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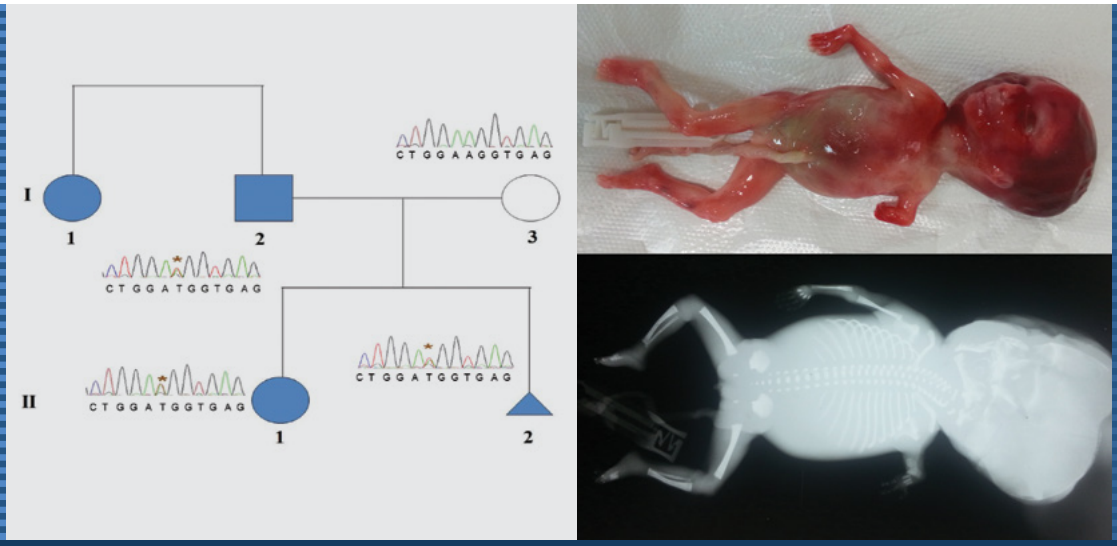

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The target audience of Journal of the Turkish-German Gynecological Association includes gynaecologists and primary care physicians interested in gynecology practice. It publishes original work on all aspects of gynecology. The aim of Journal of the 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.

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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.

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

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

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

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

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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.

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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.

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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.

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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.

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Contents

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Editorial

Dear Colleagues,

It is my great pleasure to meet with you again in the first issue of the Journal of the Turkish - German Gynecological Association (J Turk Ger Gynecol Assoc) in the publishing year of 2016.

I would like to remind you that the archive of our journal starting with September 2009 issue has been indexed in PubMed Central and available for access. It is important to remember the journal is an Open Access publication and the full text content of its archive is available free of charge. We hope that this will improve the journal's international visibility and recognition which will in turn positively affect our citation activity. I kindly ask all of my colleagues to keep this in mind while working on a new project.

In this issue you will read many interesting international papers, from Greece to Egypt, from Brasil to Germany, India and USA. As you know, gestational diabetes mellitus is associated with significant maternal and neonatal complications. In this issue, you will read a paper from Germany, explaining how a new screening test was implemented in German Gynecologists' routine practice. A paper from Turkey investigates the utility of preoperative neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, and lymphocyte count as biomarkers to distinguish malignant from benign ovarian masses. Emergency peripartum hysterectomy is performed as a life-saving procedure in cases of intractable obstetric hemorrhage. You will read an evaluation on emergency peripartum hysterectomy cases over a 14-year period in a tertiary center in Turkey. Ovarian cancer is still one of the most difficult types of cancer to combat, and the mortality rate is high. An alternative approach to the current therapy of ovarian carcinoma is the individualization of treatment by determining the sensitivity of tumoral tissue to chemotherapeutic agents before the initiation of chemotherapy. The objectives of one of the study that you will read are to determine the efficacy of in vitro chemosensitivity assays in ovarian carcinoma and to measure the correlation of three leading assays. Besides all of these manuscripts, you will also find other very interesting articles, case reports, a review and a quiz in this issue. Enjoy reading!

I wish to extend my heartfelt gratitude and appreciation to everyone who dedicated their time to deliver expertise, effort, and contribution to this publication and evaluation process, and I would welcome your participation and contributions in this journal as the loyal readers.

Dear Colleagues,

Eleventh Turkish German Gynecology Congress on 11-15 May 2016 will be held in Belek, Antalya, Turkey. We are happy to invite all our dear friends and colleagues to this forthcoming scientific event. This time our congress venue will be the newly constructed Sueno Hotels Deluxe's Convention Center in Belek. Our 11th congress will both act as a means for our colleagues to catch up with the current developments in our field in the most efficient way and also as a means of escaping the routine and stresses of the daily life, meeting with friends and fellows in a relaxing atmosphere. We are pretty confident that you will be happy to benefit from the rich scientific agenda of the congress and also will enjoy the atmosphere and fascinating social program.

Our foundation which has been organizing one of the biggest congresses of Turkey for the past twenty years has worked hard to organize a unique and memorable meeting. At this year's congress every morning we will be having keynote lectures with the world's most reputable speakers; May 11, Wednesday Prof. Sara Brucker (Surgical Gynecology - Quo Vadis?) and Prof. Camran Nezhat (Future Surgeons, Future Surgeries and Future Training), May 12, Thursday Prof. Serdar Bulun (Targeting stem cells to treat uterine leiomyomas), May 13, Friday Prof. Karl Oliver Kagan (Pregnancy as window for future health) and May 14, Saturday Prof. Uzi Beller ("Preventing Ovarian Cancer" – A strategy with hope). In addition, our congress will have two live surgeries, one from France the other from USA, with the moderation by Prof. Ceana Nezhat.

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Editorial

In our forthcoming 11th congress, besides having an enriched scientific program, we are also having pre-congress courses. Even though we have newly announced our courses there seems to be an overwhelming demand, due to this we kindly ask you to realize your registration without further delay. Hope to see you all in Antalya on May 11, 2016.

Dear Researchers,

The best 3 abstracts submitted to the Congress Scientific Committee within the scope of the XI. Turkish-German Gynaecology Congress will be rewarded with the Bayer Abstract Award. The best abstract in the field of "Contemporary Treatments in Endometriosis" will be rewarded with 5000 TL. As traditional, the best abstracts submitted in the field of Endoscopic Surgery will receive a 4000 TL Aysun – Cihat ÜNLÜ reward. The aim of these awards is to appreciate the prolificacy of our colleagues in research projects and to encourage especially our young colleagues for the forthcoming years.

Please do not forget to mark 11-15 May 2016 on your calendars in order to not to miss this scientific festival.

Your sincerely,
Cihat Ünlü, M.D.
Editor in Chief of JTGGA President of TAJEV

Efficacy and safety of post-cesarean section incisional infiltration with lidocaine and epinephrine versus lidocaine alone in reducing postoperative pain: A randomized controlled double-blinded clinical trial

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Abstract

Objective: Aim was to assess the efficacy and safety of incisional infiltration of lidocaine and epinephrine vs. lidocaine only to reduce postcesarean section (C/S) pain.

Material and Methods: It was a prospective, randomized, controlled, double-blinded clinical trial that was conducted in two tertiary hospitals in Egypt and included 153 women undergoing C/S under general anesthesia. They were randomly divided into the following two groups: Group I (control group, number=78), in which the wound was infiltrated before skin closure with 20 mL of 2% lidocaine, and Group II (study group, number=75), in which the wound was infiltrated before skin closure with 20 mL of 2% lidocaine and epinephrine. The primary outcomes were the time to first analgesic (TFA) request (minutes) and the postoperative pain scores that were measured using a visual analogue scale (VAS). The secondary outcomes included the duration of C/S, onset of mobilization, onset of breastfeeding, duration of hospital stay, local or systemic side effects of lidocaine and epinephrine, postoperative pyrexia, and postoperative wound infection.

Results: The pain score determined using VAS after 1 and 2 h was significantly decreased in Group II than in Group I. However, at 4.8 and 16 h, these results were significantly reversed in Group II than in Group I. The cumulative postoperative opioid consumption was significantly less in Group II than in Group I (50 vs. 90 mg). The onset of mobilization, onset of breastfeeding, and duration of hospital stay was significantly shorter in Group II than in Group I, whereas the TFA request was significantly longer in Group II.

Conclusion: Administering epinephrine with 2% lidocaine prolongs the anesthetic effect and reduces the opioid analgesic dose postoperatively required, thereby enhancing patient recovery. (J Turk Ger Gynecol Assoc 2016; 17: 1-5)

Keywords: Local anesthetic, lidocaine, epinephrine, cesarean section

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Introduction

With approximately one of three babies surgically born, cesarean section (C/S) is the most frequent surgery performed worldwide (1). The World Health Organization has advised all healthcare bodies worldwide to maintain the C/S rate well under 15%. Nevertheless, recently, the rate has reached up to 46% in China and well above 25% in other countries in Asia, Africa, and Latin America and in the United States (2); such an increase might be related to the changes in the physicians' practice patterns, financial incentives, and patients' preferences (3).

Pain is an upsetting feeling that usually delays a patient's recovery and can be accompanied by tissue damage. Proper pain assessment provides crucial information that helps in diagnosing various types of pain, such as somatic, neuropathic, or visceral pain (4).

Unfortunately, to date, postoperative pain is not properly controlled because of many factors. One of these is the inability to efficiently put into action pain management protocols, together with the lack of precision of pain assessment techniques (5). Others factors include wrong beliefs and the patients' high expectations. There is usually a lack in customizing analgesic strategies to satisfy the patients' requirements. Acute pain has detrimental effects if left untreated because it results in acute neurohumoral changes, neuronal re-modeling, depression, anxiety, insomnia, loss of control, inability to sense and communicate with others, and long-lasting psychological and emotional illness and may also end up in prolonged chronic pain states (6, 7). In contrast, sufficient control of post-C/S pain is imperative to relieve the patients' discomfort and to enhance breastfeeding performance and infant care (8).

