed by a first trimester ultrasound. BMI was presented as a measure of body fat based on height and weight. BMI was categorized based on the World Health Organization (WHO) classification into underweight (less than 18.5), normal weight (18.5–24.9), overweight (25–29.9), obese (more than 30), and morbidly obese (more than 40).

In our hospital, the biophysical profile (BPP) and Bishop score were used to determine the stage of labor. The Bishop score is a quantitative means of describing the cervical status to decide the necessity of cervical ripening agents. The parameters in this score are cervical dilatation and effacement, the position and consistency of the cervix, and the station of the fetal head. If the Bishop score is ≤6, we accept the cervix as unfavorable for vaginal delivery and apply agents such as prostaglandin E2 ovules (Propess ovule; Ferring Medical, İstanbul, Turkey) for cervical ripening. Labor induction, when indicated, is performed using oxytocin infusion (Synpitan forte; Deva Medical, İstanbul, Turkey) with or without amniotomy. It was performed in women with no effective contractions accompanying cervical dilatation and effacement to promote delivery.

Labor pain, rupture of membranes, post-term pregnancy, suspicious non-stress test (NST), extreme vaginal bleeding, preeclampsia/chronic hypertension, growth restriction, or oligohydramnios were included as indications for admission to the delivery room. The reasons to proceed with a C-section included fetal distress, malpresentation, macrosomia, multiple pregnancies, failure to progress in labor or failed induction, fetal anomalies, and maternal clinical conditions.

Our study was designed as a retrospective cohort study and conducted according to the Helsinki Declaration. There was no ethical approval needed because we collected data of the patients from the records in the archives and we did not document any personal information. Also, in our hospital, informed consent was obtained from every patient for the use of medical information in scientific publications.

Statistical analysis

Statistical analysis was performed with the Statistical Package for the Social Sciences (IBM Corp.; Armonk, NY, USA) statistics 22.0 version for Windows. Differences in the mean values and characteristics between the groups were analyzed with the independent samples t-test and chi-square test. The means are presented herein with the standard deviation. $p<.05$ was considered statistically significant. The correlation coefficients and their significance were calculated using the Pearson test. For the multivariate analysis, the possible factors identified with univariate analyses were further entered into the logistic regression analysis to determine the independent predictors of patient outcome.

Results

Two hundred thirty-eight multiparous women with a history of vaginal birth at 37–42 gestational weeks were enrolled in our study. Of these, 46.2% of patients had given birth through a C-section. The mean age of all the women was 29.8±5.5 years. The mean BMI of all the women was 30.4±4.6. Of all the women, 96 (40.3%) had spontaneous labor, whereas 142 (59.7%) women received labor induction. All the demographic and clinical characteristics of the patients are represented in Table 1. The most frequent indication for admission to the delivery room was labor pain, whereas that for C-section was fetal distress (Table 2).

Table 3 shows the mean levels in terms of the characteristics of the women according to the route of delivery. There was a statistically significant difference between groups based on a history of operative delivery, that of labor induction and pres-

Table 1. Demographic and clinical characteristics of patients

Characteristics	Number (%) or mean±standard deviation
Maternal age	29.8±5.5
BMI (kg/m^2)	30.4±4.6
Gravida	3.3±1.5
Parity	2.0±1.4
Maternal health problems	
Absent	204 (85.7)
Present	34 (14.3)
Weight gain (kg)	10.5±4.4
Time interval from previous birth (years)	5.4±3.7
Gestational week (weeks)	38.0±3.0
Placental location	
Anterior	139 (58.4)
Posterior	70 (29.4)
Left lateral	7 (2.9)
Right lateral	13 (5.5)
Fundal	9 (3.8)
Bishop score	7.7±3.5
BPP	3.5±1.0
Induction	
Absent	96 (40.3)
Present	142 (59.7)
Birth weight (g)	3116.3±763.7
Meconium	
Absent	226 (95)
Present	12 (5)
Induction of previous birth	
Absent	108 (45.4)
Present	130 (54.6)
Operative intervention of previous birth	
Absent	234 (98.3)
Present	4 (1.7)
Birth weight of previous child (g)	3165.9±571.3

BMI: body mass index; BPP: biophysical profile

Table 2. Indications on admission and for C-section

Indication on admission		Indication for C-section	
Labor pain	167 (70.2)	Fetal distress	42 (38.2)
Rupture of membranes	29 (12.2)	Malpresentation	16 (14.5)
Post-term pregnancy	11 (4.6)	Macrosomia	12 (10.9)
Preeclampsia/chronic hypertension	10 (4.2)	Multiple pregnancies	12 (10.9)
Oligohydramnios/IUGR	9 (3.8)	Obstructed labor	10 (9.1)
Suspicious NST	9 (3.8)	Preeclampsia	7 (6.4)
Vaginal bleeding	3 (1.3)	Placentation anomalies	3 (2.7)
		Fetal anomalies	3 (2.7)
		Maternal conditions	3 (2.7)
		Cord problems	2 (0.8)

IUGR: intrauterine growth restriction; C-section: cesarean section; NST: non-stress test
Data are presented as n (%).

Table 3. Comparison of characteristics between patients according to the route of delivery

Characteristics	Vaginal delivery (Patient number: 128)	C-section (Patient number: 110)	p
Maternal age	29.4±5.6	30.4±5.3	NS
BMI (kg/m^2)	30.1±4.3	30.8±5.0	NS
Gravida	3.2±1.3	3.4±1.7	NS
Parity	1.8±1.2	2.1±1.6	NS
Weight gain (kg)	10.0±3.8	11.0±5.0	NS
Time interval from previous birth (years)	4.7±3.8	6.1±3.5	.003
Gestational week (weeks)	38.9±2.0	37.0±3.6	<0.001
Bishop score	9.5±2.7	5.6±3.0	<0.001
BPP	3.8±0.6	3.1±1.2	<0.001
Birth weight (kg)	3270.2±516.7	2937.3±947.1	.001
Birth weight of previous child (kg)	3168.1±555.5	3163.5±591.7	NS

BMI: body mass index; BPP: biophysical profile; C-section: cesarean section; NS: not significant
Used independent samples t-test; $p<0.05$ accepted as statistically significant.

ence of meconium, and the indication for admission to the delivery room (Table 4).

Bivariate correlation analysis demonstrated that a lower Bishop score and biophysical profile, smaller gestational period, and lower birth weight were associated with the group requiring a C-section, whereas older age and a long time interval from the previous birth were associated with the group not requiring a C-section. Table 5 shows the results of the logistic regression analysis. The presence of meconium was identified as the strongest factor to proceed with C-section in our study population.

Discussion

We analyzed the predictors for C-section in multiparous women with a history of vaginal delivery. Approximately 40% of all the women gave birth spontaneously without labor induction, whereas 76.1% of the women requiring induction gave birth by the vaginal route. Many studies claimed that induction was a significant risk factor for C-section, especially in nulliparous women (3-7). Rattigan et al. (8) also reported that the rates of operative delivery increase in women who receive labor induction regardless of parity. According to these studies, nulliparity is an independent risk factor for C-section in women who receive labor induction. However, the difference between these studies and our study is the study population, which is entirely multiparous. On the other hand, induction did not increase the risk of surgical delivery in multiparous women according to our results. Lee et al. (9) proposed that the rate of C-section was associated with the Bishop score but not impacted by labor induction; this is consistent with to our results. As a result, there is no certainty that induction increases the risk for C-section. There are also some situations in which labor induction is required for maternal and infant health (10).

In our study, a longer time interval from the previous birth, smaller gestational week, lower Bishop score and BPP, and a lower birth weight were determined as predictors for C-section. Similar to our results, Ennen et al. (11) accepted that an advanced maternal age, high BMI, and low Bishop score

Table 4. Comparison of characteristics between patients according to the route of delivery

Characteristics	Vaginal delivery (Patient number: 128)	C-section (Patient number: 110)	p
Maternal health problems			
Absent	112 (54.9)	92 (45.1)	NS
Present	16 (47.1)	18 (52.9)	
Placental location			
Anterior	80 (57.6)	70 (45.8)	NS
Posterior	33 (47.1)	38 (53.5)	
Left lateral	8 (61.5)	5 (38.5)	
Right lateral	2 (28.6)	5 (71.4)	
Fundal	5 (55.6)	4 (44.4)	
Induction			
Absent	20 (20.8)	76 (79.2)	<0.001
Present	108 (76.1)	34 (23.9)	
Meconium			
Absent	126 (55.8)	100 (44.2)	.014
Present	2 (16.7)	10 (83.3)	
Induction of previous birth			
Absent	56 (51.9)	52 (48.1)	NS
Present	72 (55.4)	58 (44.6)	
Operative intervention of previous birth			
Absent	128 (54.7)	106 (45.3)	.044
Present	0	4 (100)	
Indication on admission			
Labor pain	105 (62.9)	62(37.1)	<0.001
Rupture of membranes	10 (34.5)	19 (65.5)	
Post-term pregnancy	7 (63.6)	4 (36.4)	
Preeclampsia/chronic hypertension	2 (20)	8 (80)	
Oligohydramnios/IUGR	3 (33.3)	6 (66.7)	
Suspicious NST	1 (11.1)	8 (88.9)	
Vaginal bleeding	0	3 (100)	

IUGR: intrauterine growth restriction; C-section: cesarean section; NST: non-stress test; NS: not significant
Data are presented as n (%).
Used independent samples t-test; $p<0.05$ accepted as statistically significant.

increased the possibility of C-section. We matched the ages of women between the control group and the study group to eliminate the effect of age on our results. Pregnancies with a smaller gestational week usually consult with an unfavorable cervix, which indicates a lower Bishop score and BPP, resulting in surgical delivery (12). Because of this, the duration of labor prolongs until full cervical dilatation and effacement. As the number of gravida increases, the possibility of many adverse outcomes, such as malpresentation, increases, which could be an indication for a C-section. We found a difference between the vaginal birth group and C-section group according to the number of gravida and parity.

Interpregnancy interval is also important for predicting C-section during labor. A longer time interval between pregnancies increased the risk for C-section. The most effective reason was thought to be advancing maternal age.

Many studies showed that there is an increased risk for surgical deliveries among women with chronic health problems in contrast to our results (13). Because the age of our study population was relatively younger, maternal health problems did not affect the risk for C-section.

We did not find any difference between the groups according to placental location, similar to many other studies. If the woman had a history of operative delivery with vacuum or forceps, the risk for surgical delivery increased.

The most important parameter that increased the rate of C-section was the presence of meconium in our results. If we encounter a pregnant woman with these factors, we should

Table 5. Results of the logistic regression analysis

Risk factors	RR (95% CI)	p
Maternal health problems	1.21 (0.94–1.56)	NS
Gestational weeks	0.80 (0.70–0.90)	.002
Indication on admission	1.04 (0.86–1.25)	NS
Bishop score	0.67 (0.60–0.75)	<0.001
Presence of meconium	12.94 (2.30–73.24)	.004

RR: risk ratio; CI: confidence interval; NS: not significant
Used binary logistic regression analysis, p<0.05 accepted as statistically significant.

ensure a careful planning during follow-up of their pregnancy.
If we look at the indications on admission, there was significant difference between the vaginal birth group and the C-section group. Rupture of membranes, preeclampsia or chronic hypertension, oligohydramnios with or without growth restriction, fetal distress with suspicious NST, or any vaginal bleeding were associated with a higher rate of C-section, because these conditions require a shorter delivery time and emergency interventions if necessary, resulting in surgical deliveries (14).
Labor pain and post-date pregnancy as indications on admission did not make a difference to the route of delivery. Many studies show that there is not sufficient evidence available about whether induction should be applied or not in these conditions (15).
Derbent et al. (16) identified that decreased physical activity is statistically associated with an increased risk of C-section besides the other factors. We did not conclude that result. Besides this, we also did not compare the perinatal morbidity and mortality, because of limited data from our hospital's database and due to the retrospective study design. This can be accepted as a weakness of our study. But the important distinction of our study from the others is the investigation of the predictors for C-section in multiparous women with a history of vaginal birth. To identify criteria for the standardization of labor management, further studies are needed.

Ethics Committee Approval: *N/A.*

Informed Consent: *Written informed consent was obtained from patients who participated in this study.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Concept - H.G.Ç., E.Ç.; Design - H.G.Ç., E.Ç.; Supervision - A.A.A.; Resources - H.G.Ç., A.B.; Materials - H.G.Ç., A.B.; Data Collection and/or Processing - H.G.Ç., A.B.; Analysis and/or Interpretation - H.G.Ç.; Literature Search - H.G.Ç.; Writing Manuscript - H.G.Ç.; Critical Review - E.Ç., A.A.A.*

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

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

References

1. Hacettepe University Institute of Population Studies (2014), "2013 Turkey Demographic and Health Survey". Hacettepe University Institute of Population Studies, T.R. Ministry of Development and TÜBİTAK, Ankara, Turkey.
2. Ferrari AP, Carvalhaes MA, Parada CM. Association between prenatal and parturition in the supplementary health network andelective cesarean section. Rev Bras Epidemiol 2016; 19: 75-88. [CrossRef]
3. Yeast JD, Jones A, Poskin M. Induction of labor and the relationship to cesarean delivery: A review of 7001 consecutive inductions. Am J Obstet Gynecol 1999; 180: 628-33. [CrossRef]
4. Seyb ST, Berka RJ, Socol ML, Dooley SL. Risk of cesarean delivery with elective induction of labor at term in nulliparous women. Obstet Gynecol 1999; 94: 600-7. [CrossRef]
5. Johnson AM. Evaluating outcomes of labor inductions beyond 39 weeks of gestation. Obstet Gynecol 2014; 123: 58S. [CrossRef]
6. Thorsell M, Lyrenas S, Andolf E, Kaijser M. Inducion of labor and the risk for emergency cesarean section in nulliparous and multiparous women. Acta Obstet Gynecol Scand 2011; 90: 1094-99. [CrossRef]
7. Levine LD, Hirshberg A, Srinivas SK. Term induction of labor and risk of cesarean delivery by parity. J Matern Fetal Neonatal Med 2014; 27: 1232-6. [CrossRef]
8. Rattigan MI, Atkinson AL, Baum JD. Delivery route following elective induction of labor at term: Analysis of 807 patients. J Clin Med Res 2013; 5: 305-8. [CrossRef]
9. Lee HR, Kim MN, You JY, Choi SJ, Oh SY, Roh CR, et al. Risk of cesarean section after induced versus spontaneous labor at term gestation. Obstet Gynecol Sci 2015; 58: 346-52. [CrossRef]
10. Caughey AB, Sundaram V, Kaimal AJ, Cheng YW, Gienger A, Little SE, et al. Maternal and neonatal outcomes of elective induction of labor. Evid Rep Technol Assess (Full Rep) 2009; 176: 1-257.
11. Ennen CS, Bofill JA, Magann EF, Bass JD, Chauhan SP, Morrison JC. Risk factors for cesarean delivery in preterm, term and post-term patients undergoing induction of labor with an unfavorable cervix. Gynecol Obstet Invest 2009; 67: 113-7. [CrossRef]
12. Vahratian A, Zhang J, Troendle JF, Sciscione AC, Hoffman MK. Labor progression and risk of cesarean delivery in electively induced nullipars. Obstet Gynecol 2005; 105: 698-704. [CrossRef]
13. Teixeira C, Correia S, Barros H. Risk of caesarean section after induced labour: do hospitals make a difference? BMC Research Notes 2013; 6: 214. [CrossRef]
14. Horowitz KM. Induction of labor for fetal growth restriction. Obstet Gynecol 2014; 123: 56S. [CrossRef]
15. Wennerholm UB, Hagberg H, Brorsson B, Bergh C. Induction of labor versus expectant management for post-date pregnancy: Is there sufficient evidence for a change in clinical practice? Acta Obstet Gynecol Scand 2009; 88: 6-17. [CrossRef]
16. Derbent AU, Karabulut A, Yıldırım M, Simavlı SA, Turhan NÖ. Evaluation of risk factors in cesarean delivery among multiparous women with a history of vaginal delivery. J Turkish-German Gynecol Assoc 2012; 13: 15-20.

Novel applications of COX-2 inhibitors, metformin, and statins for the primary chemoprevention of breast cancer

Darren Micallef, Sarah Micallef, Pierre Schembri-Wismayer, Jean Calleja-Agius
Department of Anatomy, Faculty of Medicine and Surgery, University of Malta, Tal-Qroqq, Msida, Malta

Abstract

Recent evidence shows that commonly prescribed drugs, such as non-steroidal anti-inflammatory drugs (NSAIDs), metformin, and statins, may have beneficial roles in the primary chemoprevention of breast cancer. Therefore, these drugs could potentially be used in addition to the hormonal drugs currently used for this purpose (namely, selective estrogen receptor modulators and aromatase inhibitors) due to their alternative mechanisms of action. (J Turk Ger Gynecol Assoc 2016; 17: 214-23)

Keywords: Chemoprophylaxis, breast neoplasms, hydroxymethylglutaryl-CoA reductase inhibitors, metformin, anti-inflammatory agents, non-steroidal

Received: 30 June, 2016 **Accepted:** 27 September, 2016

Introduction

Chemoprevention is defined as the use of pharmacological or natural agents that inhibit the development of a disease. In the case of breast cancer, the main chemopreventive agents used are selective estrogen receptor blockers (SERMs) and aromatase inhibitors (AIs) (1, 2).

Cyclooxygenase 2 (COX-2) inhibitors, non-steroidal anti-inflammatory drugs (NSAIDs), metformin, and statins are drugs that have long been in clinical use. NSAIDs are used as anti-inflammatories, analgesics, and, in the case of aspirin, antithrombotics (3); metformin is used as an anti-hyperglycemic (4) and in the treatment of metabolic syndrome (5) and polycystic ovarian syndrome (PCOS); and statins are used for the primary and secondary prevention of cardiovascular disease by lowering cholesterol levels (6). Therefore, the safety profiles and adverse reaction profiles of these drugs are well understood. Thus, these drugs are good clinical candidates for further exploration of their mechanisms of action as applied to chemoprevention of breast cancer (7-9). Current studies regarding the novel application of these drugs have been critically analyzed with respect to their potential use in breast cancer chemoprevention. However, more research is needed to prove that these studies were adequately powered and thus are of good statistical significance, so that the use of these drugs can be considered as an option for chemoprevention of breast cancer in clinical practice.