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Usually, high doses of opioid analgesics are necessary to ease severe postoperative pain; however, this strategy has many disadvantages, such as evident disruption of mother–newborn bonding (9). Local anesthesia is of help because of the decreased opioid consumption, and it can be used because of its affordability as part of the smart strategic protocol for pain relief (10).
This study aimed to assess the efficacy and safety of incisional infiltration of lidocaine and epinephrine vs. lidocaine only to reduce post-C/S pain, and thus, enhance the patient's recovery.

Material and Methods

This was a prospective, randomized, controlled, double-blinded clinical trial that was conducted in two tertiary hospitals in Egypt (Ain Shams University Maternity Hospital and Mis Al-Gededa Military Hospital) in the period from August 2014 to February 2015.
The study was approved by the research ethical committee of Ain Shams University Maternity Hospital in March 2014 according to the World Medical Association Declaration of Helsinki and was registered on clinicalTrails.gov. with the study registration number (NCT 02274974).
A total of 160 women undergoing C/S under general anesthesia for various indications were included. Women with known or suspected sensitivity to local anesthesia, medical disorders that were induced by pregnancy as pre-eclampsia, medical disorders aggravated by pregnancy as cardiovascular diseases, hemodynamically unstable patients, or patients lacking adequate verbal communication were excluded. Of 160 women, 153 met the inclusion criteria. All participants signed a written informed consent after the benefits and risks of the trial were explained, and they had the right to withdraw from the study at any time.
The patients were randomly divided into the following two groups: Group I (control group, n=78), in which the wound was infiltrated before skin closure with 20 mL of 2% lidocaine (Arab Drug Company for pharmaceuticals; AL Amireya, Cairo, Egypt), and Group II (study group, n=75), in which the wound was infiltrated before skin closure with 20 ml of 2% lidocaine and epinephrine in a dose-related manner, i.e., 1:200,000 [by adding 1/4 of an ampoule of 1 mg/mL adrenaline (Misr pharmaceutical industries; El Matareya, Cairo, Egypt) to a bottle of lidocaine HCL 2% 50 mL (20 mg/mL)].
Patients were randomly assigned to either Group I or II using a computer-generated random number list, which was generated using Medcalc© version 12.5 (Medcalc Software; Ostend, Belgium). The randomization list was concealed and accessed by sequentially numbered, opaque, sealed envelopes, immediately before the intervention. The patient, surgeon, and personnel involved in the assessment of postoperative pain were blinded to both of the groups. Two identical bottles for masking were prepared, one containing lidocaine only and the other containing lidocaine and epinephrine.
All C/S were performed by surgeons with the same level of training and experience (senior registrars). The technique and suture materials used in all cases were the same. The skin was closed by subcuticular sutures using delayed absorbable synthetic suture [vicryl (2-0)®]. Prior to the infiltration of the solution in both groups, the anesthetist was informed to check for any side effects.
Pain severity was measured using a 10-point visual analogue scale (VAS) at 15 min, 1, 2, 4, 8, 16, and 24 h after recovery from anesthesia. The amount of opioids (pethidine) (Misr pharmaceutical industries; El Matareya, Cairo, Egypt) consumed after 1, 2, 4, 8, 16, and 24 h was recorded.
The primary outcomes were the time to first analgesic (TFA) request (minutes) and the postoperative pain scores that were measured using VAS. The secondary outcomes included the duration of C/S in minutes, onset of mobilization in minutes, onset of breastfeeding in minutes, duration of hospital stay in hours, local and systemic side effects of infiltration of lidocaine and epinephrine, postoperative pyrexia (≥38°C) on two occasions, postoperative wound infection, liver function tests, and renal function tests pre- and postoperatively.

Sample size verification

The sample size required was estimated using the G*Power software version 3.1.7 (Institute of Experimental Psychology, Heinrich Heine University, Dusseldorf, Germany). A previous study by Fouladi et al. (11) reported that in women receiving postincisional lidocaine alone, the maximum pain scores were observed at 3 and 6 h postoperation with mean VAS scores±standard deviation (SD) of 5.1±(1.4) and 5.0±(1.3), respectively. According to the same study, the TFA request that was associated with postincisional infiltration of lidocaine was 3.3±(2.0) h.
Since there was no previous available data regarding the effect of adding epinephrine to lidocaine for postincisional wound infiltration after C/S on postoperative pain and duration of analgesia, the sample size required for this study was estimated on the basis of targeting a standardized effect size, which was considered clinically relevant. Thus, it was estimated that a sample size of 64 patients per group would achieve a power of 80% (type II error, 0.2) to detect a medium effect size (Cohen's d=0.5) with regard to the outcome measures. The effect size (d) was calculated as follows: $d=(m_1–m_2)/s$, where m_1=mean of the control group, m_2=mean of the experimental group, and s=pooled standard deviation. Seventy patients were included in each group (estimating a 10% drop out).
The statistical test performed for sample size estimation was the two-sided unpaired t-test, and the type I error was set at a conventional value of 0.05 (confidence level=95%).

Statistical analysis

Data were analyzed using IBM© SPSS Statistics version 22 (IBM Corp.; Armonk, New York, USA) and MedCalc© version 13 (MedCalc Software; Ostend, Belgium).
Normally distributed numerical variables were interpreted as mean and SD, and intergroup differences were compared using the unpaired student's t-test.
Non-normally distributed numerical variables were presented as median (interquartile range), and between-group differences were compared using the Mann–Whitney test.
Categorical variables were presented as a number and percentage, and the Pearson chi-square test was used to compare

intergroup differences. Ordinal variables were compared using the chi-square test for trend. p value of <0.05 was considered statistically significant.

Results

Figure 1 shows the CONSORT flow chart of this study. A total of 160 patients were assessed for their eligibility to participate in the study. Of 160 patients, only 153 were actually randomized to participate in the study. Because of the loss of contact and some other factors, 145 patients actually participated in the study, with 70 patients in Group II and 75 in Group I (the numbers in each group were higher than the anticipated sample size as the anticipated losses were less).

Table 1 shows the demographic data in both groups, and it revealed that both groups were matched with regard to age, weight, height, body mass index, and parity, with no significant difference.

Table 2 shows that the signs of early recovery following C/S was highly significantly quicker in Group II than in Group I, as demonstrated by an earlier onset of mobilization ($p<0.0001$) and breastfeeding ($p<0.0001$) and a shorter duration of hospital stay ($p<0.0001$). The duration of C/S did not significantly differ between both the groups ($p=0.289$). The TFA request was significantly longer in Group II than in Group I ($p<0.0001$).

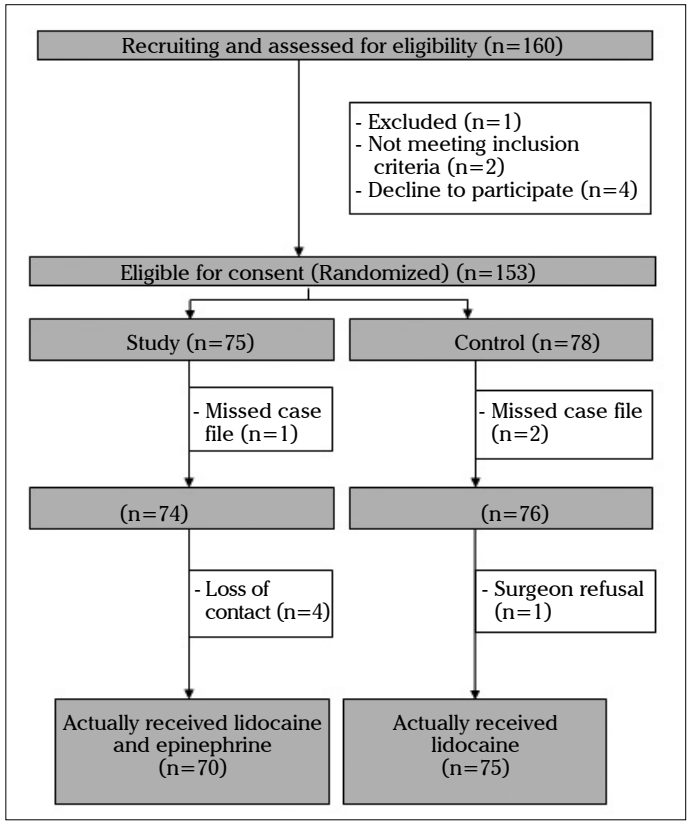

Figure 1. A consort flow chart of both the groups of the study
At the beginning of the study, 160 patients were recruited and assessed for eligibility. Of them, only 153 patients were included and randomized in the study (lidocaine–epinephrine) and control (lidocaine only) groups; however, because of various reasons, after dropouts, only 145 patients participated in the study; 70 patients in the study group and 75 in the control group.

Table 3 shows the cumulative postoperative opioid consumption in both the groups, and the consumption was highly significantly less in Group II than in Group I ($p<0.0001$), which means that administering epinephrine with lidocaine is more effective in postoperative pain control. Furthermore, Table 3 showed a significant decrease in pain score at 1 and 2 h in Group II than that in Group I ($p<0.0001$). These results were significantly reversed at 4 and 16 h ($p=0.011$ at 4 h, $p=0.028$ at 8 h, and $p=0.023$ at 16 h). At 24 h, there was no significant difference in pain scores between both the groups ($p=0.938$).

There was no significant difference between both the groups regarding the pre- and postoperative renal and liver functions. There was a significant decrease in the hemoglobin level of 1 g/dL and hematocrit value of 3% in both the groups with respect to the pre- and postoperative results, with no significant difference between both the groups.

In both the groups, none of the patients developed postoperative infection, postoperative pyrexia, allergic reactions, or complications from general anesthesia, local anesthesia, or epinephrine.

Discussion

C/S is the most common laparotomy currently performed worldwide; thus, any useful refinement in the operative technique, however minimal, is likely to yield substantial benefits and cause early recovery (1, 12). The surgical technique for cesarean delivery has changed from time to time and surgeon to surgeon, and these changes involved both the uterine and skin incisions (12).