Search strategy

The papers used as references in this review were identified using relevant keywords in related search engines such as Pubmed and Google Scholar, after performing a broader search regarding the chemoprevention of breast cancer and finding mentions of these drugs in other documents. The search terms used to identify these sources include “breast neoplasms,” “breast cancer,” “NSAIDs,” “aspirin,” “COX-2 inhibitors,” “COX2 inhibitors,” “cyclooxygenase 2 inhibitors,” “metformin,” “statins,” and “HMG-CoA reductase inhibitors.” The search results were then filtered, analyzed, and used as references in compiling this review.

Non-steroidal anti-inflammatory drugs (NSAIDs)

Various epidemiological studies have been conducted to establish a relationship between the use of NSAIDs and the incidence of breast cancer (10-12). The results of these studies are inconsistent; however, this inconsistency is most likely due to the fact that tumors have variable molecular properties (13).

All NSAIDs, such as aspirin and ibuprofen, inhibit both cyclooxygenase enzymes, largely with little or no selectivity for either enzyme. Aspirin irreversibly inhibits cyclooxygenase, while ibuprofen and other NSAIDs are reversible inhibitors.

A meta-analysis has been conducted, focusing mainly on the effects of aspirin and ibuprofen (14). This meta-analysis included 16 case-control studies, 18 cohort studies, 3 case-

Address for Correspondence: *Jean Calleja-Agius*
e.mail: jean.calleja-agius@um.edu.mt

DOI: 10.5152/jtgga.2016.15200

control studies nested in well-defined cohorts, and one clinical trial, all of which were performed between 1966 and 2008, examining the association between the use of NSAIDs and the risk of breast cancer. The results of these studies were pooled and statistically analyzed separately for aspirin and ibuprofen. The conclusions of this meta-analysis were that aspirin use decreased the risk of breast cancer. It was also noted that high intake of aspirin did not strengthen this relationship. Similarly, use of ibuprofen led to a decrease in the risk of breast cancer; however, higher intake of ibuprofen did not strengthen this association (14).

This analysis also considered the effects that genetic polymorphisms of the COX-2 gene might have on these results; for example, the COX-2.847 mutation is associated with an even lower risk of breast cancer among patients using aspirin. Overall, NSAID use was associated with a lower risk of developing breast cancer.

More recently, the relationship of aspirin and ibuprofen use with the risk of breast cancer has been re-examined in the context of the different molecular subtypes of cancer (15). A total of 26,580 menopausal women aged 59 to 77 years were involved in this analysis. During follow-up through 2005, 1581 cases of breast cancer were observed. Estrogen receptor (ER) status was available for 1262 of these patients; 1060 were ER positive, and 202 were ER negative. Progesterone receptor status was available for 1237 cases; 910 were progesterone receptor positive, and 327 were progesterone receptor negative. The women were divided into groups based on frequency of NSAID intake. It was found that women who regularly took aspirin had an approximately 20% lower risk of breast cancer than those who did not. In this study, higher frequency of aspirin intake was associated with lower risk (15), which contrasts with the results from the 2008 meta-analysis. These inverse associations of aspirin use were observed for ER+, ER-, PR+, and PR- tumors, with the greatest correlations observed for ER+ and PR+ tumors. However, in this study, no association was found between intake of non-aspirin NSAIDs and risk of breast cancer. One major limitation of this study was that the type of non-aspirin NSAID used was not specified; this may have masked any association between the intake of particular non-aspirin NSAIDs, such as ibuprofen, and the risk of breast cancer (15). It was further suggested that this difference is due to the fact that aspirin is an irreversible inhibitor of COX-2, while non-aspirin NSAIDs are reversible inhibitors.

The results of these various studies, although inconsistent and controversial, suggest that aspirin has chemopreventive potential; meanwhile, the chemopreventive potential of other NSAIDs remains to be clarified. Furthermore, in-depth examination of the relationship of NSAID consumption with various tumor subtypes, such as those in which COX-2 or epidermal growth factor receptor (EGFR) is upregulated, may shed light on the types of tumors that can be prevented with NSAIDs. These drugs would then be combined with other agents to achieve greater chemopreventive efficacy through combination treatment.

COX-2 inhibitors

NSAIDs not only inhibit COX-2, but also cyclooxygenase 1 (COX-1), which is responsible for regulating normal physiological functions; therefore, various adverse effects are associated with NSAID use. Side effects include stomach ulcers, nausea, vomiting, and prolonged bleeding after injury (16). For this reason, specific inhibitors of COX-2 have been developed. These include celecoxib and nimesulide, which are potent anti-inflammatory agents that lack the associated adverse effects (16). However, a different side effect profile was noted, mostly related to the cardiovascular system. This includes a higher predisposition to hypertension, atherosclerosis, and thrombosis, resulting in a higher risk of myocardial infarctions and strokes (17); these are conditions for which risk is already increased in peri- and post-menopausal females.

COX-2 is induced in inflamed tissue from its constitutively active isoenzyme, COX-1. It is involved in the metabolism of arachidonic acid into prostaglandins and thromboxanes specific to the inflamed tissue; this mediates local vasodilation, edema, pain, and fever (16).

Because prostaglandins are crucial in mediating inflammatory response and the associated pain, various inhibitors of the cyclooxygenase enzymes have been identified or developed; their main target is pain relief.

Persistent inflammation may cause DNA damage, induce increased cellular proliferation to repair damaged tissue, and create an environment that is rich in cytokines and growth factors; all of these lead to tumorigenesis (18). Molecular links between cytokines and tumorigenesis have already been demonstrated for breast cancer and other conditions (19). In fact, COX-2 is highly overexpressed in numerous cancers, including breast, liver, colorectal, lung, and esophageal cancers (20, 21). Blocking persistent inflammation with NSAIDs and specific COX-2 inhibitors may therefore prove useful in the prevention of tumorigenesis.

Furthermore, a lower degree of prostaglandin synthesis leads to inhibition of the enzyme aromatase, which is responsible for the synthesis of estrogen. In fact, one of the major prostaglandins, PGE_2, specifically induces the promoter II region on the aromatase gene (22). COX-2 inhibition has been shown to prevent estrogen-induced breast tumor formation to a greater extent than ibuprofen (a non-selective NSAID); thus, it demonstrates selective chemopreventive potential for ER-positive tumors (22).

The effects of ibuprofen were compared to those of celecoxib, particularly in their ability to inhibit carcinogenesis induced by 7,12-dimethylbenz(a)anthracene (DMBA) (Sigma-Aldrich; Darmstadt, Germany) in female Sprague-Dawley rats (22). These 50-day old rats were randomized into three groups, with 40 rats in each group. One group received powdered placebo with a standard diet, another group received 1500 mg/kg celecoxib with a standard diet, and the last group received 1500 mg/kg ibuprofen with a standard diet. After seven days, all rats were given an intragastric dose of 15 mg DMBA in 1.0 mL of sesame seed oil. The experimental and control diets were

Table 1. Biochemical and molecular associations between type 2 diabetes mellitus and breast cancer

	Biochemical Mechanisms (24)
Insulin	Insulin, which is secreted in increased amounts in type 2 diabetes, was shown to be mitogenic in breast tissue. This is compounded by the fact that insulin receptors tend to be over-expressed in breast cancer cells (25, 26). In fact, circulating level of C-peptide as a marker for insulin secretion has been shown to be positively associ ated with risk of breast cancer in some studies (27-30).
Insulin-like growth factor-1 (IGF-1)	Increase in insulin secretion is accompanied by an increase in the serum level of IGF-1, which may also contribute to tumor growth and thus can predict the risk of breast cancer in premenopausal women (31).
Estrogens and androgens	Increased levels of insulin also lead to higher levels of serum estrogens and androgens (32, 33) through inhibition of sex hormone-binding globulin (34). Increased levels of estrogen and testosterone have been associated with an increased risk of breast cancer in post-menopausal women (35, 36).
	Molecular Mechanisms (37)
Insulin Receptor (IR)	IR is heterotetrameric protein consisting of four subunits; two subunits bind insulin, while the other two subunits span the membrane, protrude into the cytosol, and have tyrosine kinase activity. Two isoforms of the insulin receptor are produced by alternative splicing: IR-A (the fetal isoform) and IR-B. In most cancers, fetal IR-A predominates because it mediates mitogenic rather than metabolic effects (37).
Insulin-like growth factor-1 receptor (IGF-1R)	(IGF-1R) is 60% homologous with IR and also has tyrosine kinase activity upon ligand binding by IGF-1. It promotes mitogenic, metastatic, and anti-apoptotic processes in breast cancer cells through the PI3K/Akt pathway. Because insulin and IGF-1 can bind to both IR and IGF-1R with different affinities, both ligands can enhance growth and survival (38).
Insulin receptor substrate-1 (IRS-1)	In type 2 diabetes, insulin resistance arises from the upregulation of cytokines and derivatives of free fatty acids. These lead to activation of protein kinase C-zeta (PKC-zeta), which phosphorylates insulin receptor substrate-1 (IRS-1), impairing its ability to activate the PI3K/Akt pathway upon ligand binding (39). Eventually, hyperglycemia and high insulin levels develop. Activation of IGF-1R by these high insulin levels can therefore lead to activation of the mitogenic and anti-apoptotic pathways, leading to an increased risk of cancer. Metabolic syndrome very often results in these patients; this is characterized by hypertension, insulin resistance, obesity, and dyslipidemia (40).

IGF-1: insulin-like growth factor-1; IR: insulin receptor; IR-A: insulin receptor isoform A (fetal); IR-B: insulin receptor isoform B; IGF-1R: insulin-like growth factor-1 receptor; PI3K: phosphatidylinositol 3-kinase; Akt: protein kinase B; IRS-1: insulin receptor substrate-1; PKC-zeta: protein kinase C-zeta

continued for 105 days before the experiment was stopped. The time of appearance of the first tumor in rats from each group was noted, while the size and location of the tumor was also assessed. At the end of the experiment, 127 palpable tumors were excised from the control rats (all adenocarcinomas) and 61 tumors were excised from rats treated with ibuprofen (all adenocarcinomas), while only 18 tumors were excised from rats treated with celecoxib (15 were adenocarcinomas, while 3 were non-malignant fibro-adenomas). Moreover, celecoxib was found to reduce the incidence of mammary cancer by 68%, tumor burden (tumors/rat) by 86%, and tumor volume by 81% compared to the control group. Only 13 of the 40 (32%) rats treated with celecoxib developed tumors, while all of the control rats (100%) developed tumors. Ibuprofen was also effective, but not as much as celecoxib; ibuprofen caused a 40% reduction in cancer risk, a 52% reduction in tumor burden, and a 57% reduction in tumor size. Moreover, the time for tumor development was prolonged with COX-2 inhibitor use. In the control group, the median time for detection of a tumor was 58 days after DMBA administration. In the celecoxib group, the median time was 95 days; in the ibuprofen group, it was 86 days.

It was also noted that celecoxib and ibuprofen appeared to have no adverse effects on rat liver, kidneys, stomach, and intestines. In humans, however, there are concerns that drugs that inhibition of COX-2 can lead to severe cardiovascular adverse effects. Nonetheless, the use of COX-2 inhibitors in patients at low risk for heart disease appears to be safe (21).

The results from this experiment show that celecoxib may be a very useful chemopreventive agent; they also support the role that COX-2 inhibitors, including the general NSAID ibuprofen, may play in reducing the risk of breast cancer.

Metformin

Diabetes has been associated with an increased risk of developing cancer; a recent meta-analysis involving 20 studies demonstrated the actual relationship between diabetes and breast cancer (23). This meta-analysis showed that women with diabetes have a 20% increased risk of developing breast cancer compared to non-diabetic women. A more recent meta-analysis suggested that diabetic women have a 23% higher risk of breast cancer, particularly menopausal women, while diabetes was also found to increase breast cancer mortality overall (24). Interestingly, the association between diabetes and breast cancer was strongest in Europe, followed by America, while it was non-significant in Asia (24). The biochemical and molecular associations between type 2 diabetes mellitus and breast cancer are outlined in Table 1 (25-42), while the structure, uses, outcomes, and adverse reactions of metformin are shown in Table 2 (4, 5, 38, 42, 43).

Metformin increases the effectiveness of neoadjuvant chemotherapy in breast cancer patients (44). In one particular study,

Table 2. Metformin: Structure, uses, outcomes, and adverse reactions

	Metformin
Structure	Semi-synthetic biguanide with two methyl groups attached to its nitrogen nucleus.
Uses	Approved for use to treat hyperglycemia (3), metabolic syndrome (4), and PCOS (41).
Outcomes (37)	Decreases glucose absorption from the gut.
	Inhibits gluconeogenesis in the liver without stimulating insulin secretion.
	Mediates increased uptake of glucose by skeletal muscle and adipose tissue, leading to a decrease in blood glucose levels.
	Increases the affinity of the insulin receptor for insulin, thus improving insulin resistance and leading to reduction in insulin levels of approximately 25% to 33% after several days.
	Decreases circulating levels of cholesterol, LDLs, and triglycerides.
Side effects (37, 42)	Mild gastrointestinal effects (30%) and metallic taste (3%), which are reversible with persistent use, and decreased levels of vitamin B12 (6%).

PCOS: polycystic ovarian syndrome; LDLs: low density lipoproteins

the pathologic complete response (pCR), i.e., the absence of residual tumor at the time of surgery, was assessed for these patients (44). The difference in pCR between the non-diabetic group (16%) and the non-metformin group (8%) was significant, as was the difference between the metformin (24%) and non-metformin (8%) groups. In contrast, the difference between the non-diabetic (16%) and metformin (24%) groups, although numerically evident, did not attain clinical significance. This proved that the anti-proliferative characteristics of metformin impair tumor development. Moreover, because insulin use was twice as great in the non-metformin group compared to the metformin group (33% and 16%, respectively), it was observed that higher insulin levels were associated with decreased pCR (44). However, overall disease recurrence did not differ significantly between the groups, and both diabetic groups had worse overall survival than the non-diabetic group (45).

Various epidemiological studies have demonstrated a lower incidence of mortality from cancer in diabetic patients receiving low-dose metformin. A large meta-analysis (46) demonstrated that the overall cancer rate decreased by 31% in patients taking metformin compared to patients taking other anti-diabetic drugs. This difference was significant for pancreatic and liver cancer but was not significant for colon, breast, and prostate cancer.

This supports earlier results that showed that cancer incidence decreased by more than 50% in patients who had been taking metformin for over 4 years (47). In 2012, another meta-analysis confirmed the beneficial effects of metformin for decreasing the risk of cancer and reducing overall cancer mortality (48).

The mechanisms underlying the action of metformin are complex and are far from fully understood. The beneficial effects of metformin may be indirect (through insulin), or it may directly affect the proliferation and growth of cells (45). Many mechanisms of metformin action have been proposed, as outlined in Table 3 (3, 45, 49-54).

Preclinical models show that metformin can lower the incidence of breast cancer (55). Metformin affects ER+ and ER- cell lines as well as human epidermal growth factor receptor 2 (HER-2) normal and abnormal cancer cell lines; it inhibits cell proliferation and causes cell cycle arrest at the G1 checkpoint, probably through reduction of cyclin D1 and E2F1 expression. It also inhibits MAPK, Akt, and mTOR activity in all of these cell lines. However, metformin does not induce apoptosis. Furthermore, at high doses, metformin was found to reduce HER-2 expression in cancer cells overexpressing HER-2; at lower doses, it was found to inhibit HER-2 tyrosine kinase activity (55).

As demonstrated in preclinical models, metformin at a low dose can inhibit the tyrosine kinase activity of the HER-2 receptor; also, a high dose of metformin can downregulate HER-2 (38). Thus, therapeutically combining metformin with the anti-HER-2 monoclonal antibody trastuzumab may be very efficient to eliminate stem/progenitor cell populations with amplified HER-2. This is particularly due to the fact that metformin can prevent resistance to trastuzumab treatment; this is very often mediated by high levels of IGF and insulin, which bind to their respective receptors and induce cellular proliferation, inhibition of apoptosis, angiogenesis, and metastasis. Metformin can decrease circulating insulin and IGF levels and can thus disrupt this alternative pathway for tumor development.

In the prevention setting, metformin can therefore regulate the rate of proliferation of tumor progenitor cells in premalignant lesions, thus preventing or delaying malignant tumor formation (38). Furthermore, by regulating proliferation of dormant cancer stem cells, metformin can also prevent recurrence of breast cancer; thus, it may be effective for secondary prevention of breast cancer.

Cancer is the second leading cause of death worldwide, while diabetes is the twelfth (48). Considering that the prevalence of diabetes is constantly increasing, the use of metformin as both an anti-diabetic drug as well as a chemopreventive agent for cancer will have numerous beneficial implications and positive results.

Table 3. Proposed mechanisms of action of metformin

Metformin	Increases AMP: ATP ratio by targeting complex I in the mitochondrial electron transport chain (48).
Activation of AMPK	Increased AMP: ATP ratio induces activation of AMPK, mediated through a variety of proteins, including the tumor suppressor protein LKB1, CaMKK, and TAK1.
	The tumor suppressor gene LKB1 is very commonly mutated in lung and pancreatic cancers as well as melanomas. The absence of functional LKB1 in breast cancer is associated with a poor prognosis, suggesting that sensitivity to metformin may be mediated by LKB1 (37). Metformin resistance may also be due to genetic polymorphisms in the organic cation transporter 1, a transporter crucial for the transport of metformin, which is not metabolized (49).
Activated AMPK	Inhibits processes that consume ATP, such as gluconeogenesis, protein synthesis, and fatty acid synthesis.
	Promotes processes that generate ATP, such as glycolysis and oxidation of fatty acids; because tumor cells require high levels of fatty acid synthesis, this shift from anabolic pathways toward catabolic pathways partly explains the anti-proliferative activity of metformin.
	Inhibits cellular proliferation (44). AMPK is involved in a complex regulatory network, integrating extracellular and intracellular stimuli to control processes that are crucial to maintain tissue size. AMPK phosphorylates and stabilizes the tumor suppressor protein TSC2, which interacts with the mammalian target of rapamycin (mTOR), the master regulator of cellular protein synthesis (50). In this way, AMPK inhibits mTOR activity, thus inhibiting tumorigenesis, cancer progression, angiogenesis, and metastasis. This provides a theoretical basis for combining metformin and agents targeting the PI3K/Akt pathway to efficiently inhibit the PI3K/Akt/mTOR interactive pathway and prevent tumorigenesis.
	Inhibits expression of the aromatase gene, thus decreasing local production of estrogen and reducing the risk of developing hormone-dependent breast cancers.
	Chronic activation of AMPK is also believed to lead to activation of p53 and cellular senescence (51). p53 acts as a metabolic checkpoint, inducing cell cycle arrest in situations of low cellular energy (52). Furthermore, active p53 can inhibit glycolysis and enhance oxidative phosphorylation, preventing the Warburg effect (i.e., the reprogramming of cellular metabolism toward aerobic glycolysis and away from oxidative phosphorylation, a phenomenon common to approximately 60% to 90% of tumors) (52). The shift towards aerobic glycolysis in the Warburg effect is required to meet the needs of constant proliferation due to enhanced metabolism and uptake of glucose. In fact, this shift provides the basis for positron emission tomography, using a radioactive analog of glucose to identify tumors with increased uptake and metabolism of the glucose analog. Metformin may inhibit this shift if p53 is functional. However, the fact that some studies show that metformin can actually enhance glycolysis through mitochondrial poisoning creates controversy, which has yet to be resolved through further research. Furthermore, mutations in p53 cause metabolic alterations, leading to the Warburg effect (52, 53).

AMP: adenosine monophosphate; ATP: adenosine triphosphate; AMPK: 5' adenosine monophosphate-activated protein kinase; LKB1: liver kinase B1; CaMKK: calcium/calmodulin-dependent protein kinase kinase; TAK1: transforming growth factor beta-activated kinase 1; TSC2: tuberous sclerosis complex 2; mTOR: mammalian target of rapamycin; PI3K: phosphatidylinositol 3-kinase; Akt: protein kinase B; p53: cellular tumor antigen p53

Statins

The anti-atherosclerotic decrease of plasma LDL caused by statins through inhibition of the conversion of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) to mevalonate in cholesterol synthesis has been well studied and documented (56). In fact, statins are the most potent class of drugs for primary and secondary prevention of cardiovascular injury.

Considering the concept that combining statins and estrogen may have synergistic effects for improving lipid profiles in postmenopausal women, investigations have been conducted to determine whether statins can reduce the incidence of breast cancer, the most controversial adverse effect associated with estrogen therapy (57).

Interestingly, a variety of preclinical studies show that statins can not only prevent cardiovascular diseases but may also inhibit cancer growth and development, although the actual mechanisms have not yet been completely elucidated (57, 58). A proposed mechanism of action for statins is outlined in Figure 1 (59); this shows the mevalonate pathway on which statins (as HMG CoA inhibitors) act. This is also linked to various *in vitro* and *in vivo* studies, which are summarized in Table 4 (57-59).

Statins can also interfere with microdomain formation in endothelial cells and inhibit oxidative stress pathways, both enzymatically and non-enzymatically. Furthermore, statins can upregulate endothelial nitric oxide synthase, eNOS, improving endothelial function (60).

In an *in vitro* study, the effects of statins on the cellular proliferation of breast cancer cell lines were studied, both alone and in combination with estradiol (57). The breast cancer cell lines, MCF-7 (ER+) and MDA-MB 231 (ER-), were cultured in the presence of the lipophilic statins atorvastatin, lovastatin, fluvastatin, simvastatin, and hydrophilic pravastatin, both alone and in combination with estrogen. The results showed that all statins, with the exception of pravastatin, significantly inhibited cellular proliferation in both cell lines after four days of culture; this association was dose-dependent. The inhibitory values

Table 4. Effects of statins as outlined in Figure 1

Endothelial cells	Interfere with microdomain formation and inhibit oxidative stress pathways, both enzymatically and non-enzymatically. Statins can also upregulate endothelial nitric oxide synthase, eNOS, improving endothelial function (59).
In vitro breast cancer cells	In an *in vitro* study, the effects of statins on cellular proliferation of breast cancer cell lines were studied, both alone and in combination with estradiol (56). The breast cancer cell lines, MCF-7 (ER+ cells) and MDA-MB 231 (ER-), were cultured in the presence of the lipophilic statins atorvastatin, lovastatin, fluvastatin, and simvastatin, as well as the hydrophilic statin pravastatin, both alone and in combination with estrogen.
	The results showed that all statins, with the exception of pravastatin, significantly inhibited cellular proliferation in both cell lines after four days of culture; this association was dose-dependent. The inhibitory values ranged from 10% to 90%, and the inhibition was greater in ER- cells. In ER+ cells, atorvastatin was a less potent inhibitor than the other statins.
	In the presence of estrogen, all statins equally inhibited cellular proliferation in ER+ cells. However, the statins were not completely successful in preventing cellular proliferation in the presence of stimulating estradiol (56).
	Further *in vitro* studies demonstrate the ability of the lipophilic statins to prevent cellular proliferation in tumor cell lines that are hormone receptor-positive and HER-2-negative (MCF-7), hormone receptor-negative and HER-2-positive (SKBr3), and double negative (MDA-231) (57).
	In vitro, the hydrophilic statin pravastatin shows no inhibitory effect on any cell lines. In contrast, the lipophilic statins fluvastatin, lovastatin, and simvastatin show significant cell growth inhibition, particularly in cells with activated Ras and HER-2 pathways.
	Furthermore, response to statins appears to be associated with cellular levels of NF-κB, an anti-apoptotic mediator and transcription factor complex. Within four hours of statin treatment, all three cell lines showed significant reductions in cellular p-MEK1/2 levels; these are key factors in the Ras-Raf-MEK-MAPK pathway, which drives cellular proliferation. However, this decrease was transient, and the levels began to increase after 12 hours. In SKBr3 cells, levels of activated NF-κB decreased to approximately 70% after 48 hours, and AP-1 levels also decreased. Moreover, cyclin D1 levels were seen to decrease, thus halting the cell cycle, while the levels of apoptotic mediators, mainly caspases (57).
In vivo (mice inoculated with MCNeuA cells)	*In vivo*, mice inoculated with MCNeuA cells (HER-2+, ER-), benefit from oral treatment of simvastatin at 1 to 2 mg/kg body weight daily (equivalent to approximately 5 to 10 mg daily in humans) (57). Tumors from mice treated with statins appeared to be richer in caspases, showing enhanced apoptosis (57).

eNOS: endothelial nitric oxide synthase; ER: estrogen receptor; MCF-7: Michigan Cancer Foundation-7 cell line; MDA-MB 231: cell line with breast adenocarcinoma metastases derived from pleural effusion; HER-2: human epidermal growth factor receptor 2; SKBr3: cell line with breast adenocarcinoma metastases derived from pleural effusion; Ras: rat sarcoma gene; NF-κB: nuclear factor κB; Raf: rapidly accelerated fibrosarcoma kinase; MEK: mitogen-activated protein kinase kinase; MAPK: mitogen-activated protein kinase; MCNeuA: mammary carcinoma from Neu transgenic mouse A

ranged from 10% to 90%; the inhibition was greater in ER- cells. In ER+ cells, atorvastatin was a less potent inhibitor than the other statins. In the presence of estrogen, all statins showed equal inhibition of cellular proliferation in ER+ cells. However, the statins were not completely successful in preventing cellular proliferation in the presence of stimulating estradiol (57).

Further *in vitro* studies have demonstrated the ability of the lipophilic statins to prevent cellular proliferation in tumor cell lines that are hormone receptor-positive and HER-2-negative (MCF-7), hormone receptor-negative and HER-2-positive (SKBr3), or double negative (MDA-231) (58). *In vitro*, the hydrophilic statin pravastatin showed no inhibitory effects on any cell line. In contrast, the lipophilic statins fluvastatin, lovastatin, and simvastatin showed significant inhibition of cell growth, particularly in cells with activated Ras and HER-2 pathways. Furthermore, response to statins appeared to be associated with cellular levels of NF-κB, an anti-apoptotic mediator and transcription factor complex. Within four hours of statin treatment, all three cell lines showed significant reductions in cellular p-MEK1/2 levels, a key player in the Ras-Raf-MEK-MAPK pathway that drives cellular proliferation. However, this decrease was transient, and p-MEK1/2 levels began to increase after 12 hours. In SKBr3 cells, levels of activated NF-κB decreased to approximately 70% after 48 hours; AP-1 levels also decreased. Moreover, levels of cyclin D1 were seen to decrease, thus halting the cell cycle, while the levels of apoptotic mediators, mainly caspases (58).

In an *in vivo* study, mice inoculated with MCNeuA cells (HER-2+, ER-) benefitted from oral treatment of simvastatin at 1 to 2 mg/kg body weight daily (equivalent to approximately 5 to 10 mg daily in humans) (58). Tumors from mice treated with statins appeared to be richer in caspases, showing enhanced apoptosis (58).

Epidemiological studies provide conflicting evidence. Numerous studies have demonstrated that statin use is associated with a lower risk of developing cancer (61-64), while other studies describe an increased risk (65, 66). Bonovas *et al.* (67) conducted a meta-analysis of 7 randomized trials and 9 observational trials; they concluded that statin use did not affect the risk of developing breast cancer. The overall incidence of breast cancer was calculated to be 1.55% in the treatment group receiving

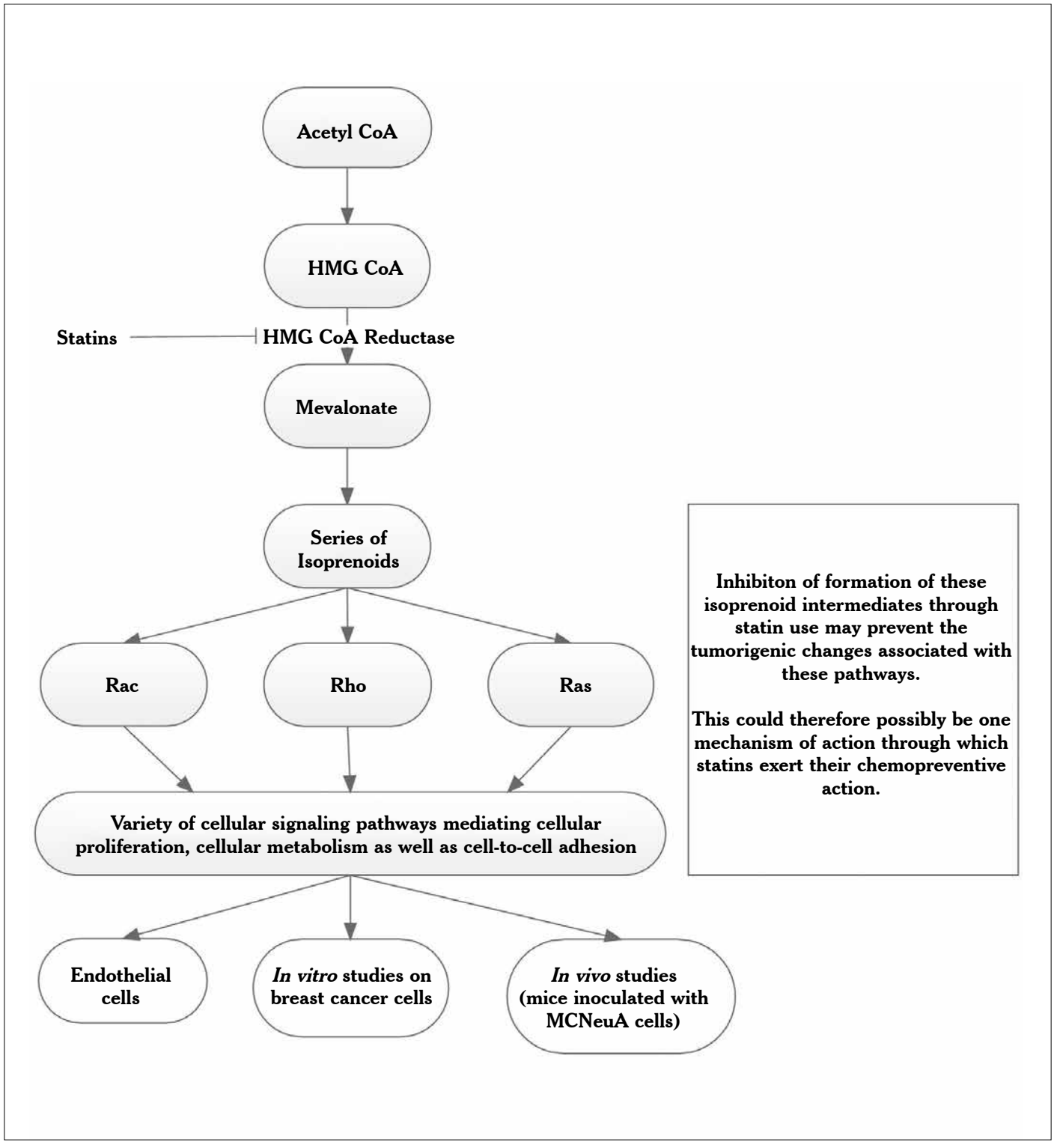


Figure 1. Proposed mechanism of action of statins (59)

statins (132 cases) and 1.43% in the non-treatment group (122 cases). This difference was not found to be statistically significant. However, the trials included in this meta-analysis mainly focused on the effects of statins on cardiovascular disease; the dosages were set only in this regard, and follow-up was relatively short. The effects of long-term statin use and the incidence of breast cancer could not be identified, which may have affected the observed association between statin use and risk of breast cancer. Furthermore, this meta-analysis included the use of both lipophilic and hydrophobic statins; the latter cannot

permeate the cell membrane and thus do not exert activity on cellular proliferation and motility. This may have also led to the calculated lack of association between statin use and risk of breast cancer.

More recently, a trial was performed to test the chemopreventive abilities of fluvastatin in women with diagnosed DCIS or stage 1 breast carcinoma. Patients were randomized to receive either 80 mg daily or 20 mg daily of fluvastatin for 3 to 6 weeks prior to surgery. The results showed that fluvastatin was most effective in patients with high grade (poorly differentiated) tumors. The proliferation of these tumors decreased by a median of 7.2%, while in low grade tumors, this decrease was only 0.3%; the difference between the two was statistically significant. Overall, tumor apoptosis increased in 38% of patients, remained the same in 41%, and decreased in 21%; high grade tumors showed an increase in apoptosis (68).

The preclinical, clinical, and epidemiological results show that statins can likely reduce the incidence of breast cancer and may have anti-tumor potential. However, controversy persists. Further investigation is still required to identify whether statin use is truly associated with reduced incidence of breast cancer, the magnitude of this association, and the class of patients in which it is most likely to be prevalent.

In conclusion, because the abovementioned drugs have different mechanisms of action than the endocrine drugs currently used as chemopreventive agents (mainly SERMs and AIs), they may be useful as chemopreventive agents in non-responders to conventional chemoprevention. This makes sense, especially when considering that these same drugs are also being used to treat concurrent, prevalent conditions such as diabetes and hyperlipidemia. However, further studies to evaluate the chemopreventive potency of these drugs compared to current chemopreventive strategies are required to clarify this controversial issue. These studies may associate these drugs with chemopreventive benefits in breast cancer and may also identify which patient groups are likely to benefit from this novel application of these drugs. The desired result would be the clinical implementation of these further studies, thus providing another mechanism of chemoprevention of breast cancer and reducing polypharmacy in the respective patients; thus, the instance of adverse drug reactions and drug-drug interactions would decrease.

Ethics Committee Approval: *N/A.*

Informed Consent: *N/A.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Concept - J.C.A.; Design - D.M., S.M.; Supervision - J.C.A.; Resources - D.M., S.M., J.C.A.; Materials - D.M., S.M.; Data Collection and/or Processing - D.M., S.M.; Analysis and/or Interpretation - D.M., J.C.A., P.S.W.; Literature Search - D.M., S.M.; Writing Manuscript - D.M., S.M.; Critical Review - J.C.A., P.S.W.*

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

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

References

1. Cazzaniga M, Bonanni B. Breast cancer chemoprevention: old and new approaches. J Biomed Biotechnol 2012; 2012: 985620. [CrossRef]
2. Micallef S, Micallef D, Schembri-Wismayer P, Brincat MP, Calleja-Agius J. Chemoprevention of breast cancer among women at elevated risk as defined by Gail Score. Minerva Ginecol 2015; 67: 335-52.
3. Russell R. Non-steroidal anti-inflammatory drugs and gastrointestinal damage-problems and solutions. Postgrad Med J 2001; 77: 82-8. [CrossRef]
4. Nathan DM, Buse JB, Davidson MB, Heine RJ, Holman RR, Sherwin R, et al. Management of hyperglycemia in type 2 diabetes: A consensus algorithm for the initiation and adjustment of therapy a consensus statement from the American Diabetes Association and the European Association for the study of diabetes. Diabetes Care 2006; 29: 1963-72. [CrossRef]
5. Emili A, Abushomar H, Nair K. Treating metabolic syndrome Lifestyle change or medication? Can Fam Physician 2007; 53: 1203-5.
6. Taylor F, Huffman MD, Macedo AF, Moore T, Burke M, Davey Smith G, et al. Statins for the primary prevention of cardiovascular disease. Cochrane Database Syst Rev 2013; CD004816. [CrossRef]
7. Santa-Maria CA, Stearns V. Statins and breast cancer: future directions in chemoprevention. Curr Breast Cancer Rep 2013; 5: 161-9. [CrossRef]
8. Cazzaniga M, Bonanni B, Guerrieri-Gonzaga A, Decensi A. Is it time to test metformin in breast cancer clinical trials? Cancer Epidemiol Biomarkers Prev 2009; 18: 701-5. [CrossRef]
9. Yiannakopoulou EC. Aspirin and NSAIDs for breast cancer chemoprevention. European Journal of Cancer Prevention: 2015; 24: 416-21. [CrossRef]
10. Harris RE, Alshafie GA, Abou-Issa H, Seibert K. Chemoprevention of breast cancer in rats by celecoxib, a cyclooxygenase 2 inhibitor. Cancer Res 2000; 60: 2101-3.
11. Harris RE, Namboodiri KK, Farrar WB. Nonsteroidal antiinflammatory drugs and breast cancer. Epidemiology 1996; 7: 203-5. [CrossRef]
12. Agrawal A, Fentiman I. NSAIDs and breast cancer: a possible prevention and treatment strategy. Int J Clin Pract 2008; 62: 444-9. [CrossRef]
13. Perou CM, Sørlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, et al. Molecular portraits of human breast tumours. Nature 2000; 406: 747-52. [CrossRef]
14. Takkouche B, Regueira-Mendez C, Etminan M. Breast cancer and use of nonsteroidal anti-inflammatory drugs: a meta-analysis. J Natl Cancer Inst 2008; 100: 1439-47. [CrossRef]
15. Bardia A, Olson JE, Vachon CM, Lazovich D, Vierkant RA, Wang AH, et al. Effect of aspirin and other NSAIDs on postmenopausal breast cancer incidence by hormone receptor status: results from a prospective cohort study. Breast Cancer Res Treat 2011; 126: 149-55. [CrossRef]
16. Lieberman M, Marks AD. Marks' Basic Medical Biochemistry: A Clinical Approach: Wolters Kluwer Health/Lippincott Williams & Wilkins; 2009.p 847-62.
17. Funk CD, FitzGerald GA. COX-2 inhibitors and cardiovascular risk. J Cardiovasc Pharmacol 2007; 50: 470-9. [CrossRef]
18. Schottenfeld D, Beebe-Dimmer J. Chronic inflammation: a common and important factor in the pathogenesis of neoplasia. CA Cancer J Clin 2006; 56: 69-83. [CrossRef]
19. Pollacco J, Sacco K, Portelli M, Schembri-Wismayer P, Calleja-Agius J. Molecular links between endometriosis and cancer. Gynecol En-