A major objective in C/S is to attempt to reduce the postoperative pain, and thus, enhance the maternal recovery. This will have an immense effect on the neonate. This was a pilot study (a prospective, randomized, controlled clinical trial) that compared the efficacy and safety of incisional infiltration of lidocaine only vs. lidocaine and epinephrine in reducing post-C/S pain.

Lidocaine and epinephrine combined was more effective than lidocaine only in controlling post-C/S pain as shown by the decrease in the pain score using VAS=1 vs. 3 after 1 h and VAS=3 vs. 7 after 2 h in Group II vs. Group I, respectively. However, these results were reversed at 4.8 and 16 h (VAS=7 vs. 6, 6 vs. 5, 4 vs. 3) in Group II vs. Group I.

The earlier requirement for administration and the significantly higher amount of consumption of pethidine during the first 24 h in Group I compared with those in Group II indicated that administering epinephrine with lidocaine in Group II helped in reducing pain; however, at the same time, higher pethidine doses participated in improving the degree of pain control in Group I compared with Group II at 4, 8, and 16 h. After 24 h, both the groups had the same degree of pain according to VAS.

There was an earlier onset of lactation in Group II than in Group I, with a difference of 43 min. Mobilization was earlier in Group I, with a difference of 45 min. Patients in Group II had a shorter duration of hospital stay, and 97.1% of women in Group II stayed for 24 h compared with 62.7% in the control. None of the patients in Group II stayed for 36 h compared with 14.7% of patients in Group I; only 2.9% of patients stayed for 48 h in Group II compared with 22.7% in Group I.

Table 1. Patients' demographic data in both the groups

Variable	Control group (number=75) Mean (SD)	Study group (number=70) Mean (SD)	p	Significance
Age (years)	28.3 (4.4)	28.0 (4.8)	0.731*	NS
Weight (kg)	78.4 (5.1)	76.8 (6.1)	0.087*	NS
Height (cm)	166.8 (5.8)	168.0 (4.3)	0.161*	NS
BMI (kg/m^2)	28.2 (3.8)	27.3 (2.9)	0.113*	NS
Parity	**Number (%)**	**Number (%)**		
P0	30 (40.0%)	25 (35.7%)	0.474**	NS
P1	15 (20.0%)	13 (18.6%)		
P2	20 (26.7%)	22 (31.4%)		
P3	8 (10.7%)	6 (8.6%)		
P4	2 (2.7%)	4 (5.7%)		

SD: standard deviation; NS: non-significant; BMI: body mass index; P: parity
*Analytical test used is independent student's t-test.
**Analytical test used is Chi-square (χ^2) test.

Table 2. Duration of cesarean section, time to first analgesic request, time of onset of mobilization, time of onset of breastfeeding, and duration of hospital stay in both the groups

Variable	Control group (number=75) Mean (SD)	Study group (number=70) Mean (SD)	p	Significance
Duration of C/S (minutes)	43.13 (10.77)	46.42 (24.37)	0.289*	NS
Time to first analgesic request (minutes)	87.57 (41.7)	122.33 (56.8)	<0.0001*	HS
Time of onset of mobilization (minutes)	127.53 (72.33)	82.57 (26.14)	<0.0001*	HS
Time of onset of breastfeeding (minutes)	132.13 (58.04)	89.42 (25.47)	<0.0001*	HS
Duration of hospital stay (hours)	31.8 (10.6)	22.4 (4.8)	<0.0001*	HS
	Number (%)	**Number (%)**		
24 h	47 (62.7)	68 (97.1)	<0.0001**	HS
36 h	11 (14.7)	0 (0.0)		
48 h	17 (22.7)	2 (2.9)		

SD: standard deviation; C/S: cesarean section; NS: non-significant; HS: highly significant
*Analytical test used is independent student's t-test.
**Analytical test used is Chi-square (χ^2) test.

Many studies in the literature focused on injecting local anesthetic at the incision site to reduce postoperative pain and enhance recovery, but none concentrated on the role of adding epinephrine to the local anesthetic. In a study assessing the maternal and fetal outcomes of local wound infiltration with lidocaine alone either preincisionally, postincisionally or combined in elective C/S, it showed that combined pre- and postincisional local wound infiltration is superior to each one alone in pain relief (11).

Table 3. Postoperative pain scores and cumulative opioid (pethidine) consumption in both the groups

Variable	Control group (number=75) Mean (SD)	Study group (number=70) Mean (SD)	p	Significance
VAS score at 15 min	0 (0-1)	0 (0-1)	>0.05*	NS
VAS score at 1 h	3 (2-7)	1 (0-1)	<0.0001*	HS
VAS score at 2 h	7 (6-8)	2 (1-5)	<0.0001*	HS
VAS score at 4 h	6 (6-7)	7 (6-8)	0.011*	S
VAS score at 8 h	5 (4-6)	6 (5-7)	0.028*	S
VAS score at 16 h	3 (2-5)	4 (3-5)	0.023*	S
VAS score at 24 h	1 (0-2)	1 (0-2)	0.938*	NS
Cumulative opioid (pethidine) consumption (mg)	90 (80-100)	50 (40-70)	<0.0001*	HS

SD: standard deviation; VAS: visual analogue scale for pain; NS: non-significant; S: significant; HS: highly significant
*Analytical test used is the Mann–Whitney test.

A Cochrane database review in 2009 that included 20 studies demonstrated that regional and general anesthesia if combined with local analgesia infiltration and abdominal nerve blocks could be of major benefit in C/S aiming to minimize the use of opioids. Non-steroidal anti-inflammatory drugs may also offer additional pain control (13).

Tumescent anesthesia, which is injecting a very dilute solution of local anesthetic combined with epinephrine and sodium bicarbonate into tissue until it becomes firm and tense, has been widely preoperatively used in many surgical applications, such as liposuction; vascular surgery; breast surgery; plastic surgery; and ear, nose, and throat procedures (14). It reduces blood loss through epinephrine-induced vasoconstriction and hydrostatic compression from the tumescent effect. Sodium bicarbonate minimizes pain occurring from the injection of an acidic local anesthetic solution. Lidocaine is very slowly absorbed from the subcutaneous tissues producing lower and a more delayed peak blood levels compared with other routes, thereby extending the postoperative duration of analgesia. Slow systemic absorption enables rapid hepatic plasma clearance of lidocaine to maintain safe local anesthetic blood levels (15).

Previous trials regarding the use of the combination of lidocaine and epinephrine were related to the ear and nose surgery as in Häfner et al. (16), which demonstrated that epinephrine prolonged the duration of action of lidocaine with a decrease in pain degree without any complications.

Another study of local anesthesia using buffered 0.5% lidocaine with 1:200,000 epinephrine for surgical excision of tumors of the digits by Firoz et al. (17) concluded that epinephrine prolonged the duration of action of lidocaine with a decrease in pain perception without any ischemic complication for the digits.

The following are some limitations of this study:

1) Small sample size. We will require further studies on larger numbers of patients to confirm the results.
2) This study did not include obese pregnant patients with body mass index of >35 and patients receiving regional anesthesia.
3) This study did not compare pre- vs. postincisional or combined injection of lidocaine and epinephrine in reducing post-C/S pain.

We conclude from this study that administering epinephrine with local anesthetics (such as 2% lidocaine in a dose-related manner, 1:200,000) prolongs its anesthetic effect and reduces the opioid analgesic dose postoperatively required. This enables early onset of mobilization and breastfeeding and shorter duration of hospital stay with no systemic or local complications.

Ethics Committee Approval: *Ethics committee approval was received for this study from the ethics committee of Ain Shams University Maternity hospital.*

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

Peer-review: *Externally peer-reviewed.*

Author Contributions*: Concept - A.T., A.A.; Design - A.Y., K.W.; Supervision - A.T., A.Y.; Materials - A.A., A.Y., K.W.; Data Collection and/or Processing - A.A., K.W.; Analysis and/or Interpretation - K.W.; Literature Review - A.A, K.W., A.Y.; Writer - K.W., A.A.; Critical Review - A.T., A.Y.;*

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Comparison of efficacy of metformin and oral contraceptive combination of ethinyl estradiol and drospirenone in polycystic ovary syndrome

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Abstract

Objective: The 2013 Endocrine Society guidelines state that hormonal contraceptives should be used for treating both menstrual irregularity and hirsutism in patients with polycystic ovary syndrome (PCOS). Metformin should be reserved for the treatment of women presenting with only menstrual irregularity because it has limited benefits in treating hyperandrogenism associated with PCOS. A high prevalence of insulin resistance is noted among the South Asians, and these guidelines may not hold good for this population. Thus, this study was conducted to investigate and compare the effects of metformin and an oral contraceptive containing drospirenone on menstrual pattern, body mass index, serum testosterone levels, and dehydroepiandrosterone sulfate (DHEAS) levels at baseline to 6 months of therapy in the treatment groups.

Material and Methods: This was a prospective observational study that was conducted over a year in patients visiting the Endocrinology outpatient department at a tertiary care center in a south Indian city. Forty-six subjects diagnosed with PCOS as per the Rotterdam criteria were included. They received either metformin twice daily or an oral contraceptive containing drospirenone once daily as a monthly regimen for 6 months.

Results: Metformin regularized menstrual cycles in 72% of patients who were followed up at 6 months. No significant difference was observed between the two treatment groups with respect to decreasing the body mass index, serum testosterone levels, and DHEAS levels ($p=0.40$, $p=0.65$, and $p=0.22$, respectively).

Conclusion: Metformin is effective in regularizing menstrual cycles, decreasing body mass index, and treating hyperandrogenism in Indian women diagnosed with PCOS. (J Turk Ger Gynecol Assoc 2016; 17: 6-9)

Keywords: Polycystic ovary syndrome, metformin, oral contraceptive containing drospirenone, menstrual cycle, testosterone

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Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disorder with a prevalence of 15%–20% (1) and is characterized by oligomenorrhea, hyperandrogenism, and ultrasonographic evidence of PCOS (2).