docrinol 2012; 28: 577-81. [CrossRef]
20. Brasky TM, Bonner MR, Moysich KB, Ambrosone CB, Nie J, Tao MH, et al. Non-steroidal anti-inflammatory drugs (NSAIDs) and breast cancer risk: differences by molecular subtype. Cancer Causes Control 2011; 22: 965-75. [CrossRef]
21. Uray IP, Brown PH. Chemoprevention of hormone receptor-negative breast cancer: new approaches needed. Recent Results Cancer Res 2011; 188: 147-62. [CrossRef]
22. Harris RE, Alshafie GA, Abou-Issa H, Seibert K. Chemoprevention of breast cancer in rats by celecoxib, a cyclooxygenase 2 inhibitor. Cancer Res 2000; 60: 2101-3.
23. Larsson SC, Mantzoros CS, Wolk A. Diabetes mellitus and risk of breast cancer: a meta-analysis. Int J Cancer 2007; 121: 856-62. [CrossRef]
24. Liao S, Li J, Wei W, Wang L, Zhang Y, Li J, et al. Association between diabetes mellitus and breast cancer risk: a meta-analysis of the literature. Asian Pac J Cancer Prev 2011; 12: 1061-5.
25. Larsson SC, Mantzoros CS, Wolk A. Diabetes mellitus and risk of breast cancer: A meta-analysis. Int J Cancer 2007; 121: 856-62. [CrossRef]
26. Belfiore A, Frittitta L, Costantino A, Frasca F, Pandini G, Sciacca L, et al. Insulin receptors in breast cancer. Ann N Y Acad Sci 1996; 784: 173-88. [CrossRef]
27. Papa V, Belfiore A. Insulin receptors in breast cancer: biological and clinical role. J Endocrinol Invest 1996; 19: 324-33. [CrossRef]
28. Hirose K, Toyama T, Iwata H, Takezaki T, Hamajima N, Tajima K. Insulin, insulin-like growth factor-I and breast cancer risk in Japanese women. Asian Pac J Cancer Prev 2003; 4: 239-46.
29. Lawlor DA, Smith GD, Ebrahim S. Hyperinsulinaemia and increased risk of breast cancer: findings from the British Women's Heart and Health Study. Cancer Causes Control 2004; 15: 267-75. [CrossRef]
30. Verheus M, Peeters PH, Rinaldi S, Dossus L, Biessy C, Olsen A, et al. Serum C-peptide levels and breast cancer risk: Results from the European prospective investigation into cancer and nutrition (EPIC). Int J Cancer 2006; 119: 659-67. [CrossRef]
31. Yang G, Lu G, Jin F, Dai Q, Best R, Shu XO, et al. Population-based, case-control study of blood C-peptide level and breast cancer risk. Cancer Epidemiol Biomarkers Prev 2001; 10: 1207-11.
32. Renehan AG, Harvie M, Howell A. Insulin-like growth factor (IGF)-I, IGF binding protein-3, and breast cancer risk: eight years on. Endocr Relat Cancer 2006; 13: 273-8. [CrossRef]
33. Nyholm H, Djursing H, Hagen C, Agner T, Bennett P, Svenstrup B. Androgens and estrogens in postmenopausal insulin-treated diabetic women. J Clin Endocrinol Metab 1989; 69: 946-9. [CrossRef]
34. Quinn M, Ruffe H, Brown J, Ennis G. Circulating gonadotropins and urinary oestrogens in postmenopausal diabetic women. Aust N Z J Obstet Gynaecol 1981; 21: 234-6. [CrossRef]
35. Plymate SR, Hoop RC, Jones RE, Matej LA. Regulation of sex hormone-binding globulin production by growth factors. Metabolism 1990; 39: 967-70. [CrossRef]
36. Kaaks R, Rinaldi S, Key T, Berrino F, Peeters P, Biessy C, et al. Postmenopausal serum androgens, oestrogens and breast cancer risk: the European prospective investigation into cancer and nutrition. Endocr Relat Cancer 2005; 12: 1071-82. [CrossRef]
37. Key T, Appleby P, Barnes I, Reeves G. Endogenous sex hormones and breast cancer in postmenopausal women: reanalysis of nine prospective studies. J Natl Cancer Inst 2002; 94: 606-16. [CrossRef]
38. Del Barco S, Vazquez-Martin A, Cufi S, Oliveras-Ferraros C, Bosch-Barrera J, Joven J, et al. Metformin: multi-faceted protection against cancer. Oncotarget 2011; 2: 896-917. [CrossRef]
39. Vigneri P, Frasca F, Sciacca L, Pandini G, Vigneri R. Diabetes and cancer. Endocr Relat Cancer 2009; 16: 1103-23. [CrossRef]
40. Ravichandran LV, Esposito DL, Chen J, Quon MJ. Protein kinase C-zeta phosphorylates insulin receptor substrate-1 and impairs its ability to activate phosphatidylinositol 3-kinase in response to insulin. J Biol Chem 2001; 276: 3543-9. [CrossRef]
41. Pasanisi P, Berrino F, De Petris M, Venturelli E, Mastroianni A, Panico S. Metabolic syndrome as a prognostic factor for breast cancer recurrences. Int J Cancer 2006; 119: 236-8. [CrossRef]
42. Lashen H. Role of metformin in the management of polycystic ovary syndrome. Ther Adv Endocrinol Metab 2010; 1: 117-28. [CrossRef]
43. Hermann LS. Metformin: a review of its pharmacological properties and therapeutic use. Diabete Metab 1979; 5: 233-45.
44. Jiralerspong S, Palla SL, Giordano SH, Meric-Bernstam F, Liedtke C, Barnett CM, et al. Metformin and pathologic complete responses to neoadjuvant chemotherapy in diabetic patients with breast cancer. J Clin Oncol 2009; 27: 3297-302. [CrossRef]
45. Goodwin PJ, Ligibel JA, Stambolic V. Metformin in breast cancer: time for action. J Clin Oncol 2009; 27: 3271-3. [CrossRef]
46. Decensi A, Puntoni M, Goodwin P, Cazzaniga M, Gennari A, Bonanni B, et al. Metformin and cancer risk in diabetic patients: a systematic review and meta-analysis. Cancer Prev Res 2010; 3: 1451-61. [CrossRef]
47. Evans JM, Donnelly LA, Emslie-Smith AM, Alessi DR, Morris AD. Metformin and reduced risk of cancer in diabetic patients. BMJ 2005; 330: 1304-5. [CrossRef]
48. Noto H, Goto A, Tsujimoto T, Noda M. Cancer risk in diabetic patients treated with metformin: a systematic review and meta-analysis. PloS One 2012; 7: e33411. [CrossRef]
49. Zhu Z, Jiang W, Thompson MD, McGinley JN, Thompson HJ. Metformin as an energy restriction mimetic agent for breast cancer prevention. J Carcinog 2011; 10: 17. [CrossRef]
50. Takane H, Shikata E, Otsubo K, Higuchi S, Ieiri I. Polymorphism in human organic cation transporters and metformin action. Pharmacogenomics 2008; 9: 415-22. [CrossRef]
51. Inoki K, Zhu T, Guan KL. TSC2 mediates cellular energy response to control cell growth and survival. Cell 2003; 115: 577-90. [CrossRef]
52. Vazquez-Martin A, Oliveras-Ferraros C, Lopez-Bonet E, Menendez JA. AMPK: Evidence for an energy-sensing cytokinetic tumor suppressor. Cell Cycle 2009; 8: 3679-83. [CrossRef]
53. Pecorino L. Molecular Biology of Cancer: Mechanisms, Targets, and Therapeutics: OUP Oxford; 3rd edition 2012. p. 123-48.
54. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science 2009; 324: 1029-33. [CrossRef]
55. Alimova IN, Liu B, Fan Z, Edgerton SM, Dillon T, Lind SE, et al. Metformin inhibits breast cancer cell growth, colony formation and induces cell cycle arrest in vitro. Cell Cycle 2009; 8: 909-15. [CrossRef]
56. Stancu C, Sima A. Statins: mechanism of action and effects. J Cell Mol Med 2001; 5: 378-87. [CrossRef]
57. Mueck AO, Seeger H, Wallwiener D. Effect of statins combined with estradiol on the proliferation of human receptor-positive and receptor-negative breast cancer cells. Menopause 2003; 10: 332-6. [CrossRef]
58. Campbell MJ, Esserman LJ, Zhou Y, Shoemaker M, Lobo M, Borman E, et al. Breast cancer growth prevention by statins. Cancer Res 2006; 66: 8707-14. [CrossRef]
59. Laufs U, Liao JK. Direct vascular effects of HMG-CoA reductase inhibitors. Trends Cardiovasc Med 2000; 10: 143-8. [CrossRef]

60. Mason RP, Walter MF, Jacob RF. Effects of HMG-CoA reductase inhibitors on endothelial function: role of microdomains and oxidative stress. Circulation 2004; 109(Suppl 1): II34-41. [CrossRef]
61. Blais L, Desgagne A, LeLorier J. 3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors and the risk of cancer: a nested case-control study. Arch Intern Med 2000; 160: 2363-8. [CrossRef]
62. Boudreau DM, Gardner JS, Malone KE, Heckbert SR, Blough DK, Daling JR. The association between 3-hydroxy-3-methylglutaryl conenzyme A inhibitor use and breast carcinoma risk among postmenopausal women: a case-control study. Cancer 2004; 100: 2308-16. [CrossRef]
63. Cauley JA, Zmuda JM, Lui LY, Hillier TA, Ness RB, Stone KL, et al. Lipid-lowering drug use and breast cancer in older women: a prospective study. J Womens Health (Larchmt) 2003; 12: 749-56. [CrossRef]
64. Katz MS. Therapy insight: Potential of statins for cancer chemoprevention and therapy. Nat Clin Pract Oncol 2005; 2: 82-9. [CrossRef]
65. Beck P, Wysowski DK, Downey W, Butler-Jones D. Statin use and the risk of breast cancer. J Clin Epidemiol 2003; 56: 280-5. [CrossRef]
66. Kaye JA, Meier CR, Walker AM, Jick H. Statin use, hyperlipidaemia, and the risk of breast cancer. Br J Cancer 2002; 86: 1436-9. [CrossRef]
67. Bonovas S, Filioussi K, Tsavaris N, Sitaras NM. Use of statins and breast cancer: a meta-analysis of seven randomized clinical trials and nine observational studies. J Clin Oncol 2005; 23: 8606-12. [CrossRef]
68. Garwood ER, Kumar AS, Baehner FL, Moore DH, Au A, Hylton N, et al. Fluvastatin reduces proliferation and increases apoptosis in women with high grade breast cancer. Breast Cancer Res Treat 2010; 119: 137-44. [CrossRef]

Robotic surgery in gynecology

Ibrahim Alkatout, Liselotte Mettler, Nicolai Maass, Johannes Ackermann

Department of Obstetrics and Gynecology, University Hospitals Schleswig-Holstein, Campus Kiel, Kiel, Germany

Abstract

Robotic surgery is the most dynamic development in the sector of minimally invasive operations currently. It should not be viewed as an alternative to laparoscopy, but as the next step in a process of technological evolution. The advancement of robotic surgery, in terms of the introduction of the Da Vinci Xi, permits the variable use of optical devices in all four trocars. Due to the new geometry of the "patient cart," an operation can be performed in all spatial directions without re-docking. Longer instruments and the markedly narrower mechanical elements of the "patient cart" provide greater flexibility as well as access similar to those of traditional laparoscopy.

Currently, robotic surgery is used for a variety of indications in the treatment of benign gynecological diseases as well as malignant ones. Interdisciplinary cooperation and cooperation over large geographical distances have been rendered possible by telemedicine, and will ensure comprehensive patient care in the future by highly specialized surgery teams. In addition, the second operation console and the operation simulator constitute a new dimension in advanced surgical training. The disadvantages of robotic surgery remain the high costs of acquisition and maintenance as well as the laborious training of medical personnel before they are confident with using the technology.

(J Turk Ger Gynecol Assoc 2016; 17: 224-32)

Keywords: Robotic surgery, laparoscopy, gynecological oncology, surgical training, cost efficiency

Received: 30 June, 2016 **Accepted:** 27 September, 2016

Robotic surgery in gynecology

Robotic surgery is a dynamic development for minimally invasive procedures. The specialty of gynecology consistently offers new opportunities for innovative surgical techniques and the advancement of existing therapy approaches (Figure 1).

Ever since the American FDA granted approval of the Da Vinci operation robot for gynecological operations in 2005, about three million robotic operations have been performed worldwide. 3500 Da Vinci systems are currently in use: 586 of these in Europe and 77 in Germany (4th quarter of 2015). According to the figures of Intuitive Surgical, about 600,000 interventions were performed on a worldwide basis in the year 2014, of which 50% were performed in gynecology, approximately 30% in urology, and about 20% in general and chest surgeries. In 2011, the proportion of robotic hysterectomies performed for benign indications in the USA was as high as 27% (1).

Currently, we have experience in robotic surgery for the majority of gynecological operations and fields of application. The known advantages of minimally invasive surgery, such as less blood loss, shorter durations of hospital stay, and lower patient morbidity compared to open procedures, have been observed here as well. Better exposure of the operating field by 3D technology and the extension of surgical instruments to 7 degrees of freedom permit the use of minimally invasive surgery, even for complex indications. Robot-assisted manipulation of the instruments permits tremor-free handling and reduces work fatigue for the surgeon, which is very advantageous for the surgeon as well as the patient in long and complex interventions. The possibility of working simultaneously on two parallel consoles shortens the learning curve, reduces complication rates, and facilitates the training of surgeons (2).

The advancement of robotic surgery in terms of the Da Vinci Xi permits the variable use of optics in all four trocars (paraaortic lymphadenectomy, omentectomy, or interdisciplinary surgery in the upper abdomen can be performed without re-docking) and ensures markedly greater flexibility due to the optimized geometry of the so-called patient cart. Robotic surgery has been criticized for the fact that it requires the use of larger trocars compared to conventional laparoscopy, and is therefore associated with more numerous and larger cosmetic scars; this is avoided by the smaller trocars now used in robotic surgery (3). The development of the single-site systems signifies further new options for the gynecological surgeon (Figure 2). For instance, freedom of movement is now maximized by the introduction of one or more additional working trocars (4, 5).

The low level of postoperative pain appears to be another advantage. It is accompanied by a lesser need for analgesics and even shorter hospital stays compared to traditional laparoscopic surgery. One explanation could be the fact that the abdominal wall need not be used as a counter bearing. The absence of irritation and the advantage of tissue protection seem to display very positive effects (6, 7).

The disadvantages, on the other hand, are the still high costs of installation and maintenance, the longer operating times

Address for Correspondence: *Ibrahim Alkatout*
e.mail: kiel.school@uksh.de

DOI: 10.5152/jtgga.2016.16187

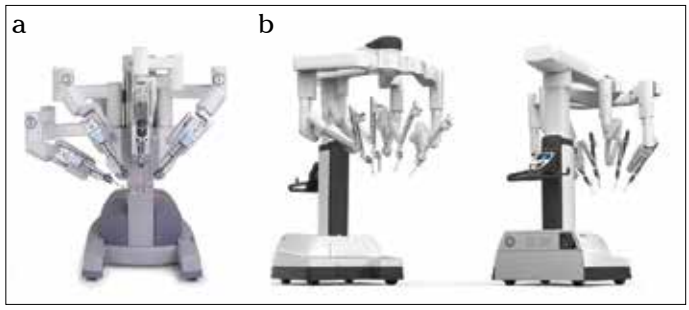


Figure 1. a, b. Da Vinci Si system with its central optical arm (12 mm diameter) and 3 working trocars, each 8 mm in diameter (a). The Da Vinci Xi system is markedly smaller with four identical flexible arms (8 mm each) (b). The camera and working trocars can be exchanged as desired. The simpler handling of the console and the instruments is not visible here.

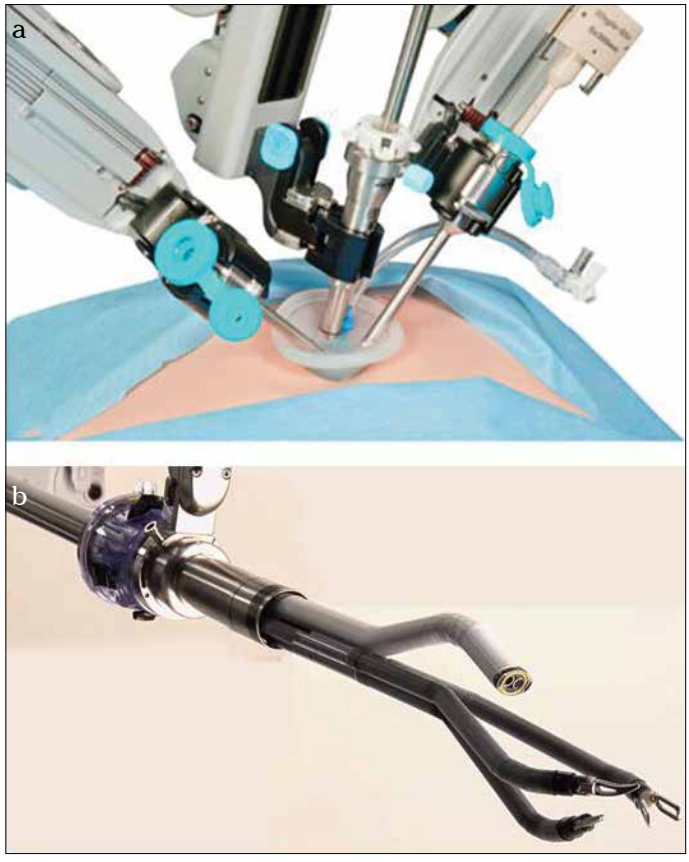


Figure 2. a, b. Single-site laparoscopy with the Da Vinci Xi system. An overview of the robot arms lying close to each other and the better distance because of the much smaller Xi system (a) Intraabdominal operations can be performed with angulated optical instruments and surgical instruments within a very small space without loss of quality (b). The single port is placed in the navel and additional working trocars can be placed at various sites in the abdominal wall to support the operation.