Combined oral contraceptives have been the traditional choice for patients not desirous of conception (3) and patients who have a major component of hyperandrogenism. Metformin for treating insulin resistance has also been found to be beneficial in restoring ovulation in women suffering from PCOS (4), and thus, can be used as a therapeutic option. However, there are not many studies demonstrating its benefit in treating hyperandrogenism (5).

Guidelines released by the Endocrine Society state that metformin has limited or no benefit in treating hyperandrogenism and should be used if patients presenting with menstrual irregularity as the chief complaint (6). The applicability of these guidelines in South Asia, particularly India, is uncertain because of the high prevalence of insulin resistance amongst this population (7). The relevance of this observation concerning therapy for patients with PCOS is highlighted by a study where patients with PCOS of the South Asian ethnicity had more severe symptoms, higher fasting insulin concentrations, and lower insulin sensitivity compared with Caucasians (8). Thus, insulin-sensitizing drugs may have a more beneficial role to play in this population. Hence, this study was conducted to compare the efficacy of metformin and oral contraceptive containing drospirenone on menstrual pattern, body mass index (BMI), serum testosterone levels, and dehydroepiandrosterone sulfate (DHEAS) levels in Indian women with PCOS.

Material and Methods

This study was conducted at a tertiary care hospital in a south Indian city among patients visiting the Endocrinology outpatient department over a 1-year period. The study was approved by the Institutional Ethics Committee.

Patients aged between 18 and 45 years who were diagnosed with PCOS as per the Rotterdam criteria (2) with a modified

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Ferriman–Gallwey score of >8 were included. Patients with contraindications to oral contraceptives; patients desirous of conception within 6 months of inclusion in the study; patients with a diagnosis of concomitant hypothyroidism, hyperprolactinemia, diabetes mellitus, renal, or adrenal insufficiency; history of drug use for PCOS before inclusion in the study, and history of smoking and substance abuse were excluded from the study.

Forty-six patients were included, and informed consent was obtained. Demographic data, baseline parameters, such as menstrual pattern and BMI, were recorded. Baseline laboratory parameters, such as serum testosterone and DHEAS levels were recorded. The patients at the physician's discretion received either 30 μg ethinyl estradiol+3 mg drospirenone (one tablet daily) as a monthly regimen of 21 days for 6 months or 2 g metformin sustained release tablet in two divided doses daily for 6 months.

The patients were followed up at 3 and 6 months of treatment, and changes in menstrual pattern, BMI, and serum androgen levels (testosterone and DHEAS) were recorded and compared between the two groups.

A priori sample size calculation was performed using a priori sample size calculator for Student's t-tests (software) version 3.0 (9) and considering an α value of 0.05, β value of 0.20, and an anticipated effect size of 0.85. The total sample size required was calculated to be 46 with 23 patients in each group. Student's t-test (two tailed, independent) was used to determine the significance of study parameters on a continuous scale between the two groups and chi-square test was used to determine the significance of study parameters on a categorical scale between the two groups. Student's t-test (two tailed, dependent) was used to determine the significance of study parameters on a continuous scale within each group. The analyses were performed using the Statistical Package for Social Sciences software version (SPSS) software version 17 (SPSS Inc.; Chicago, USA).

Results

Forty-six patients (23 in each group) were enrolled in the study, with 72% follow-up at 3 months and 48% follow-up at 6 months (Figure 1).

The baseline characteristics of patients in both the groups were comparable (Table 1). Regarding the menstrual pattern, nine of the 16 patients in the metformin group and all 17 patients who were analyzed in the oral contraceptive containing drospirenone group had attained regular menstrual cycles at the 3-month follow-up (p=0.002 between the two groups), and eight of the 11 patients in the metformin group and all 11 patients in the oral contraceptive containing drospirenone group had attained regular menstrual cycles at the 6-month follow-up (p=0.06 between the two groups). BMI, serum testosterone levels, and serum DHEAS levels significantly declined within the individual groups, but no significant difference was observed between the two groups at the 6-month follow-up (Table 2).

Both the drugs were well tolerated with no serious adverse events being recorded during the study period.

Discussion

Therapy for patients with PCOS is targeted toward regularization of menstrual cycles and suppression of ovarian testosterone production, which is conventionally, achieved using combined oral contraceptives. However, side effects, such as increased risk of venous thrombosis and weight gain, make the use of combined oral contraceptives inappropriate in obese women and women with prediabetes. Thus, alternative options are explored. Metformin is an insulin-sensitizing drug that has demonstrated promise in therapy for patients with PCOS. This study assessed the efficacy of metformin and oral contraceptive containing drospirenone in patients with PCOS.

All patients enrolled in the study had oligomenorrhea. As expected, oral contraceptive containing drospirenone regularized menstrual cycles in all patients. However, 72% of patients receiving metformin also had regular menstrual cycles after 6 months of therapy, with no significant difference being observed between

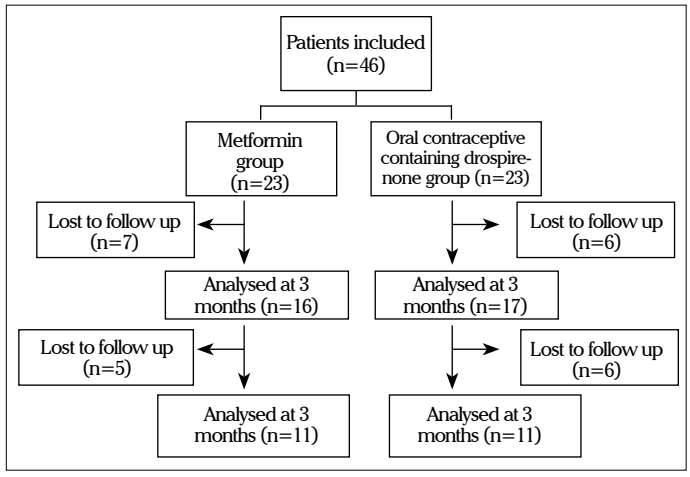

Figure 1. Patient recruitment chart

Table 1. Baseline characteristics of patients

Baseline characteristics	Metformin group (n=23)	Oral contraceptive containing drospirenone group (n=23)	p
Age (years)	24.52±4.37	23.52±6.04	0.52
BMI (kg/m^2)	27.72±5.04	26.48±4.56	0.25
Patient symptoms and clinical signs			
1. Oligomenorrhea	23 (100%)	23 (100%)	1.00
2. Hirsutism (FG score>8)	23 (100%)	23 (100%)	1.00
3. Infertility	4 (17.39%)	2 (8.69%)	0.33
4. Acne	6 (26.08 %)	6 (26.08%)	0.85
USG for PCOS features			
1. Present	19 (82.60%)	19 (82.60%)	1.00
2. Absent	4 (17.39%)	4 (17.39%)	

BMI: body mass index; FG score: modified Ferriman-Gallwey score; USG: ultrasonography; PCOS: polycystic ovary syndrome

Table 2. Comparison of efficacy of metformin and oral contraceptive containing drospirenone on body mass index, serum testosterone and DHEAS levels at baseline, 3 and 6 months of treatment

Parameters	Baseline (Mean±SD)			3 months of therapy (Mean±SD)			6 months of therapy (Mean±SD)		
	Met	Drosp	p	Met	Drosp	p	Met	Drosp	p
BMI (kg/m^2)	27.72± 5.04	26.48± 4.56	0.38	26.27± 4.60***	25.57± 4.30**	0.65	25.61± 3.19 **	24.28± 3.98 **	0.40
Serum Testosterone (ng/dL)	56.52± 11.70	62.23± 13.59	0.15	51.75± 10.43***	53.94± 15.40***	0.68	49.67± 13.68 **	52.57± 14.17***	0.65
Serum DHEAS (mcg/dL)	197.30± 60.35	227.16± 66.34	0.30	175.00± 50.02	200.61± 47.03*	0.37	166.50± 43.12	195.45± 32.73*	0.22

SD: standard deviation; Met: metformin; Drosp: oral contraceptive containing drospirenone; BMI: body mass index; DHEAS: dehydroepiandrosterone sulphate

*,** and*** denote statistical significance at $p<0.05$, $p<0.01$ and $p<0.001$ respectively within the individual group (Met versus Met; Drosp versus Drosp) for decline in BMI, serum testosterone and serum DHEAS levels from baseline at 3 and 6 months of treatment.

The p-values mentioned in the table denote the p-values between the two treatment groups (Met versus Drosp) for BMI, serum testosterone and serum DHEAS levels at baseline, 3 and 6 months of treatment.

the two groups. Bobde et al. (10) reported a significant regularization of menstrual cycle in patients who received oral contraceptive containing drospirenone compared with those who received metformin ($p=0.001$). This is also reported in a review by Costello et al. (11), where 18 of 21 patients on metformin and 20 of 24 patients on oral contraceptives had oligomenorrhea. Metformin was less effective compared with oral contraceptives in improving menstrual pattern ($p=0.004$). In this study, the results observed at 3 months are consistent with earlier studies. The mechanism for regularization of cycles in oral contraceptive containing drospirenone users is withdrawal bleeding that occurs because of the hormone-free interval in the monthly cycle. In this study, the delayed effect of metformin on the menstrual cycle is possibly because of the lag period of 4–6 months that is required for the plasma luteinizing hormone (LH) levels to return to normal following decreased LH pulse secretion when the patient is initiated on metformin therapy (10).