(at least in the beginning), and the initial learning curve that has to be traversed again (even by experienced laparoscopists) in order to work safely and gently. Doctors (anesthetists and surgeons) as well as nursing staff have to be trained for working with the robot system (2, 5). The advantages and limitations of robotic laparoscopy are shown in Table 1, 2.

Table 1. Advantages of the Da Vinci robot system compared to conventional laparoscopy

Advantages of the Da Vinci robot system compared to conventional laparoscopy
Ergonomics
Intuitive handling of instruments
3-D optics without additional equipment
7 degrees of freedom
Faster learning curve
Digital networking
Dual console
Integrated fluorescence visualization (Firefly)
Less postoperative pain

Table 2. Disadvantages of the Da Vinci robot system compared to conventional laparoscopy

Limitations of the Da Vinci robot system compared to conventional laparoscopy
Higher costs of disposable materials and maintenance
Absence of tactile feedback
Additional learning curve
Additional time for docking
Usually requires more numerous and larger puncture sites (Si System)

Application in benign gynecological diseases

The use of robotic surgery for gynecological diseases extends to nearly all surgical therapy options. However, owing to the high cost of robotic surgery, it will probably not become a common procedure for the treatment of benign diseases. Currently, robotic surgery is focused on malignant diseases whose costs are borne more easily by the health care system. Nevertheless, it would be necessary to develop innovative operation concepts that make robotic surgery a viable option in benign disease treatment as well. (1, 8). The new technical advancements of the surgery robot may help to extend the use of minimally invasive procedures to complex fields of application that were previously accessible only to laparotomy while utilizing the advantages of the minimally invasive approach. Potential areas of use for robotic surgery in benign gynecological diseases are shown in Table 3.

Robotic hysterectomy for benign diseases is currently the alternative to conventional laparoscopy in many developed countries. The frequent use of robotic surgery has further reduced the number of abdominal hysterectomies, especially in the USA (Figure 3). Cases of complex comorbidities, such as severe adhesions, obesity, or deep infiltrating endometriosis, are well suited for robot-assisted surgery (9). Lim et al. (10)

Table 3. Applications of Da Vinci robotic surgery for benign gynecological disease

Use of robotic surgery for malignant gynecological disease	
Cervical cancer	Radical hysterectomy
	Total mesometrial resection (TMMR)
	Trachelectomy
Endometrial cancer	Hysterectomy
	Peritoneal mesometrial resection (PMMR)
Lymphadenectomy and sentinel biopsy (dye and fluorescence)	Pelvic lymphadenectomy
	Paraaortic lymphadenectomy
Exenteration	Anterior exenteration
	Posterior exenteration
Ovarian cancer	Staging of early ovarian carcinoma
	De-bulking advanced ovarian carcinoma
Omentectomy	

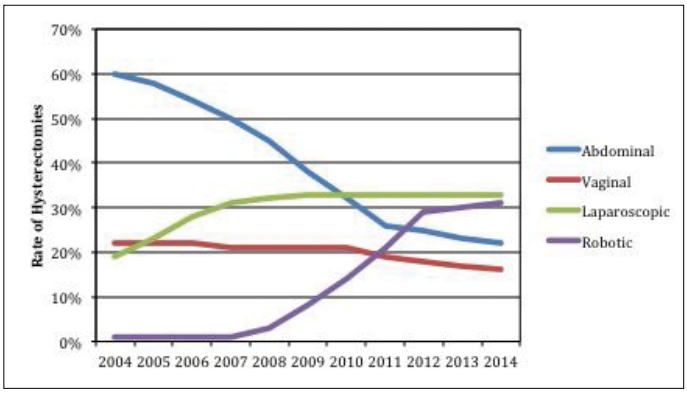


Figure 3. Trends in the use of hysterectomy methods in Western countries

published the first comprehensive multicenter study on this subject, which was only focused on experienced surgeons (>60 operations), thus circumventing the distorting effect of the learning curve. The authors analyzed more than 30,000 hysterectomies with a 30-day follow-up period. Robotic hysterectomy for benign disease was compared with alternative surgical modalities. Even in complex operations, robotic hysterectomies were associated with a lower intraoperative complication rate compared to abdominal and vaginal hysterectomies, and a significantly lower postoperative complication rate compared to other surgical procedures, especially laparoscopy.

In patients with a symptomatic myomatous uterus of reproductive age, or in women who undergo the surgical procedure because they wish to have children, enucleation of the myoma is an organ-preserving therapy option (11). One of the most dreaded complications of this operation is rupture of the uterus. Due to its simplified suturing method, and the possibility of uterine reconstruction, robotic surgery yields similar results and is associated with similar rupture rates as the open procedure, especially in this setting. In a population of 107 patients, Pitter et al. (12) reported 92 births and only one case of uterine rupture. Since these patients are still young, they benefit greatly from the minimally invasive approach in terms of long-term sequela, such as adhesions or weakness of the abdominal wall. Deep infiltrating endometriosis is one of the most suitable indications for robotic surgery in benign gynecological disease treatment. Since the patients are still young and wish to have children, they require organ-preserving and tissue-protective surgery (13). Continuity is usually preserved during excision of the endometriosis from the rectum and the ureter. However, more extensive interventions with partial resection of the bowel or bladder can also be performed with lower complication rates by this minimally invasive approach. Furthermore, the excellent view and the precise navigation of instruments permit preservation of the ovarian reserve, optimal restoration of anatomical conditions, and the prevention of postoperative adhesions (13). Recent investigations have shown that robotic surgery achieves these goals in the vast majority of cases, such as the fulfillment of a woman's wish for children, a significant reduction of pain, and the improvement of gastrointestinal symptoms. Further studies in large cohorts of patients will show whether, and to what extent, robotic surgery is superior to a traditional laparoscopic procedure (14).

Urogynecological interventions can be performed safely, precisely, and rapidly with the robot. The results of sacrocolpopexy are similar to those obtained by open surgery. The robot is especially useful for better visualization and handling in the presacral region, as well as in the insertion of a mesh and its secure fixation to the vaginal stump, the cervical stump, or the presacral area (15).

Re-anastomosis of the fallopian tubes for the purpose of re-fertilization is associated with a success rate of 67.6% in open surgery, and a 5.6% incidence of ectopic pregnancies. (16). Compared to conventional laparoscopy, the operation robot permits more "delicate" handling and a better view of the anatomical/histological layers, which is required for precise suturing and exact restoration of anatomical conditions (17). The disadvantage compared to the open procedure is the longer operating time. However, the duration of the hospital stay and the period of convalescence are markedly reduced (18). Long-term investigations will show whether robot-assisted surgery provides similar success rates as the open procedure. Notably, recent investigations revealed a re-anastomosis rate higher than 90% (19) (Figure 4, 5).

In obstetrics, cervical cerclage is another field of application for robotic surgery in order to avoid preterm births in patients with cervical insufficiency (20). The most common method is operation by vaginal access. When this is rendered impossible because of an excessively short cervix (caused by extensive conization, for instance) or for other technical reasons, abdominal access serves as an alternative (21). Here the advantages of the robotic procedure compared to the traditional laparoscopic approach include a much simpler intracorporeal suturing and more precise intraoperative exposure with less injury to the adjoining structures. A cerclage may be applied before, as well as during, a pregnancy. Successful pregnancies, near-term

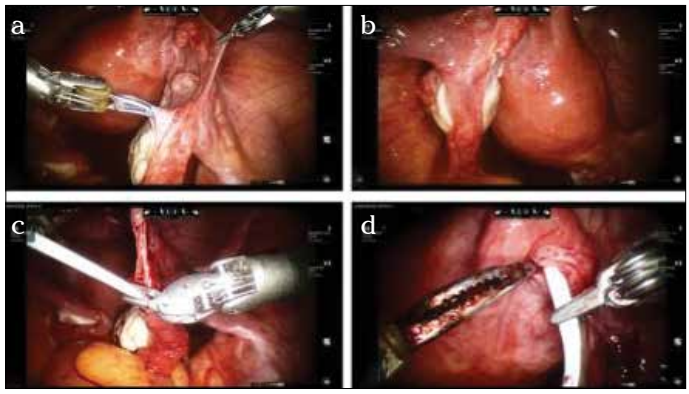


Figure 4. a-d. Operation site after laparoscopic sterilization on the right side (a) and the left side (b). After freshening the wound edges in the distal aspect and mobilization of the mesentery, a thin and blunt catheter is inserted into the distal end; the catheter protrudes from the fimbrial funnel of the fallopian tube (c). The other end of the catheter is pushed into the freshened proximal end so that it almost serves as a bridge (d).

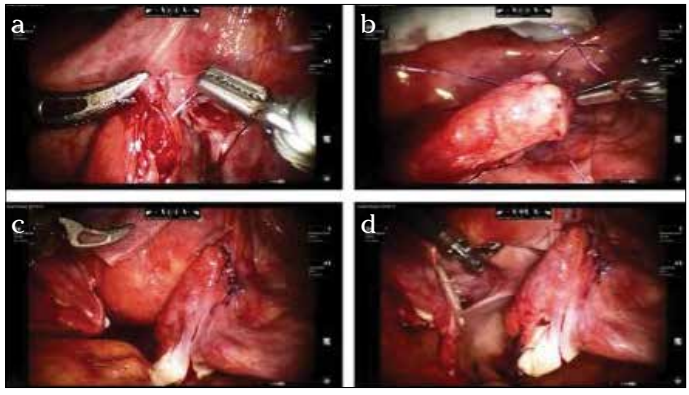


Figure 5. a-d. The first suture is made, strictly in the seromuscular aspect, omitting the mucosa. Very thin suture material or even monofilament sutures (at least 4–0) are suitable for this purpose (a). Four to eight sutures (b) that may then be knotted consecutively (c). Final situs on the left side after complete re-anastomosis (d). Bland suture and successful chromopertubation; no contrast medium emerges from the suture area and contrast medium is drained promptly through the fimbriae.

births, and low rates of preterm births have been reported for both procedures (22, 23). As the removal of the cerclage at the end of pregnancy is rendered very difficult by its intraabdominal positon, in these cases, a primary cesarean section is usually the only possible mode of delivery. Basically, the procedure is still in the experimental stage and should therefore be confined to individual cases after an exact estimation of the risks involved. It should be performed at centers specialized in the technique and in the course of clinical studies.

Application in malignant gynecological disease

The use of robotic surgery for malignant gynecological diseases is increasing in proportion to centralization and the availability of the robot. In the meantime, several retrospective and comparative studies have shown that the oncological outcome of minimally invasive surgical procedures is at least equivalent to that of open surgery. This is true of the general advantages of minimally invasive surgery as well as specific oncological aspects, although long-term studies on this subject are largely unavailable yet. A few investigations have shown that, for instance, robotic radical hysterectomies appear to be equivalent to surgery by the abdominal approach in terms of long-term survival. In fact, the robotic procedure is possibly superior to the conventional procedure in terms of the number of resected lymph nodes (7, 24).

Despite the success of screening programs and HPV vaccination, cervical carcinoma continues to be a global problem. The feasibility of radical hysterectomy with the aid of the robot has been confirmed on several occasions. Compared to abdominal and conventional laparoscopic radical hysterectomy, the authors observed lesser blood loss, shorter operating times, lower complication rates, and shorter hospital stays (25). Nevertheless, the existing body of data is still heterogeneous. Besides, large randomized controlled studies that would eliminate bias on the part of individual surgeons and permit generalizations are still lacking (26).

The use of the robot may be meaningful even in fertility-conserving operations like trachelectomy. However, the majority of these operations are performed at individual centers. The three-dimensional image, large magnification, and tremor-free work ensure optimal visualization of vascular structures and the parametria, which must be partially preserved during the operation. The largest study published so far, based on two centers in Sweden, showed a pregnancy rate of 81% and a low rate of preterm births (71% delivered their children after the 36th gestational week) in 56 women (27).

The technique of total mesometrial resection (TMMR) of the uterus was first described by Michael Höckel and is now an established alternative in robot-assisted surgery for the treatment of cervical cancer. In contrast to the Wertheim-Meigs operation, it is not oriented to traditional anatomical margins, but integrates the principles of embryonic development into the concept of the morphogenetic uterovaginal unit. The data reported thus far indicate that the new therapy approach is associated with a large recurrence-free interval, better five-year survival rates, and lower morbidity rates compared to the traditional approach. These advantages are largely attributable to the absence of locoregional recurrence and the avoidance of additional adjuvant radiotherapy (28). TMMR can now also be performed by a minimally invasive approach (29).

Endometrial carcinoma is the most common malignant genital tumor among women in the Western world. At the time of diagnosis, the tumor is limited to the uterus in about 70% of cases. The primary therapy approach is surgery, consisting of hysterectomy with bilateral adenectomy. Surgery in endometrial cancer can be performed by the open approach, the minimally invasive approach (laparoscopic or robotic) or, in early tumor stage cases, even by the vaginal approach. Robotic hysterectomy with bilateral adenectomy was evaluated in a Danish study in regards to its impact on quality of life. A significant limitation of quality of life was noted one week postoperatively (56% of the

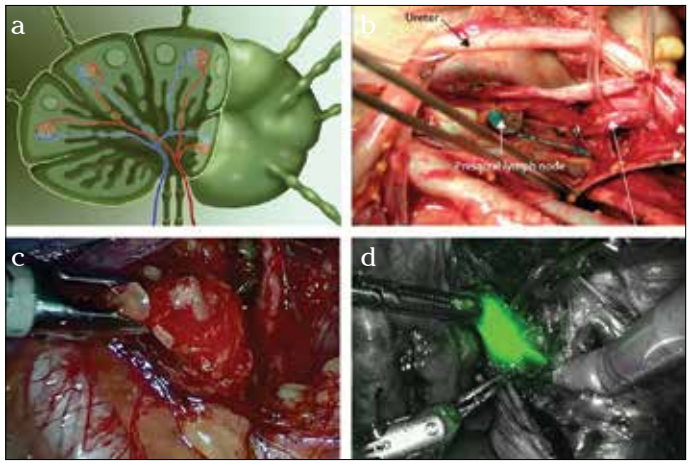


Figure 6. a-d. Schematic diagram of a lymph node. Several afferent lymph vessels from the peripheral aspect, central blood supply, and one or two efferent lymphatic vessels (a). Sentinel lymph nodes in endometrial cancer, here at the level of the common iliac artery on the right side, color-marked with methylene blue (b). In comparison: sentinel lymph node intraoperatively in endometrial cancer (c), partly marked with the fluorescent dye indocyanine green (d), here at the level of bifurcation of the common iliac artery on the right side.

baseline value). However, just 5 weeks post-surgery, the mean activity level of the patients were as high as 84%, thus reaching the preoperative baseline value (30). Especially the depth of tumor infiltration and histological grading determine the need for simultaneous or two-stage pelvic and paraaortic lymphadenectomy. Robotic surgery achieved equivalent or better oncological results compared to laparoscopy. A Finnish investigation of 99 patients revealed no difference in the number of resected lymph nodes; but robotic surgery was associated with shorter operating times, fewer conversions to laparotomy, as well as fewer minor and major complications (31). In an American study, the robot-assisted approach was found to be superior to the laparoscopic approach (32). The increasing use of minimally invasive surgical procedures also permits the application of sentinel node concepts. Sentinel nodes can be marked in color or by a fluorescent dye with the robot, and signify a further advancement compared to traditional laparoscopy (Figure 6) (33). Indocyanine green can also be used for intraoperative navigation, during ontogenetic compartment resection, in order to visualize the compartmental lymphatic system (34). Based on ontogenetic development, a new surgical concept has now been developed for compartment-based radical peritoneal mesometrial resection (PMMR) and therapeutic lymphadenectomy, according to Höckel, for the purpose of reducing locoregional recurrence. The efficacy of mesometrial resection of endometrial cancer is a part of ongoing studies in open as well as robotic surgery (35).

A large number of patients with endometrial cancer are overweight and obese. About 40% of the patients are overweight. The effort involved in the treatment of obese individuals is significantly greater than that for the treatment of non-obese patients with the same disease. Approximately one half of all endometrial cancers are operated upon with the minimally invasive approach. The limits of conventional laparoscopy become especially evident here. The general view of the lesser pelvis and the limited mobility of instruments have been reported as the primary limitations. These patients could especially benefit from the advantages of robotic surgery. Low complication rates have already been reported in many cases (36).

Other meaningful applications include anterior/posterior exenteration as interdisciplinary interventions. First described by Brunschwig in 1949, exenteration is still the treatment of choice for advanced or recurrent malignancies in the central compartment. Exenteration is frequently the sole potentially curative surgical method for recurrent cervical or endometrial cancer. Especially because of the ubiquitous use of laparoscopy in advanced urological disease and diseases calling for visceral surgery, the interdisciplinary approach permits the clinician to utilize the advantages of minimally invasive surgery (37).

The use of robotic surgery for the treatment of ovarian cancer is a debated issue, but should be viewed in the same context as the laparoscopic operation. At least for staging early carcinomas, as well as completion surgery for borderline tumors, the advantages of the minimally invasive approach are obvious. Simultaneously, there appears to be no evidence of any drawbacks. Attempts have also been made to use the robot for de-bulking surgery in ovarian cancer (38, 39). The necessity to operate in all four quadrants implies re-docking and rotation of the operating table. Since the body of data on this subject is still rather scarce, the patients should be selected individually; the procedure should undoubtedly be regarded as experimental (40).

Table 4 provides an overview of recent reports on the use of robotic surgery for malignant gynecological diseases.

Application in complex situations

In an age of increasing medical specialization and professionalization, the interdisciplinary approach is gaining importance. Even in surgical specialties, the use of a multimodality therapy concept and good cooperation between the individual specialties are becoming increasingly important (41). Especially, these facts offer new opportunities for robotic surgery. The direct division of the operation between surgeons of various specialties is rendered possible by two operation consoles that can be used simultaneously. This permits the specialists, who have different points of focus, to complement each other in symbiotic fashion. Furthermore, operating far from the field of operation enables a more rapid exchange of surgeons. Modern communication systems and telemonitoring have made it possible to exchange information over a large distance (42). In addition to saving resources and reducing costs, it is possible to work more effectively and provide better patient care.

Overweight patients constitute one of the greatest problems in health care and health care costs in the modern Western world. In Germany, approximately every second woman is overweight (BMI>25) and every fourth woman is obese (BMI>30) (43). In addition to the generally high health risk and resulting diseases, such as arterial hypertension, arteriosclerosis, and type II diabetes mellitus, obesity is a challenge in surgical specialties as well. On the one hand, overweight patients benefit from a minimally invasive approach in terms of morbidity and hospitalization. On

Table 4. Selected studies on the use of robotic surgery in malignant gynecological disease

Authors/Year	Disease	Number of patients	Study design	Study results
Margina et al. 2008 (25)	Cervical cancer, hysterectomy	129	Prospective/comparison of the abdominal, laparoscopic, and robotic approach.	Less blood loss, shorter operating time, and shorter hospital stay.
Wright et al. 2012 (41)	Cervical cancer, hysterectomy	1894	Retrospective/comparison of the abdominal, laparoscopic, and robotic approach.	Less blood loss, shorter hospital stay, and costs similar to the laparoscopic procedure.
Chen et al. 2014 (42)	Cervical cancer, hysterectomy	100	Retrospective/comparison of the abdominal, laparoscopic, and robotic approach.	Less blood loss, shorter operating time, shorter hospital stay, lower conversion rates to laparotomy, less postoperative pain, and earlier return to a normal diet.
Boggess et al. 2008 (32)	Endometrial cancer, hysterectomy with staging	322	Retrospective/comparison of the abdominal laparoscopic and robotic approach.	Resection of more numerous lymph nodes, shorter operating time compared to TLH, and lower complication rates compared to the abdominal procedure.
Gehrig et al. 2008 (43)	Endometrial cancer, hysterectomy with staging	81	Retrospective/comparison of the abdominal, laparoscopic, and robotic approach in overweight patients.	Less blood loss, shorter operating time, shorter hospital stay, and higher rate of resected lymph nodes.
Gaia et al. 2010 (44)	Endometrial cancer, hysterectomy with staging	1591	Systematic meta-analysis of 8 studies/comparison of the abdominal, laparoscopic, and robotic approach.	Less blood loss with similar rates of perioperative complications for robotic and laparoscopic operations.
Stephan et al. 2015 (45)	Endometrial cancer, hysterectomy in patients with morbid obesity, BMI>50	168	Retrospective/robotic hysterectomy: Comparison of patients with morbid obesity (BMI>50) and patients with a lower BMI,	Same result in regards to blood loss, complications, hospital stay, and removal of lymph nodes.
Feuer et al. 2013 (38)	Ovarian cancer	89	Retrospective/robotic, abdominal, initial staging, or debulking after neoadjuvant chemotherapy	Longer operating time, less blood loss, shorter hospital stay, similar rates of complications, residual tumor, 1-year survival
Magrina et al. 2013 (39)	Ovarian cancer	52	Retrospective/Cytoreduction in case of tumor recurrence, comparison of the abdominal, laparoscopic, and robotic approaches.	Less blood loss, shorter hospital stay, similar operating time, complication rates, residual tumor, and survival time.

the other hand, these patients impose high demands on the surgeon and the operation system. Traditional laparoscopy reaches its limits here (4). Thanks to the robust surgical tools and 7 degrees of freedom (which dispense with the need for using the abdominal wall as a counter bearing) in robotic surgery, it can also be used in severely obese patients. The limitations imposed on the anesthetist by the extremely low head-down position have been largely overcome. Furthermore, shorter operating times are now possible with a comparable number of complications and hospital stays of similar duration (44, 45).

Possibilities to improve surgical training in the age of minimally invasive surgery

In order to obtain a good result with the Da Vinci operation robot, the surgeon first requires targeted training and sufficient initial practice. Knowledge of open surgery, vaginal surgery, and conventional laparoscopy are, of course, extremely important. Working at the operation console, at a distance from the patient, and the limited tactile feedback call for some readjustment on the part of the surgeon. However, the merits of the system create new opportunities in training and advanced training at all levels. The paradigm shift in the last few years has caused a growing generation of surgeons to primarily use laparoscopy instead of traditional open surgery. Classical training of anatomy-based fundamental surgical techniques and the positioning of the teacher's hand next to the student's hand are hindered to an increasing degree by mechanization. Thus, a basic understanding of anatomy and the specific properties of tissue, resulting from palpatory perception, is lacking in many cases and must be developed gradually. Hence, all surgical approaches retain their value. However, the basic mechanical skills of laparoscopy and robotic surgery can be acquired by introducing the surgeon early to virtual training programs and

Table 5. Selected studies on the cost efficiency of robotic surgery in gynecology

Study	Intervention for	Comparison of methods	Variables	Costs
Desille-Gbaguidi et al. 2013 (52)	Endometrial and cervical cancer	Laparoscopic vs. robotic	Overall cost of treatment	Endometrial cancer: 6,666 € (laparoscopic) vs. 10,816 € (robotic) Cervical cancer: 7,803 € (laparoscopic) vs. 12,211 € (robotic)
Ind et al. 2015 (53)	Endometrial cancer	Abdominal vs. laparoscopic vs. robotic	Overall cost of treatment	Abdominal: 12,462 £ Laparoscopic: 9,979 £ Robotic: 8,481 £
Wright et al. 2012 (41)	Cervical cancer	Abdominal vs. laparoscopic vs. robotic	Overall cost of treatment	Abdominal: 9,618 $ Laparoscopic: 11,774 $ Robotic: 10,176 $
Wright et al. 2014 (57)	Hysterectomy for benign and malignant indications	Laparoscopic vs. robotic	Overall cost of treatment	Benign indication: 6,535$ (laparoscopic) vs. 8,152 $ (endoscopic) Malignant indication: 8,237 $ (laparoscopic) vs. 9,691 $ The cost difference is strongly dependent on the number of interventions
Reynisson and Persson 2013 (54)	Radical hysterectomy with pelvic lymphadenectomy	Abdominal vs. robotic	Overall cost of treatment	At a rate of 400 interventions per year, the cost of the robotic operation is similar to that of open surgery.

the pelvitrainer. Furthermore, the excellent imaging quality provides a clear view and enables the surgeon to learn about the relevant functional anatomy from a new perspective. The dual console of the robot signifies a quantum leap in practical surgical training. Two surgeons can work simultaneously, similar to the "driving school concept," and perform an operation together. Telemedicine will permit training and advanced training independent of space and time in the future (46).

Cost efficiency of robotic surgery

The most expensive aspect of robotic surgery, compared to all other surgical procedures, is the high cost of the equipment and the annual fixed costs (running costs and materials). In a study conducted by Desille-Gbaguidi, the cost of operations with the robot for endometrial cancer was 2.7-fold higher than that of conventional laparoscopy, and the cost of operations for cervical cancer was 2.6-fold higher than that of conventional laparoscopy. The overall cost of patient care was 1.6-fold higher (47). However, these calculations include the high acquisition cost of the equipment and, especially, the prolonged operating times. Furthermore, a variety of cost factors were included in the calculations. A careful analysis of endometrial cancer from Great Britain, however, has recently reported the contrary (48). There is consensus about the fact that maximum utilization of the system and a well-coordinated team with efficient processes of selection and organization can reduce the costs to the level of open surgical techniques. This is primarily achieved by progressively shorter operating times, fewer complications, and markedly shorter hospital stays. In the long term, more rapid convalescence and an earlier return to work also play a decisive role for the health care system (49). Attempts made thus far to compare conventional laparoscopy with robotic surgery in regards to costs have yielded very diverse results. The use of disposable laparoscopic instruments, for instance, was not taken into account (50). Statistical projections about the required number of robotic interventions per year in order to achieve profitability range between 165 and 400 operations (49, 51). Critical studies on the cost efficiency of robotic surgery are shown in Table 5.

Future perspectives

The entry of alternative manufacturers in the field of robotic surgery will transform the cost situation in a groundbreaking manner. Increasing specialization on the one hand, and centralization on the other, will advance technological progress and digital networking of innovative surgical procedures more rapidly. Preliminary results obtained with the new Xi system have shown that the new system has been adopted rapidly and without difficulties in gyneco-oncology as well as in the interdisciplinary sector. Its high flexibility and satisfactory complication rates will lead to a larger volume of data in terms of results in the near future, which must then be sustained from the oncological point of view as well.
The fact that robot-assisted surgery complements, but does not replace, conventional laparoscopy is an undisputed fact.

Ethics Committee Approval: *Ethics committee approval was received for this study from the ethics committee of the Christian Albrechts University in Kiel.*

Informed Consent: *Written informed consent was obtained from all patients who participated in this study.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Concept - I.A., L.M.; Design - J.A., I.A.; Supervision - L.M., N.M.; Resources - I.A., N.M.; Materials - J.A., L.M., N.M., I.A.; Data Collection and/or Processing - J.A., I.A.; Analysis and/or Interpretation*

- J.A., L.M., N.M., I.A.; Literature Search - J.A., I.A.; Writing Manuscript - J.A., Critical Review - L.M., N.M.

Acknowledgements: *The authors would like to thank Dawn Rüther for editing the manuscript.*

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

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

References

1. Liu H, Lu D, Wang L, Shi G, Song H, Clarke J. Robotic surgery for benign gynaecological disease. Cochrane Database Syst Rev 2012; 2: CD008978. [CrossRef]
2. Schollmeyer T, Mettler L, Alkatout I. Roboterchirurgie in der Gynäkologie - Der Operateur am Schreibtisch. Der Gynäkologe 2011: 196-201. [CrossRef]
3. Yuh B, Yu X, Raytis J, Lew M, Fong Y, Lau C. Use of a mobile tower-based robot--The initial Xi robot experience in surgical oncology. J Surg Oncol 2016; 113: 5-7. [CrossRef]
4. Sinha R, Sanjay M, Rupa B, Kumari S. Robotic surgery in gynecology. J Minim Access Surg 2015; 11: 50-9. [CrossRef]
5. Herron DM, Marohn M; SAGES-MIRA Robotic Surgery Consensus Group. A consensus document on robotic surgery. Surg Endosc 2008; 22: 313-25; discussion 311-2. [CrossRef]
6. Soliman PT, Langley G, Munsell MF, Vaniya HA, Frumovitz M, Ramirez PT. Analgesic and antiemetic requirements after minimally invasive surgery for early cervical cancer: a comparison between laparoscopy and robotic surgery. Ann Surg Oncol 2013; 20: 1355-9. [CrossRef]
7. Sinno AK, Fader AN. Robotic-assisted surgery in gynecologic oncology. Fertil Steril 2014; 102: 922-32. [CrossRef]
8. Venkat P, Chen LM, Young-Lin N, Kiet TK, Young G, Amatori D, et al. An economic analysis of robotic versus laparoscopic surgery for endometrial cancer: costs, charges and reimbursements to hospitals and professionals. Gynecol Oncol 2012; 125: 237-40. [CrossRef]
9. Nezhat C, Saberi NS, Shahmohamady B, Nezhat F. Robotic-assisted laparoscopy in gynecological surgery. JSLS 2006; 10: 317-20.
10. Lim PC, Crane JT, English EJ, Farnam RW, Garza DM, Winter ML, et al. Multicenter analysis comparing robotic, open, laparoscopic, and vaginal hysterectomies performed by high-volume surgeons for benign indications. Int J Gynaecol Obstet 2016; 133: 359-64. [CrossRef]
11. Soto E, Flyckt R, Falcone T. Endoscopic management of uterine fibroids: an update. Minerva Ginecol 2012; 64: 507-20.
12. Pitter MC, Gargiulo AR, Bonaventura LM, Lehman JS, Srouji SS. Pregnancy outcomes following robot-assisted myomectomy. Hum Reprod 2013; 28: 99-108. [CrossRef]
13. Siesto G, Ieda N, Rosati R, Vitobello D. Robotic surgery for deep endometriosis: a paradigm shift. Int J Med Robot 2014; 10: p. 140-6. [CrossRef]
14. Abo C, Roman H, Bridoux V, Huet E, Tuech JJ, Resch B, et al. Management of deep infiltrating endometriosis by laparoscopic route with robotic assistance: 3-year experience. J Gynecol Obstet Biol Reprod (Paris) 2016; pii: S0368-2315(15)00380-4.
15. Ayav A, Bresler L, Hubert J, Brunaud L, Boissel P. Robotic-assisted pelvic organ prolapse surgery. Surg Endosc 2005; 19: 1200-3. [CrossRef]
16. Abelha Mde C, Costa RR, Lopes VM, Reis RC, Silva CM. [Tubal reanastomosis: analysis of the results of 30 years of treatment]. Rev Bras Ginecol Obstet 2008; 30: 294-9.
17. Rodgers AK, Goldberg JM, Hammel JP, Falcone T. Tubal anastomosis by robotic compared with outpatient minilaparotomy. Obstet Gynecol 2007; 109: 1375-80. [CrossRef]
18. Bedaiwy MA, Barakat EM, Falcone T. Robotic tubal anastomosis: technical aspects. JSLS 2011; 15: 10-5. [CrossRef]
19. Kavoussi SK, Kavoussi KM, Lebovic DI. Robotic-assisted tubal anastomosis with one-stitch technique. J Robot Surg 2014; 8: 133-6. [CrossRef]
20. Moore ES, Foster TL, McHugh K, Addleman RN, Sumners JE. Robotic-assisted transabdominal cerclage (RoboTAC) in the non-pregnant patient. J Obstet Gynaecol 2012; 32: 643-7. [CrossRef]
21. DaCosta V, Wynter S, Harriott J, Christie L, Frederick J, Frederick-Johnston S. Laparoscopic cervicoisthmic cerclage for the treatment of cervical incompetence: case reports. West Indian Med J 2011; 60: 590-3.
22. Gocmen A, Sanlikan F. Two Live Births following Robotic-Assisted Abdominal Cerclage in Nonpregnant Women. Case Rep Obstet Gynecol 2013; 2013: 256972.
23. Walsh TM, Borahay MA, Fox KA, Kilic GS. Robotic-assisted, ultrasound-guided abdominal cerclage during pregnancy: overcoming minimally invasive surgery limitations? J Minim Invasive Gynecol 2013; 20: 398-400. [CrossRef]
24. Brudie LA, Backes FJ, Ahmad S, Zhu X, Finkler NJ, Bigsby GE 4th, et al., Analysis of disease recurrence and survival for women with uterine malignancies undergoing robotic surgery. Gynecol Oncol 2013; 128: 309-15. [CrossRef]
25. Magrina JF, Kho RM, Weaver AL, Montero RP, Magtibay PM. Robotic radical hysterectomy: comparison with laparoscopy and laparotomy. Gynecol Oncol 2008; 109: 86-91. [CrossRef]
26. Magrina, JF, Zanagnolo VL. Robotic surgery for cervical cancer. Yonsei Med J 2008; 49: 879-85. [CrossRef]
27. Johansen G1, Lönnerfors C2, Falconer H1, Persson J. Reproductive and oncologic outcome following robot-assisted laparoscopic radical trachelectomy for early stage cervical cancer. Gynecol Oncol 2016; 141: 160-5. [CrossRef]
28. Höckel M, Horn LC, Manthey N, Braumann UD, Wolf U, Teichmann G, et al. Resection of the embryologically defined uterovaginal (Mullerian) compartment and pelvic control in patients with cervical cancer: a prospective analysis. Lancet Oncol 2009; 10: 683-92. [CrossRef]
29. Kimmig R, Wimberger P, Buderath P, Aktas B, Iannaccone A, Heubner M. Definition of compartment-based radical surgery in uterine cancer: radical hysterectomy in cervical cancer as 'total mesometrial resection (TMMR)' by M Hockel translated to robotic surgery (rTMMR). World J Surg Oncol 2013; 11: 211. [CrossRef]
30. Herling SF, Møller AM, Palle C, Thomsen T. Health-related quality of life after robotic-assisted laparoscopic hysterectomy for women with endometrial cancer--A prospective cohort study. Gynecol Oncol 2016; 140: 107-13. [CrossRef]
31. Mäenpää MM, Nieminen K, Tomás EI, Laurila M, Luukkaala TH, Mäenpää JU. Robotic-assisted vs traditional laparoscopic surgery for endometrial cancer: a randomized controlled trial. Am J Obstet Gynecol 2016; 215: 588.e1-588.e7. [CrossRef]
32. Boggess JF, Gehrig PA, Cantrell L, Shafer A, Ridgway M, Skinner EN, et al. A comparative study of 3 surgical methods for hysterectomy with staging for endometrial cancer: robotic assistance, laparoscopy, laparotomy. Am J Obstet Gynecol 2008; 199: 360.e1-9. [CrossRef]
33. Morice P, Leary A, Creutzberg C, Abu-Rustum N, Darai E. Endometrial cancer. Lancet 2016; 387: 1094-108. [CrossRef]
34. Kimmig R, Aktas B, Buderath P, Rusch P, Heubner M. Intraoperative navigation in robotically assisted compartmental surgery of uterine cancer by visualisation of embryologically derived lymphatic networks with indocyanine-green (ICG). J Surg Oncol 2016; 113: 554-9. [CrossRef]
35. Kimmig R, Iannaccone A, Aktas B, Buderath P, Heubner M. Embryologically based radical hysterectomy as peritoneal mesometrial resection (PMMR) with pelvic and para-aortic lymphadenectomy for loco-regional tumor control in endometrial cancer: first evidence for efficacy. Arch Gynecol Obstet 2016; 294: 153-60. [CrossRef]