There was no significant difference in BMI observed between the two groups at 6 months. Cinar et al. (12) reported no decline in BMI among 25 patients who received oral contraceptive containing drospirenone alone, whereas a decline in BMI was observed among 20 patients who received metformin along with an oral contraceptive. In another study by Ibáñez et al. (13), oral contraceptive containing drospirenone alone was ineffective in reversing dysadipocytokinemia, hypertriglyceridemia, and adiposity, whereas patients who received add-on metformin demonstrated reversal of the aforementioned features. Weight loss because of metformin observed in this study could be because of its anorexic property and its ability to counteract adipose tissue expansion through direct inhibition of adipogenesis (14). Moreover, Oelkers et al. (15) reported reduction in body weight because of its anti-mineralocorticoid properties of drospirenone in healthy young menstruating women who received oral contraceptive containing drospirenone. The results of this study re-instate that weight loss is observed with oral contraceptive containing drospirenone. The problem of weight gain experienced with the use of conventional combined oral contraceptives is, thus, reduced with oral contraceptives containing drospirenone.

In this study, no significant difference is observed between the two groups in decreasing serum testosterone and DHEAS levels. A review by Jakubowicz et al. (16) reported a 44% decrease in serum androgens in obese women diagnosed with PCOS. A decline in serum androgens is also reported in lean women diagnosed with PCOS who were on metformin therapy. However, Bobde et al. (10) and Cosma et al. (17) reported oral contraceptive containing drospirenone to be more effective in improving hirsutism in patients with PCOS. Drospirenone being a 17-α spironolactone derivative exhibits anti-androgenic property. This property is also exhibited by metformin in this study probably because of its ability to treat insulin resistance in this inherently insulin-resistant population. Insulin resistance is postulated as a mechanism in the pathogenesis of PCOS (18, 19) because of the following hypotheses. First, hyperinsulinemia is hypothesized to increase ovarian testosterone production by stimulating insulin receptors present on the ovarian theca cells, which act by a signaling pathway different from that mediating the metabolic effects of insulin. Second, hyperinsulinemia results in decreased synthesis of sex hormone-binding globulin in the liver, and thus, free testosterone levels are increased. Third, insulin facilitates local activity of insulin-like growth factor-binding protein-1 (IGF-1) in the ovary, which stimulates ovarian androgen production. Both the drugs were well tolerated with no serious adverse events being recorded.

The results of this study suggest that the universal guidelines released by the Endocrine society may not be applicable to this population; hence, ethnicity-wise modifications may be required. Good response to metformin on hyperandrogenism in this patient subset proves that it may be beneficial for South Asians who are more insulin resistant compared with Caucasians. Further studies are required.

The limitations of the study are that the study design was observational, and there was loss to follow-up of patients. Li et

al. (20) also reported a 25.6% treatment compliance and follow-up rate among patients with PCOS in the 6-month study period. Non-compliance and subsequent failure to return for review was observed among patients with an increased BMI, and the contributing factors included the long duration of therapy, delayed beneficial effects of drug treatment, and concern regarding adverse drug reactions. This study depicts a similar scenario, and thus, reinforces the need to develop educational tools and materials to strengthen treatment education and intervention in the clinic. Moreover, drug treatment for patients with PCOS is provided on an outpatient basis. In this age of advancing technology, the development of mobile applications to provide reminders to patients to take their pill daily at home and go to the clinic for regular reviews can be a simple, yet effective step to ensure treatment compliance.

Ethics Committee Approval: *Ethics committee approval was received for this study from the ethics committee of M.S. Ramaiah Medical College and Hospitals.*

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German gynecologists' experience with a universal screening for gestational diabetes mellitus in daily practice: A qualitative study

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Abstract

Objective: In March 2012, a universal screening for gestational diabetes mellitus (GDM) was implemented in Germany. Despite international recommendations, a two-step approach was introduced [step 1: 50-g glucose challenge test (GCT); if GCT is suspicious, step 2 follows: 75-g oral glucose tolerance test with (OGTT)]. This qualitative study aimed at examining how gynecologists administer the screening for GDM in daily practice, whether they perceive any difficulties, and whether they have suggestions for improvement.

Material and Methods: Seventeen resident gynecologists were interviewed face-to-face in semi-structured interviews. The interviews were recorded, transcribed verbatim, coded, and analyzed using qualitative content techniques.

Results: We revealed differences in the screening administration. Three gynecologists directly offered the second step of the two-step screening (OGTT) instead of completing the first step before offering the second step. These gynecologists only conducted GCT if the woman (with statutory health insurance) was not willing to pay for OGTT. Critique concerns the late introduction of billing codes, lack of information from official institutions, unavailability of readymade syrup with 50-g glucose, and lack of information material for pregnant women.

Conclusion: Our results reflect that not all gynecologists appear to conduct the screening conforming to the maternity directive. However, this has to be validated in larger quantitative surveys. That some gynecologists directly conducted OGTT may fuel the discussion regarding the screening procedure. The two-step approach was already highly controversial at the time of introducing the screening because national and international organizations recommend a one-step approach. Therefore, our results are also relevant for other countries who have implemented a two-step screening and for countries planning to implement a screening. (J Turk Ger Gynecol Assoc 2016; 17: 10-5)

Keywords: Gestational diabetes mellitus, glucose tolerance test, prenatal care, pregnancy, qualitative research

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Introduction

Gestational diabetes mellitus (GDM) is associated with significant maternal and neonatal complications (1). In addition, it increases the mother's risk of developing a type 2 diabetes mellitus in long term (2). The prevalence of GDM in advanced economies ranges between 1.7% and 11.6% (3).

Because the prevalence is increasing worldwide (3), screening has been implemented in many countries worldwide. The screening aims at early detection and treatment of GDM. In particular, universal screening, i.e., an approach in which all pregnant women are screened for GDM regardless of their individual risk status, have been demonstrated to be cost-effective (4). Here, two different screening approaches are possible: GDM testing without previous screening (one-step approach) or a screening followed by definitive GDM diagnosis (two-step approach; 4).

In March 2012, a universal GDM screening was included in the German maternity directive. In contrast to the recommendations of the International Association of Diabetes and Pregnancy Study Groups (5), World Health Organization (6), German Diabetes Society, and German Society for Gynaecology and Obstetrics (7), a two-step approach was implemented. In the first step, every pregnant woman should be offered a glucose challenge test (GCT; 50-g glucose) as part of prenatal checkups. In the second step, an oral glucose tolerance test (OGTT; 75-g glucose) is offered for those with positive results in GCT.

As a result of the amendment in the maternity directive, all statutorily insured pregnant women (i.e., approximately 600.000 women/year) are entitled to GCT free of charge between gestational weeks 24+0 and 27+6. Reimbursement of the physicians is conducted by health insurances. When GCT is positive, the health insurances also pay for OGTT. If

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OGTT is performed without a verifiable reason, the pregnant woman has to pay for it herself. Before the introduction of this new guideline, gynecologists offered OGTT as a self-paid prenatal check-up. The gynecologists decided to whom OGTT was offered. An exception is pregnant women who are privately insured. Here, the private health insurance pays for OGTT, even if no GCT was previously conducted.

To date, the management of the GDM screening in daily practice for German gynecologists remains unclear. In this study, resident gynecologists were offered the opportunity to comment on 1) administration of the screening in their practice, 2) potential difficulties perceived during screening administration, 3) reimbursement of the screening, 4) cooperation with diabetologists, and 5) potential suggestions for improvement of the screening procedure. Because this was the first time for German gynecologists to express their view in a scientific study, we selected a qualitative study design so as to not constrict the participants in providing their answers. In addition, this study design enabled us in obtaining a deeper knowledge of potential problems faced by resident gynecologists regarding the GDM screening.

Material and Methods

Design

In this qualitative exploratory study, semi-structured interviews with resident gynecologists were conducted to collect the data. This type of interview was selected to provide participants the opportunity to freely phrase their answers. This enables us to achieve a deeper understanding of the ideas, opinions, and worries of gynecologists (8), and thus, to identify potential snags in daily screening practice.

Data collection

Altogether, 50 randomly selected gynecologists in the German cities Mannheim, Ludwigshafen, and Heidelberg who were stratified by sex and number of gynecologists per city were contacted via a mail. Seventeen gynecologists agreed to participate in this study. The interviews were conducted face-to-face by the first author (KD, PhD, female sociologist) in the gynecologists' practice without the presence of a third person (Dec/02/2013–Feb/06/2014).

We used a semi-structured interview guide with open-ended questions, which focused on the recent change in the German maternity directive. The interview guide was peer reviewed by experts in perinatology and obstetrics (HM) and diabetology (HK) and afterwards pre-tested in two interviews with gynecologists. These two interviews were included in the analysis because no essential changes in the interview guide were necessary.

Before the interview, the gynecologists were informed regarding the aim of the study and data protection. After the interview, sociodemographic and practice characteristics were collected. The average duration of the interviews was 33:12 min (minimum, 15 min; maximum, 58 min). They were audiotaped (Olympus DS-2500) and transcribed verbatim (191 pages).

Table 1. Examples for quotations concerning the administration of the screening

Quotation 1:	"Well, conducting the 50-g glucose challenge test is now a standard practice, but we rarely offer this test to our patients. We have been offering the 75-g glucose test as part of the prenatal medical care examinations for years and we will continue to propose this more accurate test to pregnant women as a medical service subject to an additional fee […]. We only conduct the 50-g test when the patient explicitly refuses to do the 75-g test. I mean, the price of the test is ok, at least for those with a normal income. But there are also patients who cannot afford to pay for the more comprehensive test, and we have no choice but to conduct the 50-g test with them." (G08)
Quotation 2:	"I have some patients who had gestational diabetes during their last pregnancy. In such cases, I conducted the test approximately in the 20th week of pregnancy. I mean of course that I conducted the real test not just the 50-g screening test." (G10)
Quotation 3:	"The patient receives a sachet containing glucose powder, which is weighed by a pharmacist, in the appointment preceding the glucose test. On the day of the test, she is asked to dissolve and drink the glucose powder at home and then come to the practice within an hour after drinking it to provide a blood sample." (G06)
Quotation 4:	"Honestly speaking, the fact that my assistant has to measure out the syrup in a measuring cup in order to have the exact amount of liquid for the test means a lot of extra work." (G01)
Quotation 5:	"I use a readymade syrup. On the bottle containing the syrup, I mark the point up to where pregnant women have to drink. Unfortunately, I have to throw away the rest of the bottle." (G02)
Quotation 6:	"There is only one for the 75-g test. If I used that liquid, I would have to throw away one third of it, which would be a pity. […] Admittedly, this liquid tastes better, but I think people can live with it for this one test." (G11)
Quotation 7:	"If they [the pregnant women] have an errand to run somewhere in town, they can go and do what they need to do. The main thing is that they are back within an hour to give a sample of venous blood." (G12)
Quotations are examples that derived from 17 interviews with resident gynecologists	

Ethical consideration
All gynecologists provided their written consent to participate. To reimburse them for the invested time, 80€ were paid. Approval of the Ethics Committee of the Medical Faculty Mannheim, Heidelberg University was obtained (2013-609N-MA).

Participants
The average age of the gynecologists was 52.9 years (minimum, 36 years; maximum, 70 years). They were resident for 13.9 years (minimum, 3 years; maximum, 29 years). Eleven of 17 gynecologists were female. The majority (n=10) had a single practice.

Analysis
Qualitative content analysis following Mayring (9) was performed to identify themes, patterns, and contradictions by comparing the 17 interviews. Categories were identified by reviewing the data. Overall, seven categories comprising 22 codes were inductively developed to systemize the data. The data were independently coded by two researchers (KD and TG). The comparison revealed high agreement (89.9%) (10). For coding the data, we used ATLAS.ti 7 (ATLAS.ti Scientific Software Development GmbH; Berlin, Germany). The results presented in this study are based on five categories (i.e., administration of the screening, potential difficulties perceived during screening administration, reimbursement of the screening, cooperation with diabetologists, potential suggestions for improvement of the screening procedure).

Results

Administration of the screening
Almost all participating gynecologists conducted the 50-g GCT to screen for GDM according to the recommendations set by the maternity directive. However, some participants considered GCT to be "not sensitive enough" (G13). Three gynecologists even recommended their patients to undergo the 75-g OGTT in the framework of the screening instead of GCT and allowed them to decide which test should be conducted (e.g., G08; quotation 1 in Table 1): Almost all participants (n=15) were reported to perform the GDM screening within the time frame proposed by the German maternity directive, i.e., between gestational weeks 24+0 and 27+6. However, one gynecologist reported to offer GCT between gestational week 20 and 24 (G05). In some cases, gynecologists conducted GCT before week 24+0 of pregnancy, e.g., because of irregularities during the prenatal check-ups. With regard to pregnant women at risk for developing GDM (e.g., women with GDM or macrosomia in preceding pregnancies), three interviewees stated that they directly offered them OGTT without previously conducting GCT (e.g., G10, quotation 2 in Table 1), which is in line with the recommended procedure. The majority of gynecologists (n=12) used glucose powder to prepare the glucose solution for GCT (Figure 1). While in most cases the solution was prepared in the practice just before the conduct of the test, two gynecologists gave their patients a sachet containing glucose powder and had them prepare and drink the glucose solution at home prior to the gynecologist's appointment (e.g., G06; quotation 3 in Table 1). Limited capacities in the waiting room were named as the main reason for such a proceeding (G06).

Five gynecologists used the readymade glucose syrup that was reported to taste better and be easier for the patient to drink. In Germany, the syrup is only available in bottles containing 75-g glucose. This means that when the readymade syrup is used, the amount for GCT (50-g glucose) has to be manually measured. While some gynecologists conferred great importance for accurate measurement of the syrup (e.g., G01; quotation 4 in Table 1), some were less accurate in this regard (e.g., G02; quotation 5 in Table 1).
The fact that the syrup is only available in bottles with 75-g glucose was the main reason for most participants preferring to use the glucose powder for GCT (e.g., G11; quotation 6 in Table 1).
Despite the recommended procedure, one gynecologist reported that he had his patients drink the glucose solution for GCT on an empty stomach (G05). In accordance with the guideline, the majority of participants stated that they had their patients stay in the practice after having drunk the glucose solution and sit still while waiting to provide a blood sample. However, some gynecologists conducted other examinations (e.g., cardiotocography) during the waiting time of 1 hour. Altogether, five gynecologists reported that their patients were allowed to move while waiting, e.g., walking to the practice for those who drank the liquid at home or just going for a short walk (e.g., G12; quotation 7 in Table 1).

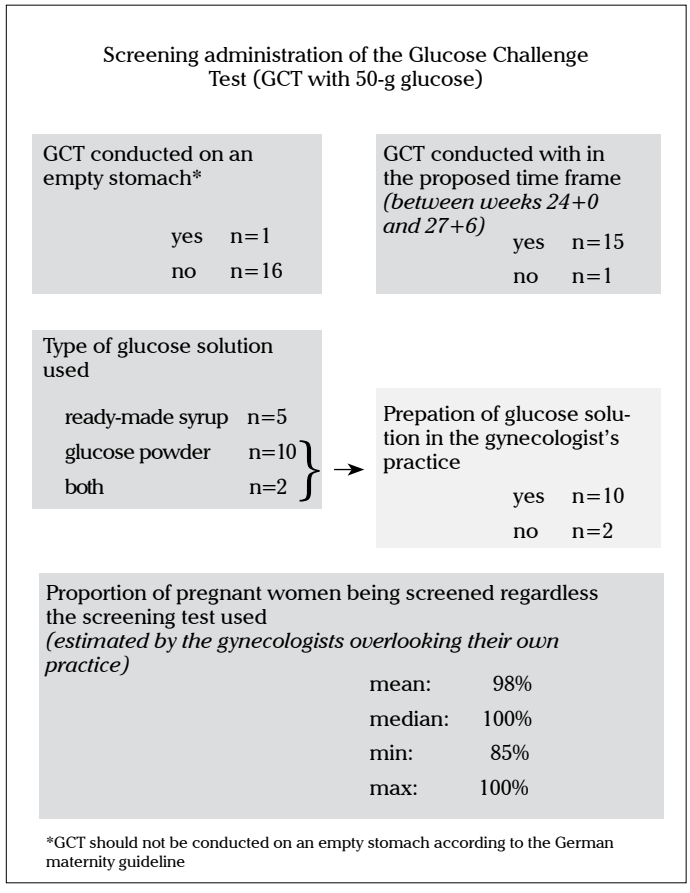

Figure 1. Information regarding the administration of the screening for gestational diabetes mellitus
Results based on 17 interviews with resident gynecologists.

Compared with OGTT, GCT was considered to be "easier to conduct (for patients) because the patients do not have to be fasting and come in the morning" (G14). When asked for their opinion regarding how patients may perceive the screening test, most participants generally emphasized the positive aspects by saying, for instance, that pregnant women perceived GCT as a sign of better care (G02, G04, and G14) or as a meaningful measure "because in every family there is someone with diabetes" (G06). However, some interviewees also mentioned negative aspects by saying that some patients might have trouble providing blood samples (G02), investing extra time in conducting the test (G08), or with the taste of the glucose solution (G14, G16).

Potential difficulties perceived during screening administration

Independent of whether GCT or OGTT was used for screening purposes, all gynecologists reported to conduct the test with nearly all of their patients. Many gynecologists explicitly emphasized that on the basis of the conventional understanding of screening measures, either of the test should be offered to all patients: "Strictly speaking, a screening includes everyone to determine those few who are affected. This is how every screening works." (G06). As a reason for non-administration of the screening test, participants most frequently named the patients themselves (n=11). In this regard, two types of pregnant women were particularly problematic: those who missed the medical check-ups during the time frame when the GDM screening should be conducted and those who rejected the screening. However, most gynecologists emphasized that such patients were "absolute exceptions" (G04). One gynecologist who was resident in a district with a high proportion of migrants emphasized language problems with patients, which sometimes hampered the appropriate health education, and thus, the administration of the test: "Well you know, I have many patients who do not speak German as fluently as you would expect them to. In those cases, it is very hard to communicate what the test is all about and why it is so important" (G10).

Some gynecologists reported that some women experienced side effects during the test, such as nausea (n=5), vomiting (n=4), or circulatory complaints (n=2). However, in general, the majority of participants stated that the conduct of the screening was unproblematic in this regard.

Reimbursement of the screening

One third of the gynecologists reported having had difficulties with the reimbursement of the screening test because the billing code for GCT in the doctors' fee schedule was approved more than 1 year after the GDM screening has been included in the maternity directive. Before this approval, the gynecologists had to submit the invoices to the health insurance and sometimes to "actively chase the payment" (G06), which was very time consuming. This shortcoming from the official institutions was sharply criticized by the gynecologists; for instance, "I personally find that from a health policy perspective, the introduction of such a regulation (the inclusion of the GDM screening in the maternity directive) should not be conducted with such poorly considered implementation strategies. If I want to introduce such a regulation, I should at least have the billing codes for the medical service in question and I should have calculated what costs to expect." (G16).

Some of the interviewees felt that they had not received sufficient information by the health insurance companies: "The health insurances offered us no help whatsoever (…) concerning the administration of the test." (G6). Therefore, every practice had to work out routines how to conduct the screening as efficiently and patient friendly as possible, which meant additional organizational efforts.

The majority of participants (n=12) reported that with the introduction of the billing codes problems with the remuneration of the screening costs came to an end. Only one gynecologist mentioned that the remuneration of the costs for GCT and time spent for the patients' care during the administration of the test were not equal (G15).

Cooperation with diabetologists

Many gynecologists cooperated with several specialists. Approximately two-thirds of the gynecologists (n=11) reported to cooperate with endocrinologists, eight with primary care physicians having an additional qualification in diabetology, and six with the women's hospitals. Most of the gynecologists (n=9) reported having had positive experiences with diabetologists. However, some of the interviewees emphasized that despite their professional qualification, the diabetologists often had little experience with pregnant women (G02, G10, G14, and G16). From the gynecologists' point of view, the consequence of this inexperience could be that patients with diabetes do not receive optimal care, e.g., unnecessary measuring of blood glucose (G16) or missing the correct initiating point for insulin therapy (G13).