36. Chan JK, Gardner AB, Taylor K, Thompson CA, Blansit K, Yu X, et al. Robotic versus laparoscopic versus open surgery in morbidly obese endometrial cancer patients - a comparative analysis of total charges and complication rates. Gynecol Oncol 2015; 139: 300-5. [CrossRef]
37. Schneider A, Köhler C, Erdemoglu E. Current developments for pelvic exenteration in gynecologic oncology. Curr Opin Obstet Gynecol 2009; 21: 4-9. [CrossRef]
38. Feuer GA, Lakhi N, Barker J, Salmieri S, Burrell M. Perioperative and clinical outcomes in the management of epithelial ovarian cancer using a robotic or abdominal approach. Gynecol Oncol 2013; 131: 520-4. [CrossRef]
39. Magrina JF, Cetta RL, Chang YH, Guevara G, Magtibay PM. Analysis of secondary cytoreduction for recurrent ovarian cancer by robotics, laparoscopy and laparotomy. Gynecol Oncol 2013; 129: 336-40. [CrossRef]
40. Chen CH1, Chiu LH, Chen HH, Chan C1, Liu WM. Comparison of robotic approach, laparoscopic approach and laparotomy in treating epithelial ovarian cancer. Int J Med Robot 2016; 12: 268-75. [CrossRef]
41. Alkatout I, Egberts JH, Mettler L, Doniec M, Wedel T, Jünemann KP, et al. [Interdisciplinary Diagnosis and Treatment of Deep Infiltrating Endometriosis.]. Zentralbl Chir 2015.
42. Sebajang H, Trudeau P, Dougall A, Hegge S, McKinley C, Anvari M. The role of telementoring and telerobotic assistance in the provision of laparoscopic colorectal surgery in rural areas. Surg Endosc 2006; 20: 1389-93. [CrossRef]
43. Institut, R.K. Übergewicht und Adipositas. 2016 21.01.2016]; Available from: http://www.rki.de/DE/Content/Gesundheitsmonitoring/Themen/Uebergewicht_Adipositas/Uebergewicht_Adipositas_node.html.
44. Scheib SA, Tanner E 3rd, Green IC, Fader AN. Laparoscopy in the morbidly obese: physiologic considerations and surgical techniques to optimize success. J Minim Invasive Gynecol 2014; 21: 182-95. [CrossRef]
45. Siesto G, Ornaghi S, Iedà N, Vitobello D. Robotic surgical staging for endometrial and cervical cancers in medically ill patients. Gynecol Oncol 2013; 129: 593-7. [CrossRef]
46. Goonewardene SS, Brown M, Challacombe B. Challacombe, Single- versus dual-console robotic surgery: dual improves the educational experience for trainees. World J Urol 2016; 34: 1337-9. [CrossRef]
47. Desille-Gbaguidi H, Hebert T, Paternotte-Villemagne J, Gaborit C, Rush E, Body G. Overall care cost comparison between robotic and laparoscopic surgery for endometrial and cervical cancer. Eur J Obstet Gynecol Reprod Biol 2013; 171: 348-52. [CrossRef]
48. Ind TE, Marshall C, Hacking M, Harris M, Bishop L, Barton D, et al. Introducing robotic surgery into an endometrial cancer service-a prospective evaluation of clinical and economic outcomes in a UK institution. Int J Med Robot 2016; 12: 137-44. [CrossRef]
49. Reynisson P, Persson J. Hospital costs for robot-assisted laparoscopic radical hysterectomy and pelvic lymphadenectomy. Gynecol Oncol 2013; 130: 95-9. [CrossRef]
50. Alkatout I, Mettler L, Günther V, Maass N, Eckmann-Scholz C, Elessawy M, et al. Safety and economical innovations regarding surgical treatment of fibroids. Minim Invasive Ther Allied Technol 2016; 25: 1-13. [CrossRef]
51. Marino P, Houvenaeghel G, Narducci F, Boyer-Chammard A, Ferron G, Uzan C, et al. Cost-Effectiveness of Conventional vs Robotic-Assisted Laparoscopy in Gynecologic Oncologic Indications. Int J Gynecol Cancer 2015; 25: 1102-8. [CrossRef]

Two successful pregnancies achieved by converting an *in vitro* fertilization cycle to an intrauterine insemination cycle in five cases with documented premature ovulation

Kubilay Vicdan, Cem Akarsu, Eran Sözen, Burcu Buluç, Deniz K. Üstündağ, Kutay Biberoğlu
Private Ankara IVF Center, Ankara, Turkey

Abstract

We here report two successful pregnancies obtained by converting an *in vitro* fertilization (IVF) cycle to an intrauterine insemination (IUI) cycle in five poor responder patients whose oocyte pick-up (OPU) procedures were canceled due to documented premature ovulation immediately before OPU. To our knowledge, this is the first article that demonstrates that switching an IVF cycle to an IUI cycle when premature ovulation occurs on the day of OPU can produce successful pregnancies, even in poor responder patients. (J Turk Ger Gynecol Assoc 2016; 17: 233-5)

Keywords: *In vitro* fertilization, intrauterine insemination, premature ovulation, poor response

Received: 14 June, 2016 **Accepted:** 27 October, 2016

Introduction

Diminished ovarian reserve (DOR) is closely associated with poor response to ovarian stimulation, detection of premature luteinizing hormone (LH) surge before ovulation trigger, premature ovulation, and failure to retrieve oocytes during timed oocyte pick-up (OPU) procedures (1, 2). It is a common and accepted strategy to cancel the cycle, to proceed to egg collection, or to convert the *in vitro* fertilization (IVF) cycle to an intrauterine insemination cycle (IUI) in these poor responder patients (2-6); however, it is very challenging for the clinician to decide on a course of action in cases when unpredicted, premature ovulation is discovered as an unwelcome surprise immediately before a timed OPU. Although cycle cancelation, puncture of smaller residual follicles, and aspiration of follicular fluid from the pouch of Douglas are recommended methods, only a few successful pregnancies have been reported with these attempts (7).

Therefore, we adopted a different strategy: performing last-minute transvaginal sonography (TVS) before anesthesia to be certain whether ovulation has occurred; if premature ovulation has occurred, the IVF cycle is changed to a rescue IUI in couples who have at least one patent fallopian tube and a total progressive sperm count of >5 million. Here, we report the cycle outcomes of five patients managed with this approach.

Case Presentation

In this article, the results obtained from five consecutive poor responder patients undergoing IVF complicated by premature ovulation are evaluated. The demographic and cycle characteristics of the patients are summarized in Table 1. In all cases, ovarian stimulation was started on day 2 of the cycle using recombinant or urinary follicle-stimulating hormone (FSH) or human menopausal gonadotropin (hMG) in conjunction with gonadotropin-releasing hormone (GnRH) antagonist. A starting dose of 300 to 450 IU/d was administered according to the patient's age, ovarian reserve, and previous cycle response. A TVS scan was performed on cycle day 5, and GnRH antagonist was started on day 6 in all cases as a fixed protocol. The gonadotropins and antagonist were both continued until the leading follicles reached ≥17 mm, at which time ovulation was triggered with 10.000 IU urinary hCG (Pregnyl, MSD) or 500 μgr recombinant human chorionic gonadotropin (hCG) (Ovitrelle, Merc Serono); the OPU procedure was scheduled for 35 hours later. On the day when OPU was scheduled, last-minute TVS before anesthesia detected no follicles in both ovaries in these cases, and a diagnosis of premature ovulation was made. Instead of canceling the cycle or attempting to puncture the remaining follicular residues, IUI was offered and performed as a rescue treatment after obtaining written consent from the couples. Semen specimens were prepared

Address for Correspondence: *Kubilay Vicdan*
e.mail: kubilayvicdan@yahoo.com

DOI: 10.5152/jtgga.2016.16118

Table 1. Demographic features and cycle characteristics of the patients

	1	2	3	5	6
Female age/years	25	39	37	37	38
Infertility duration/years	6	1.5	7	13	17
Infertility type	Primary	Primary	Primary	Secondary	Primary
FSH (mIU/mL)	11.7	21.1	20.1	9	11
Antral follicle count	2	3	3	4	4
Regular cycle	+	+	+	+	+
HSG, patent tube	+	+	+	+	+
TPMS/swim up (million)	6.48	6.64	21	6.23	16.2
Infertility reason	Unexplained	Unexplained	Unexplained	Unexplained	Unexplained
Previous OI cycle	-	6	-	-	-
Previous IUI cycle	1	-	-	3	3
Previous IVF cycle	3	-	-	-	1
Protocol type	Antagonist	Antagonist	Antagonist	Antagonist	Antagonist
Gonadotropin type	uFSH	uFSH+HMG	HMG	r-FSH	HMG
Starting dose	300	375	450	375	375
Total gonadotropins (Units)	2625	2550	4650	3375	6825
Trigger day	11	11	14	10	13
Follicle number, hCG day	1	1	2	4	2
Follicle number at pick-up	-	-	-	-	-
Pregnancy	-	-	-	+	+

FSH: follicle-stimulating hormone; TPMS: total progressive motile sperm; OI: ovulation induction; IUI: intrauterine insemination; IVF: *in vitro* fertilization; hCG: human chorionic gonadotropin; HMG: human menopausal gonadotropin; HSG: hysterosalpingogram

by density gradient centrifugation. All cases received luteal support with vaginal micronized progesterone. A blood test for hCG was performed 14 days after IUI in all 5 cases, and 2 had positive results. Transvaginal ultrasound confirmed a clinical pregnancy at 7 weeks of gestation in both patients, and both of these pregnancies are currently ongoing after 20 weeks.

Discussion

A meta-analysis has demonstrated that women with antral follicle counts of fewer than four were 8.7 times less likely to become pregnant after IVF and 37 times more likely to have their cycle canceled compared with normoresponders (8). In poor responders with stimulated IVF cycles, the decision whether to cancel the cycle, to continue with the egg collection, or to convert to an IUI cycle is very challenging. Matorras et al. (4) assessed the utility of transforming an IVF cycle with low response to an IUI cycle in 57 patients undergoing IVF due to unexplained infertility, mild to moderate male factor, or previous IUI failure. They reported a pregnancy rate of 14% in IVF poor responders with at least 2 to 4 follicles; they concluded that IUI should be considered in the management of poor responders. Contrastingly, in another study of 1350 IVF cycles with one or two mature follicles, conversion of an IVF cycle to an IUI cycle was reported to have the poorest prognosis, while proceeding with egg collection was much more successful in achieving pregnancy (2). Another multicenter comparative study which analyzed 7176 initiated cycles also suggested that IVF should be pursued for women demonstrating two follicles, while conversion to IUI was recommended for cycles with only one follicle, if sperm and tubal parameters were favorable (3). It was also clearly demonstrated that performing IUI as a rescue treatment provided no advantage over taking no further action when no oocytes were collected during a timed follicular puncture (6).

Encountering unexpected premature ovulation at the time of OPU under anesthesia is another challenge for the clinician as well as for the infertile couple; this is a totally different situation with very few treatment alternatives. Wu et al. (7) reported three cases of successful pregnancies with puncture of small or medium-sized follicles to retrieve oocytes (*in vitro* maturation was performed in two cases); however, this remains the only success story which was not reproduced by others. Furthermore, puncture of the remaining follicles and aspiration of the peritoneal fluid necessitate additional methods, such as OPU itself, and anesthesia; this imposes additional financial and emotional burdens on the couple. Cancellation of the

cycle is a more logical approach in the case of documented premature ovulation during a timed OPU procedure because it is impossible to detect the exact time of ovulation or to know if the oocytes have already been captured by the Fallopian tubes. In this article, our results demonstrate that couples with at least one patent tube and with a normal sperm count can benefit from a rescue IUI in the case of premature ovulation detected at the time of OPU, even after previous failed IUI or IVF treatment cycles. Therefore, we suggest a "rescue" IUI as an alternative approach in unexplained infertility cases with DOR who are scheduled to undergo IVF and who experience premature ovulation before a timed OPU procedure.

Ethics Committee Approval: *N/A.*

Informed Consent: *Written informed consent was obtained from patients who participated in this study.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Concept - C.A.; Design - K.V.; Supervision - K.B.; Resources - K.V.; Materials - C.A.; Data Collection and/or Processing - E.S., B.B.; Analysis and/or Interpretation - K.V.; Literature Search - D.K.Ü.; Writing Manuscript - K.V.; Critical Review - K.B.*

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

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

References

1. David ER, Zakarin L, Chao K, Meyer L, Owen KD, Rosenwaks Z. Diminished ovarian reserve is the predominant risk factor for gonadotropin-releasing hormone antagonist failure resulting in breakthrough luteinizing hormone surges in in vitro fertilization cycles. Fertil Steril 2014; 102: 99-102. [CrossRef]
2. James DM, Nicopoullos MD, Abdalla H. Poor response cycles: when should we cancel? Comparison of outcome between egg collection, intrauterine insemination conversion, and follow-up cycles after abandonment. Fertil Steril 2011; 95: 68-71. [CrossRef]
3. Quinquin M, Mialon O, Isnard V, Masin M, Parinaud J, Delotte J, et al. In vitro fertilization versus conversion to intrauterine insemination in Bologna-criteria poor responders: how to decide which option. Fertil Steril 2014: 102: 1596-601. [CrossRef]
4. Matorras R, Corcostegui B, Mendoza R, Ramon O, Aporicio V, Uriarte MT, et al. Converting an IVF cycle to IUI in low responders with at least 2 follicles. J Reprod Med 2003; 48: 10: 789-91.
5. Sönmezer M, Pelin Cil A, Atabekoğlu C, Ozkavukçu S, Ozmen B. Does premature luteinisation or early surge of LH impair cycle outcome? Report of two successful pregnancies. J Assist Reprod Genet 2009; 26: 159-63. [CrossRef]
6. Matorras R, Aparicio V, Corcostegui B, Prieto B, Mendoza R, Ramon O, et al. Failure of intrauterine insemination as a rescue treatment in low responders with adequate HCG timing with no oocytes retrieved. Reprod Biomed Online 2014; 29: 634-9. [CrossRef]
7. Wu FS, Lee RK, Hwu YM. Encountering premature ovulation during controlled ovarian hyperstimulation in IVF/ICSI cycles. Taiwan J Obstet Gynecol 2012; 51: 2: 256-9. [CrossRef]
8. Gibreel A, Maheshwari AM, Bhattacharaya S, Jhonson NP. Ultrasound tests of ovarian reserve: a systematic review of accuracy in predicting fertility outcomes. Hum Fertil 2009; 12: 95-106. [CrossRef]

What is your diagnosis?

An uncomplicated cesarean section was performed under general anesthesia on a 33-year-old woman with a gestational age of 34 weeks and 2 days due to a valuable pregnancy. At postoperative hour 4 the patient developed dyspnea, tachypnea, hypertension, and agitation, and she was transferred to the intensive care unit due to worsening general condition and alteration in consciousness. At initial assessment, the general condition of the patient was poor, with confusion and agitation, and her Glasgow Coma Scale score was 13. Also, on auscultation, she had rales and rhonchi bilaterally in her lungs, her respiratory rate was 36/min, and her cardiac rate was 160 bpm with arrhythmia. She was intubated without delay and mechanical ventilation was started (Drager Evita 4, Germany, BIPAP mode, lung recruitment maneuver). An antero-posterior chest x-ray showed bilateral diffuse infiltrations, and blood gas analysis indicated oxygen-resistant arterial hypoxemia (PaO_2/FiO_2: 75). A pre-diagnosis of severe acute respiratory distress syndrome (ARDS) was suspected based on Berlin criteria (Figure 1, Table 1). Her central venous pressure was 6 mm Hg. However, based on the presence of risk factors for pulmonary embolism and its clinical resemblance to ADRS, the patient's D-dimer level was measured and contrast-enhanced computed tomography (CT) of the chest was scheduled to rule out pulmonary embolism. The patient's D-dimer level was 2.7 mg/dL. However, CT imaging could not be performed due to her poor medical condition, which precluded transfer to the CT unit from the ICU. Despite this, enoxaparin (Cleaxane 60 mg/0.6 mL, Sanofi Aventis; İstanbul, Turkey) s.c. BID was started due to a possible occurrence of pulmonary embolism after consultation with the departments of pulmonology and obstetrics. A diagnosis of severe ARDS was primarily considered, and midazolam (Demizolam, DEM Medical; İstanbul, Turkey) infusion at a rate of 10 mg/hour was commenced to increase adaptation to mechanical ventilation. Also, esmolol (Brevibloc premiks, Eczacıbasi Baxter; İstanbul, Turkey) infusion was given due to a heart rate of 145 bpm and a blood pressure of 170/110 mmHg, and furosemide (Lasix amp, Sanofi Aventis; İstanbul, Turkey) (4 mg/h) was given due to the presence of bilateral rales on lung auscultation and pinkish-foamy endotracheal aspiration fluid, suggesting pulmonary edema. Additionally, clarithromycin (Uniklar 500 mg, Mustafa Nevzat; İstanbul, Turkey) (2x500 mg) and ceftriaxone (Rocephin 1 gr, Saba Drugs; İstanbul, Turkey)

Table 1. The patient's mechanical ventilation and arterial blood gas analysis

Time (hours)	**0**	**2**	**4**	**10**	**36**	**72**	**96**	**120**
Ventilatory mode	BIPAP	BIPAP	BIPAP	BIPAP	CPAP	CPAP	y-connection	Extubation
FiO_2	1	0.8	0.6	0.5	0.4	0.4	0.3	0.3
P_{insp}	30	30	30	30	-	-	-	-
P_{ASB}	20	20	20	20	10	10	-	-
PEEP	10	10	10	10	5	5	-	-
Respiratory rate	14	14	14	14	0	0	-	-
PaO_2/FiO_2	75	89	101	133	175	272	245	383
pH	6.98	7.07	7.27	7.48	7.53	7.42	7.45	7.47
$PaCO_2$	66	59	41.4	32.6	34.2	42.9	39.9	39.2
PaO_2	75	71.4	60.6	66.7	70.3	109	73.5	115
SaO_2	79	82.7	84.8	94.1	94.9	97.7	93.4	97.7
HCO_3	11.1	13.4	18.3	26.1	30.1	27.7	27.8	28.8
Lactate	6.7	5.7	4	2.4	1.1	1.9	2.1	1.4
Base excess	-14.6	-11.9	-6.8	1.3	6.1	3.8	3.8	4.7

BIPAP: bilevel positive airway pressure; CPAP: continuous positive airway pressure; FiO_2: fraction of inspired oxygen; P_{insp}: inspiration pressure; P_{ASB}: pressure support above PEEP; PEEP: positive end-expiratory pressure; PaO_2: partial arterial oxygen pressure; $PaCO_2$: partial arterial carbon dioxide pressure; SaO_2: arterial oxygen saturation; HCO_3: bicarbonate

Address for Correspondence: *Ayşe Belin Özer*
e.mail: abelinozer@gmail.com

DOI: 10.5152/jtgga.2016.16143

(2x1 gr) were started due to possible pneumonia. Magnesium (Magnesium sulfate amp 15%, Biofarma; İstanbul, Turkey) was infused at a dose of 60 mg/h on the basis of its neuroprotective effects as well as its therapeutic effects on pre-eclampsia. Esmolol infusion was gradually reduced as the patient's blood pressure and heart rate returned to normal values. Mechanical ventilation parameters were adjusted according to serial arterial blood gas measurements and chest x-ray findings (Table 1, Figure 1). Progressive improvement was observed clinically; sedation was stopped on day 4, and the patient was placed on a y-connector. At day 5 she was extubated, and on day 6 she was transferred to the obstetrics ward. On postoperative day 8, contrast-enhanced CT of the chest showed several bilateral pulmonary nodules, the greatest being 6 mm in diameter. The patient was discharged on the same day.