While all participants cooperated with diabetologists, differences existed with regard to the time point, i.e., as to when diabetologists were involved in the care of pregnant women. Approximately half of the gynecologists referred their patients to a diabetologist after positive OGTT, whereas the other half directly referred their patients to diabetologists with a positive result of GCT. Thus, the latter ones did not conduct OGTT themselves. A potential explanation was provided by G14: "As soon as (GCT) is suspected, any consecutive examination should be conducted by specialists. He or she should take over further tests because it is also him or her who will determine adequate glycemic control and therapy. It would be nonsense for the patient to undergo the 75-g test in my practice and then again with the internist. Moreover, I would not want the colleague to base his glycemic control and therapy conclusions on my 75-g test either."

In general, gynecologists reported not having enough time to offer their patients in-depth nutrition counseling. This was another reason why they underlined the importance of diabetologists to counsel pregnant women regarding nutrition.

Potential suggestions for improvement of the screening procedure

When asked regarding their potential suggestions to improve the screening, participants frequently mentioned the need for information that could be handed out to pregnant women. According to them, there is a lot of GDM information available on the internet for gynecologists; however, there is a lack of short and easy-to-understand information for patients (G02). Especially leaflets or brochures with some general information on GDM, with information on causes and consequences for mother and child (G17), and with information on the next

steps after a positive screening (G15) were asked for. Two interviewees emphasized that it would be very helpful to have such information material in several languages (G11 and G16).
Moreover, participants underlined the need for more specific information for pregnant women, e.g., on how to eat healthy during the pregnancy (G15). However, another interviewee, who is very engaged in the field of GDM, stated that there is a lot of material regarding this topic available from various institutions (e.g., Federal Center for Health Education). One gynecologist emphasized that currently "the aspect of long-term importance (of GDM) for women is not sufficiently discussed. As soon as pregnancy is over, the topic of gestational diabetes disappears as well"(G16). In her opinion, during the pregnancy, "there is a very strong focus on the child, which is generally ok" (G16). However, according to her, there should be an equally strong focus on contraception and health behavior counseling for women after the pregnancy.
Another aspect in need of improvement from the gynecologists' point of view was the lack of readymade glucose syrup with 50-g glucose as described above. The fact that one-third of the bottle contents has to be thrown away if the gynecologist decides to use the readymade syrup with 75-g glucose for GCT was regarded as a "wastage" (G02). The availability of readymade glucose syrup was also considered necessary by gynecologists who used glucose powder. Some of them reported that the powder did not easily dissolve in water and emphasized that readymade syrup would be easier to drink for the patients because of its better taste.

Discussion

The majority of gynecologists adhered to the procedure mentioned in the maternity directive in terms of established parameters for GCT (i.e., gestational week, non-fasting, and no physical activity during waiting time). Nonetheless, contrary to the maternity directive, the 75-g OGTT was frequently conducted as a screening test, particularly for privately insured pregnant women. However, there were also gynecologists who still offered OGTT for screening to every pregnant woman (not only privately insured) as a self-paid service and only conducted GCT, if the women were not willing to pay for OGTT.
In addition, some gynecologists did not follow the Evidence-based Guideline on Diagnostics, Therapy, and Aftercare (11), which states that "During the test, the pregnant woman must sit near the test laboratory, shall not lie or move unnecessarily, and no other examination shall be carried out at this time." We found gynecologists that allowed pregnant women to walk (e.g., go shopping), handed out sachets containing glucose powder to pregnant women to dissolve and drink the glucose powder at home before coming to the practice, and conducted other examinations (e.g., cardiotocography) during the waiting time. Therefore, our results demonstrate that the GDM screening is not consistently conducted.
Perceived difficulties were the late introduction of billing codes and cooperation with diabetologists during the introduction period of the screening. Gynecologists stated that many diabetologists had a lack of experience with pregnant women. However, as time passed, this changed, and most gynecologists are now satisfied with the cooperation, particularly those working with endocrinologists.
Another issue that appeared during the interviews was that some gynecologists directly refer pregnant women to a diabetologist after a positive GCT, while other gynecologists only refer them after a positive OGTT. This means that some pregnant women might have to undergo three tests: GCT at the gynecologist's clinic, OGTT at the gynecologist's, and OGTT at the diabetologist's. This implies a high burden for the women and additional costs for the healthcare system.
While previous empirical studies focused on the experience of women having GDM (12), experience of midwives counseling women with GDM (13), and patients' attitudes towards the screening (14), this study is the first to deal with the daily practice of GDM screening from the gynecologists' point of view. The point in time for this study was ideal for two reasons. First, since the introduction of the screening, enough time has passed for the screening to be implemented into daily routine. Second, at the same time, the screening was still in an early stage; therefore, the gynecologists were able to remember the time of implementation and reorganization.
Nonetheless, there are several limitations that should be considered when interpreting our results. First, our data focus on the specific situation of the GDM screening in Germany and may not be applicable to other countries. However, our results may be interesting for countries who have implemented a two-step screening procedure. The difficulties in the interaction between gynecologists and diabetologists may also apply to other countries. Second, we cannot exclude a potential selection bias in our study population because the majority of participating gynecologists was well informed regarding the screening and conducted it by conforming to the maternity directive. Hence, our results may reflect a best-case scenario. Therefore, larger quantitative studies are required to obtain representative data.
Our results revealed that the introduction of the GDM screening into the maternity directive did not go smoothly. In our study, those who conduct the screening as a daily routine, namely resident gynecologists, were questioned for the first time since the screening's introduction. We were able to reveal a number of critical points that had not been addressed to date. For example, gynecologists are to comply with the requirements set in the maternity directive, but at the same time, they are confronted with problems that make it difficult for them to meet these requirements. Among the problems raised, gynecologists particularly deplored the introduction period of the screening where they had to deal with financial uncertainty because of the lack of billing codes.
Following the gynecologists' suggestions, two aspects are highly important to facilitate the screening and patient counseling in the future. First, the provision of more information material covering issues such as the time after pregnancy and healthy nutrition in particular. Against the backdrop of their multiethnic clientele, the information material should be available in different languages. Second, the provision of readymade glucose syrup with 50-g glucose would make the screening procedure more bearable for patients because of its better taste compared with glucose powder being dissolved in water. To date, there only exists readymade syrup with 75-g glucose. This leads to a widespread use of glucose powder, which is less tolerated because of its extreme sweetness.

One aspect that should be considered in future research is that the gynecologists are often not really involved in the screening administration. During the field phase of our study, it became apparent that often the medical technical assistant alone prepares the glucose solution, takes care of the women, and takes the blood sample. Informal talks with the assistants revealed that many pregnant women face difficulties drinking the glucose solution because they find it disgusting. Furthermore, it was mentioned that some pregnant women experience nausea (in particular, when it comes to drinking the solution that is not readymade but needs to be dissolved in water). Another difficulty, which was not mentioned by the gynecologists, appears to be the bad dissolution of the glucose powder in water. These facts reveal that the assistants have a different, possibly deeper insight into the conduct of the test. Therefore, it might be interesting to increase their involvement in future studies.

Our results are the first to shed some light into the "black box" GDM screening in gynecological care in Germany. After a difficult start, gynecologists finally succeeded in including the screening in their daily practice. However, we revealed differences in the administration of the screening. The different handling of the screening might be tackled by offering further training to the gynecologists or by proposing a more detailed procedure in the directive. Further studies, particularly quantitative representative studies, are urgently warranted for quality assurance of the screening conduction. Such studies would be useful to determine the proportion of gynecologists who conduct the screening according to the maternity directive (e.g., no conduct of GCT on empty stomach). Because of the qualitative nature of our study and a potential participation bias with more gynecologists, who are interested in this topic, participating in this study, we cannot provide an answer.

Nonetheless, our results imply that many pregnant women in Germany directly undergo OGTT despite the two-step approach that is suggested by the maternity directive. This might fuel the discussion on the two-step approach in Germany. Prior to the introduction of the screening, there were discussions on which screening should be included in the maternity directive. The German Diabetes Society and the German Society for Gynecology and Obstetrics preferred a one-step approach that offered OGTT to every pregnant women (7), which is in line with different international institutions (5, 6). Nonetheless, a more cost-efficient two-step approach was introduced after long discussions to have a less complex and less time-consuming test for all pregnant women in the first step. The results of our study are also interesting for other countries because of recent changes in statements and recommendations on the GDM screening worldwide.

Ethics Committee Approval: *Ethics committee approval was received for this study from the ethics committee of Medical Faculty Mannheim, Heidelberg University (2013-609N-MA).*

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Reference range for uterine artery Doppler pulsatility index using transvaginal ultrasound at 20–24w6d of gestation in a low-risk Brazilian population

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Abstract

Objective: To establish reference range for uterine artery (UtA) Doppler pulsatility index (PI) using transvaginal ultrasound at 20–24w6d of gestation in a Brazilian population.

Material and Methods: A retrospective cross-sectional study in 847 low-risk pregnant women undergoing routine second trimester ultrasound examination was conducted from February 2012 through March 2015. The mean UtA PI was calculated using color Doppler ultrasound with UtA gated at the level of the internal os. Mean±standard deviation and ranges for UtA Doppler PI in relation to gestational age (GA) are reported. Polynomial regression was used to obtain the best fit using mean UtA Doppler PI and GA (weeks) with adjustments performed using determination coefficient (R^2). The 5th, 50th, and 95th percentiles for the mean UtA Doppler PI in relation to GA were determined.

Results: The mean UtA Doppler PI ranged from 1.14 at 20 weeks to 0.95 at 24 weeks of gestation. The best-fit curve of mean UtA Doppler PI as a function of GA was a first-degree polynomial regression: mean UtA Doppler PI=1.900−0.038×GA (R^2=0.01).