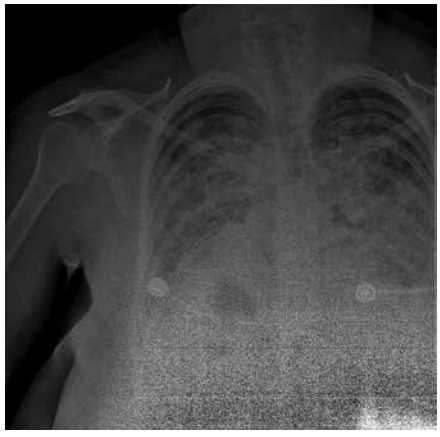


Figure 1. The patient's posterior-anterior chest x-ray immediately before admission to the intensive care unit

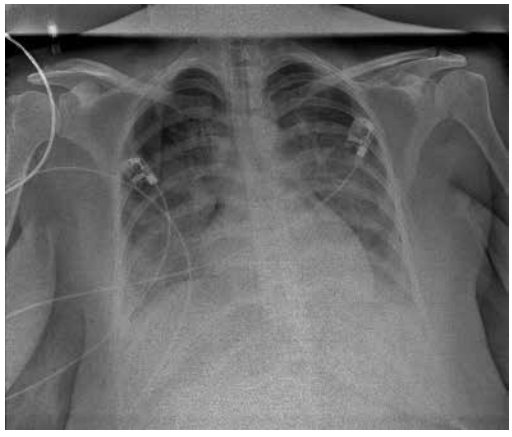


Figure 2. The patient's anterior-posterior chest x-ray on the second day

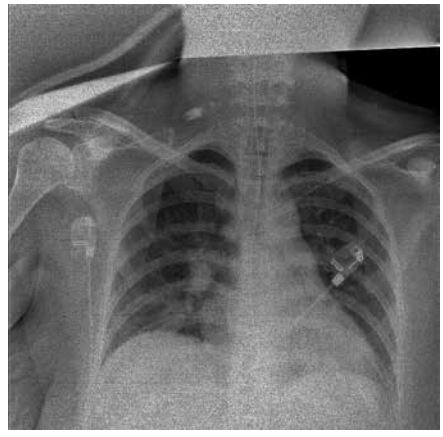


Figure 3. The patient's anterior-posterior chest x-ray on the sixth day

Answer

Acute respiratory distress syndrome is an acute, diffuse, and inflammatory lung disease characterized by increased pulmonary vascular permeability, increased lung weight, and decreased aerated lung tissue. Clinical manifestations of ARDS include hypoxemia and bilateral radiographic opacities, while the pathological manifestations include diffuse alveolar damage associated with alveolar edema and acute inflammation of the alveolar walls and hyaline membranes (1).

Although some risk factors for ARDS during pregnancy are similar to those in the general population (e.g., sepsis, aspiration, pancreatitis, trauma, inhalation injury, drowning, and pneumonia), pregnancy-specific conditions such as amniotic fluid embolism, pre-eclampsia/eclampsia, HELLP syndrome, chorioamnionitis, and endometritis may also play etiological roles in pregnant women (2-5).

Acute respiratory distress syndrome has an annual incidence of 1.5/100,000 and has a fatal outcome in 35% to 50% of cases. Despite the absence of clear data on the incidence of ARDS in obstetric patients, the incidence rate in these patients is thought to be comparable to that in the general population. In the UK and the US, the prevalence of the need for intensive care among pregnant women is around 0.9%; ARDS represents a major cause of maternal death in intensive care units (6).

Patients with acute hypoxemic respiratory failure frequently have dyspnea, tachypnea, and tachycardia. Auscultation may reveal diffuse bilateral rales in the basal lung zones and wheezing, and the patient may be cyanotic. Bilateral diffuse alveolar and interstitial infiltrations are typical in lung x-rays, although it may be challenging to differentiate these from x-ray images of patients with congestive heart failure or fluid overload (7). Our patient with a history of pre-eclampsia had dyspnea, tachypnea, and cyanosis at postoperative hour 4, followed by alteration in her mental state. She was intubated without delay due to her worsening clinical status and blood gas results, and mechanical ventilation was initiated. The patient's central venous pressure was measured to rule out hypervolemia, as the patient had bilateral rales on auscultation of the lungs and a small amount of pinkish-foamy endotracheal aspiration fluid. Central venous pressure between 4 and 7 mmHg was measured during her clinical course; this was indicative of the absence of hypervolemia. Furosemide infusion was given for possible pulmonary edema.

Adequate disinfection of the surgical field and absence of foul-smelling vaginal discharge suggested that endometritis and chorioamnionitis had no triggering roles in ARDS. A diagnosis of HELLP was also largely out of consideration due to the patient's normal bleeding-clotting time, platelet count, and liver enzyme levels. Delayed gastric emptying in pregnant women is associated with increased risk of aspiration during general anesthesia. Our patient, who had a history of panic disorder, received general anesthesia, which may have resulted in aspiration as a potential cause of ARDS. However, analysis of the endotracheal aspiration fluid showed no material suggestive of active aspiration. Also, no vomiting occurred during anesthesia or during the post-operative period. Furthermore, a diagnosis of sepsis could be readily ruled out due to the absence of a causative microbial agent in the patient's endotracheal aspiration fluid, urine, and blood samples, and also due to the absence of any foci of infection.

Acute respiratory distress syndrome induced by tocolytic agents may occur during beta-adrenoceptor agonist infusion or within 12 hours of its discontinuation. The clinical manifestations may be resolved after 12 hours with diuretics, supportive treatment, and discontinuation of the causative agent (7). However, our patient had a more severe clinical course, and no rapid improvement was observed after stopping the tocolytic agent.

Amniotic fluid embolism, however, classically presents with ARDS, hemodynamic collapse, and disseminated intravascular coagulation (7). Our patient only had ARDS; also, contrary to hemodynamic collapse, hypertension and tachycardia were detected. Also, the patient's bleeding-clotting parameters were normal.

Pre-existing pre-eclampsia was considered as the etiological factor responsible for the development of ARDS in our patient. In a study by Mabie et al. (4), 16 patients were admitted to the intensive care unit due to a diagnosis of ARDS during a 6-year period; only 4 of these cases were related to eclampsia/pre-eclampsia. Among these four cases, additional comorbidities (aspiration pneumonia, lupus nephritis, sepsis, massive transfusion, etc.) were present that could be associated with the occurrence of ARDS. Meyer and Schmidt reported a pre-eclamptic patient in whom ARDS persisted during the postpartum period despite labor and treatment of pre-eclampsia (8). No other possible causes of ARDS were present in our patient; therefore, the most likely cause of her condition was pre-eclampsia.

Treatment of ARDS during pregnancy or the postpartum period is similar to that in non-pregnant individuals. In patients admitted to the intensive care unit due to acute hypoxia and diffuse lung infiltrates during pregnancy or the postpartum period, an etiological search should be undertaken; hypervolemia and congestive heart failure should be ruled out, and amniocentesis should be conducted. For diagnostic purposes, bronchoalveolar lavage aspiration and even lung biopsy may be performed (2). Because the most probable cause of ARDS was pre-eclampsia in our patient, additional diagnostic work-up was not performed. For the management of these patients, the underlying condition should be corrected, and conventional mechanical ventilation should be performed (Vt<6 mL/kg, p plat<30 cm H_2O). Recommended strategies when this treatment is insufficient include airway pressure release ventilation (APRV), high frequency oscillatory ventilation (HFOV), prone positioning, inhaled nitric oxide, and additional lung recruitment maneuvers (2, 7). Magnesium infusion and intermittent recruitment therapy with mechanical ventilation were used therapeutically in our patient.

Acute respiratory distress syndrome may occur during pregnancy or the postpartum period, may be either associated or not associated with pregnancy, and may threaten maternal and

fetal health. Based on our experience with this patient, who had ARDS induced by pre-eclampsia, we wish to emphasize the fact that these patients may be successfully managed by early recognition, pressure-controlled ventilation and supported treatment in the intensive care unit, and with other supportive measures.

Ayşe Belin Özer, Mustafa Özdemir, Abdurrahman İleri, İsmail Demirel

Department of Anesthesiology and Intensive Care, Fırat University School of Medicine, Elazığ, Turkey

References

1. ARDS Definition Task Force, Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, et al. Acute respiratory distress syndrome: the Berlin Definition. JAMA 2012; 307: 2526e33.
2. Cole DE, Taylor TL, McCullough DM, Shoff CT, Derdak S. Acute respiratory distress syndrome in pregnancy. Crit Care Med 2005; 33: S269-78. [CrossRef]
3. Catanzarie VA, Willms D. Adult respiratory distress syndrome in pregnancy: report of three cases and a review of the literature. Obstet Gynecol Surv 1997; 52: 381-92. [CrossRef]
4. Mabie WC, Barton JR, Sibai BM. Adult respiratory distress syndrome in pregnancy. Am J Obstet Gynecol 1992; 167: 950-7. [CrossRef]
5. Catanzarite V, Willms D, Wong D, Landers C, Cousins L, Schrimmer D. Acute respiratory distress syndrome in pregnancy and the puerperium: causes, courses, and outcomes. Obstet Gynecol 2001; 97: 760-4. [CrossRef]
6. Munnur U, Bandi V, Guntupalli KK. Management principles of the critically ill obstetric patient. Clin Chest Med 2011; 32: 53-60. [CrossRef]
7. Janakiram R, Meenakshi K, Madurai J. Acute respiratory distress syndrome (ARDS) in pregnancy. In: Gandhi A, Malhotra N, Malhotra J, Gupta N, Malhotra Bora N, editors. Principles of critical care in obstetrics. New Delhi, India: Springer India; 2016; p. 9-14. [CrossRef]
8. Meyer NJ, Schmidt GA. Acute lung ınjury in pregnancy. In: Bourjeily G, Rosene-Montella K, editors. Pulmonary problems in pregnancy. New York, USA: Humana Press; 2009; p. 355-81. [CrossRef]

Acknowledgements for the Year 2016

(Reviewers contributed at the review process in 2016)

On behalf of the office staff and the Editorial Board of the Journal of *The Turkish German Gynecological Association*, we would like to thank to all of our reviewers of the past year for their outstanding contributions. Their thorough reviews and expertise enable our journal to improve its scientific quality. We certainly look forward to their ongoing support, suggestions and recommendations as to how to continue to advance the overall quality of the Journal of *The Turkish German Gynecological Association*.

Prominent Reviewers in 2016

We would like to acknowledge, with special thanks and appreciation, the following reviewers for their remarkable contributions to peer review process of the Journal of *The Turkish German Gynecological Association* in 2016. We extend our sincere gratitude for their timely, scholarly and critically evaluations.

Ali Kolusarı
Cihan Kaya
Mehmet Sühha Bostancı
Murat Seval
Mustafa Kara
Özge Yıldırım
Özlem Pata
Şebnem Özyer
Taner Usta
Tuncay Yüce
Yavuz Emre Şükür
Yılmaz Güzel

Reviewers in 2016

We are also grateful for the help of the reviewers who devoted time and effort to the Journal of *The Turkish German Gynecological Association* in 2016.

Akmal El-Mazny
Abdollah Ansari
Abdulkadir Turgut
Abdullah Karaer
Ahmet Zeki Işık
Akihiro Takeda
Alan Adno
Antonio Braga
Asangla Ao
Atossa Mahdavi
Ayla Aktulay
Ayla Sargın Oruç
Ayşe Güler Okyay
Aytekin Tokmak
Ayten Türkkanı
Azra Naseem
Behiye Pınar Göksedef
Beril Yüksel
Borut Kovacic
Bülent Haydardedeoğlu
Bülent Yılmaz
Carlos A. Petta
Cavidan Gülerman
Cavit Kart
Cem Çelik
Cem Dane
Cem Fıçıcıoğlu
Christos Iavazzo
Chutatape Anuntapon
Cihan Çetin
Cihat Ünlü
Deepti Goswami
Elham Bahrami
Emek Doğer
Emre Günakan
Emre Özgü
Eralp Baser
Ercan Baştu
Esra Esim Büyükbayrak
Fabricio Da Silva Costa
Fatemeh Nazari
Federico García Bragado
Filippo Bellati
Filiz Bilgin Yanık
Gabriele Tonni
Gautier Chene
Gian Mario Tiboni
Giuseppe Rizzo
Gökhan Açmaz
Gürkan Bozdağ
Hany Abdel-Aleem
Hakan Timur
Hasan İnal
Hasan Topçu
Hasan Ulubaşoğlu
Hasan Yüksel
Hilmiye Aksu
Hüseyin Aksoy
Inaki Lete
İbrahim Alanbay
İbrahim Egemen Ertaş
İbrahim Uyar
İbrahim Yalçın
Jan Bosteels
Konstantinos Tziomalos
Kazuki Nagai
Koji Matsumoto
Korula George
Laleh Eslamian
Leire Rodriguez Gomez
Lida Moghaddam Banaem
Luis Guedes-Martins
Ming Chen
Mohamed S. Elmahaishi
Marie Therese Camilleri Podesta
Mehmet Sıddık Evsen

Mete Çağlar
Michal Kovo
Murat Api
Murat Bakacak
Murat Öz
Murat Seval
Mustafa Kara
Mustafa Sarı
Müge Harma
Neville Calleja
Nicel Taşdemir
Oluş Api
Osman Aşıcıoğlu
Özlem Şengül
Önder Çelik
Özlem Moraloğlu
Prabha Sinha
Polat Dursun
Prasad Lele
Rosa Guarch Troyas
Sabri Çavkaytar
Sadik Sahin
Safaa Al-Hassani
Selim Büyükkurt
Serkan Kahyaoğlu
Seung-Yup Ku
Şafak Hatırnaz
Temel Ceyhan
Tien Le
Timur Gürgan
Tolga Taşçı
Toshiyuki Hata
Tuncay Yüce
Turhan Aran
Ümit Görkem
Ümran Kücükgöz Güleç
Wilfried Gyselaers
Yavuz Şimşek
Yusuf Üstün

CONGRESS CALENDAR

INTERNATIONAL MEETINGS

January 27-28, 2017	**ESHRE CAMPUS Effects of ART and Endometriosis on Pregnancy Outcome** Sofia, Bulgaria https://www.eshre.eu/sofia
February 17, 2017	**25th World Congress on Controversies in Obstetrics, Gynecology & Interfility (COGI)** Vienna, Austria http://cogi-congress.org/
October 4-8, 2017	**IVF 19th World Congress on In Vitro Fertilization in conjunction with VI. Society of Reproductive Medicine and Surgery Congress** Antalya, Turkey http://www.isivf2017.com
October 26-29, 2017	**13rd World Congress of Perinatal Medicine** Belgrade, Serbia http://www.wcpm2017.com/

NATIONAL MEETINGS

March 2-5, 2017	**13. Uludağ Jinekoloji ve Obstetri Kış Kongresi** Bursa, Turkey http://www.uludagkadindogum.org
March 17, 2017	**5. Acıbadem Kadın Doğum Günleri**
April 17, 2017	**Türkiye Maternal Fetal Tıp ve Perinatoloji (Perinatal Medicine) XI. Ulusal Kongresi**
May 17, 2017	**4. Klinik Embriyoloji Derneği Kongresi**
October 17, 2017	**15. TJOD ve EBCOG Kongresi**
October 17, 2017	**6. Üreme Tıbbı ve Cerrahisi Kongresi**
November 3-5, 2017	**Türkiye Maternal Fetal Tıp ve Perinatoloji Ultrasonografi Kursu**

JTGGA CME/CPD CREDITING

Answer form for the article titled "*Robotic surgery in gynecology*" within the scope of CME/CPD

1. What is not a technical advantage of robotic surgery?
   a) 3D-visualization of the operating field
   b) 7 degrees of freedom of the surgical instruments
   c) Tremor-free handling of the instruments
   d) Reduced work fatigue of the surgeon
   e) Automatic surgery without need of the surgeon

2. What is not an advantage of the Da Vinci Xi system compared to the current Da Vinci-Si system?
   a) The variable use of optics in all four trocars
   b) The optimized geometry of the so-called patient cart
   c) The use of smaller trocars with the result of smaller cosmetic scars
   d) The use of single-site systems
   e) No need of assistance next to the patient

3. What is not an indication for robotic-assisted surgery in benign gynecology cases and in obstetrics?
   a) Deep infiltrating endometriosis
   b) The minimal invasive performance of the caesarean section
   c) Symptomatic myomatosus uterus in patients with the wish to have children
   d) Re-anastomsis of the fallopian tubes for the purpose of re-fertilization as an alternative to open surgery
   e) Robotic hysterectomy for benign diseases of the uterus

4. What is not an indication for robotic-assisted surgery in malignant gynecological diseases?
   a) Ovarian cancer in patients with the need of radical surgery
   b) Endometrial cancer in overweight patients
   c) Fertility-conserving surgery in patients with cervical carcinoma
   d) Total mesometrial resection (TMMR) in patients with cervical cancer
   e) Anterior/posterior exenteration in interdisciplinary interventions

5. What is not a benefit of robotic surgery to improve surgical training?
   a) The use of virtual training programs in surgical education
   b) Good tactile tissue-feedback of the instruments
   c) Excellent 3D imaging quality
   d) The possibility of the use of the dual console that enables a "driving school concept"
   e) The use of telemedicine in robotic surgery

6. What is not true concerning the cost efficiency of robotic surgery in gynecology?
   a) The maximum utilization of the system and a well coordinated team with efficient processes of selection and organization can reduce the costs to the level of open surgical techniques
   b) The most expensive aspect of robotic surgery, compared to all other surgical procedures, is the high cost of the equipment and the annual fixed costs
   c) The statistical projections about the required number of robotic interventions per year in order to achieve profitability range between 165 and 400 operations
   d) Robotic surgery enables a very cost-effective surgery that can help to reduce costs of health systems in underdeveloped countries
   e) Attempts made thus far to compare conventional laparoscopy with robotic surgery in regard of costs have yielded very diverse results

JTGGA CME/CPD CREDITING

Answer form for the article titled "*Robotic surgery in gynecology*" within the scope of CME/CPD

1st Question	A	B	C	D	E
2nd Question	A	B	C	D	E
3rd Question	A	B	C	D	E
4th Question	A	B	C	D	E
5th Question	A	B	C	D	E
6th Question	A	B	C	D	E

People who answer these questions will receive "2 TMA-CME/CPD credits"

TMA-CME CREDITING BOARD ENQUIRY FORM

JTGGA MANUSCRIPT 2016/4

DATE

TR Identification Number ☐☐☐☐☐☐☐☐☐☐☐ *This form will not be reviewed if TR Identification Number is not stated.*

Name ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

Surname ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

Signature ..... **The City You Work In** .....

Your Institution .....

IMPORTANT NOTE: You may apply for Turkish Medical Association CME/CPD credits by answering the questions in the front page, filling in your personal information and sending this form to "Abdi İpekçi Cad. No: 2/7 34367 Nişantaşı, İstanbul" by post. This form should arrive to the above-mentioned address latest by January 29, 2017.