Conclusion: In summary, when the mean UtA PI Doppler values were measured by transvaginal ultrasound at 20–24w6d of gestation, decrease in UtA Doppler PI values with advancing GA was observed. Reference range for the mean UtA Doppler PI at 20–24w6d of gestation using the transvaginal ultrasound in a low-risk Brazilian population was established. We believe that this reference range may be of clinical value in daily obstetric practice. (J Turk Ger Gynecol Assoc 2016; 17: 16-20)

Keywords: Uterine Doppler artery, second trimester, reference range

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Introduction

Preeclampsia (PE) is thought to be the consequence of inadequate trophoblastic invasion of maternal uterine vessels (1-3). A correlation between the histopathological findings and increased impedance to flow and subsequent development of PE as documented by abnormal waveform analysis and Doppler indices has been well documented and reported (4, 5). There is evidence that the uterine artery (UtA) Doppler ultrasound shows more accuracy for the prediction of PE when performed alone in the second than in the first trimester of pregnancy (6).

In clinical setting, reference ranges for UtA Doppler ultrasound during pregnancy are recommended for the appropriate analysis of impedance to flow. In this regard, pulsatility index (PI) has been advocated as the best Doppler index (7). Gómez et al. (7) contributed to the construction of reference range of UtA Doppler PI using transvaginal and transabdominal ultrasound. Specifically, transvaginal approach was used at 11–14 weeks of gestation, whereas transabdominal approach was used at 15–41 weeks. Ferreira et al. (8) compared the reproducibility of UtA Doppler PI in the first and second trimesters of pregnancy using both transvaginal and transabdominal scan and observed that PI was evenly significantly higher in both trimesters using transvaginal approach.

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According to Fetal Medicine Foundation (FMF) guidelines, risk assessment for premature delivery should be evaluated by cervical length measurement at 20–24 weeks of gestation using transvaginal ultrasound. At the same time, UtA Doppler PI can be measured to screen for the development of PE, fetal growth restriction (FGR), abruptio placentae, and stillbirth (9-19).
Therefore, the aim of this study was to establish the reference range for the mean UtA Doppler PI obtained using transvaginal ultrasound in a low-risk Brazilian population at 20–24w6d of gestation.

Material and Methods

A retrospective cross-sectional study in 847 low-risk pregnant women undergoing routine second trimester ultrasound examination was conducted from February 2012 through March 2015. This study was approved by the Ethic Committee of Uberaba University (CAAE: 50412115.0.0000.5145). Low-risk pregnant women were randomly selected either from public or private health services of the metropolitan region of Uberaba, Southeast Brazil.
Inclusion criteria were the following: singleton pregnancy, gestational age (GA) determined by the last menstrual period and confirmed by an ultrasound examination performed before 22 weeks of gestation (crown–rump length between 11 and 13w6d and biparietal diameter between 14 and 22w0d), and normal fetal growth (estimated fetal weight within the 10th and the 90th percentile according to the standard curve] (20). Exclusion criteria were as follows: PE in previous pregnancy, pregestational diabetes, disease of connective tissue, renal diseases, chromosomal abnormalities, or congenital malformations detected by prenatal ultrasound. Pregnant women were assessed once, and postnatal outcomes were not available.
The sample size was calculated according to Royston's precept, which a sample of approximately 20 cases per GA is recommended to construct reference ranges for fetal biometric parameters (21).
Clinical investigation was carried out at Mário Palmério University Hospital and Radiology Clinic of Uberaba. Ultrasound examinations were performed using by two examiners (ABP and TMRCC) with FMF accreditation by means of transvaginal ultrasound. The ultrasound exams were performed using only transvaginal route in two apparatus (Accuvix V20–Samsung; Seoul, Korea and Voluson E6-General Electric; Zipf, Austria) equipped with endovaginal probes.
UtA Doppler PI was assessed by transvaginal approach according to the following steps: (i) pregnant women were required to empty their bladder and were positioned in the classical dorsal lithotomy position and (ii) a sagittal view of the uterine cervix was obtained. The probe was sweep laterally until the visualization of paracervical vessels. Color Doppler was activated to identify the UtA at the level of the internal os. UtA was gated at this point just before branching into arcuate arteries. Care has been taken not to insonate the cervicovaginal artery (which runs from cephalad to caudad) or the arcuate arteries. Velocities over 50 cm/s are typical of uterine arteries, which can be used to differentiate this vessel from arcuate arteries (22). Routinely in our center, after angle correction (<30°) when necessary, pulsed Doppler gate was placed over the whole width of the vessel to calculate PI and peak systolic velocity of UtA. When at least three similar consecutive waveforms were obtained, mean PI value of the left and right uterine arteries was calculated (7) (Figure 1).
Data were transferred to an Excel spread sheet (Microsoft Corp.; Redmond, WA, USA) and analyzed by one of the authors (WPM) using PASW program version 18.0 (SPSS Inc.; Chicago, IL, USA) and GraphPad version 5.0 (GraphPad Software; San Diego, CA, USA). Maternal demographic characteristics such as age, height, weight, body mass index (BMI), number of pregnancies, and parity were reported as mean and ranges. Ethnicity, cigarette smoking, and alcohol consumption were reported as percentage. To obtain reference values for mean UtA Doppler PI, a polynomial regression model, as recommended by Altman et al. (23) was used. Regression analysis to obtain the best-fit model polynomial equation for the measurements and their respective standard deviation (SD) values depending on the GA was calculated. Percentiles measurements were calculated

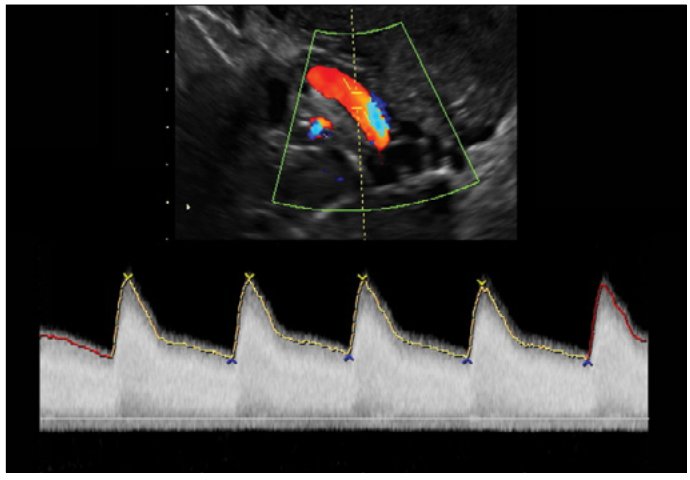

Figure 1. Transvaginal ultrasound showing the uterine artery Doppler
The uterine artery is identified by color Doppler flow mapping and Doppler velocity waveforms. When three similar consecutive waveforms were obtained, the automatic mean pulsatility index (PI) of the left and right uterine arteries was measured, and mean PI Doppler was calculated.

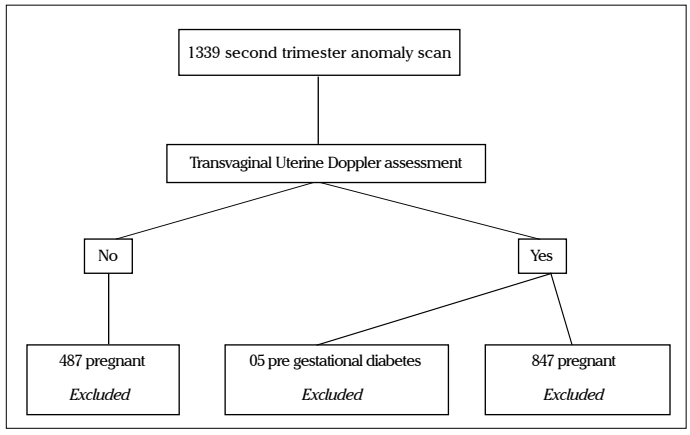

Figure 2. Flow diagram showing patient assessment process

using the following formula: =mean+(SD×K), where K is the corresponding percentile of the standard normal distribution: ±1.65 for the 5th and 95th percentile. The 5th, 50th, and 95th percentiles were determined for each GA (23).

Results

Initially, 1339 pregnant women were selected; however, 492 (36.8%) were excluded because not met the inclusion criteria: five (0.4%) with pregestational diabetes and 487 (36.4%) have declined transvaginal uterine Doppler assessment. This left 847 pregnant women for final statistical analysis (Figure 2). The distribution of pregnant women in each GA period (weeks) was the following: 20–20w+6d (72); 21–21w+6d (204); 22–22w6d (384); 23–23w6d (16), and 24–24w6d (41).

The demographic characteristics of study population are shown in Table 1.

The relationship between mean UtA Doppler PI and GA (weeks) was described by a first-degree polynomial regression by the following formula: mean UtA Doppler PI=1.900−0.038×GA (R^2=0.01). Figure 3 shows the scatterplot of mean UtA Doppler PI as a function of GA (weeks). Table 2 shows the 5th, 50th, and 95th percentiles of mean UtA Doppler PI as a function of GA, from 20 to 24w6d of gestation.

Discussion

This study has established reference ranges for the mean UtA Doppler PI in a low-risk Brazilian pregnant women using transvaginal ultrasound from 20 to 24w6d of gestation.

In a study by Kurdi et al. (16) in 946 women with known outcome measures (PE, birth weight, abruptio placentae, and stillbirth), the presence of bilateral notches at UtA Doppler investigation was associated with an odds ratio (OR) of 12.8 for developing early PE and an OR of 52.6 for PE requiring delivery before 37 weeks of gestation. Opposite, pregnant women with normal UtA Doppler studies had an OR for developing PE of 0.11 (95% confidence interval 0.04–0.28) and 0.3 for delivery of small for gestational age newborns <5th centile. In addition, this study demonstrated that in pregnant women with bilateral notches and mean resistance index (RI) of >0.55, the positive predictive value for the main outcome measures was 46%. Persistence of a diastolic notch (around 24 weeks of gestation) or abnormal flow velocity ratio has been associated with inadequate trophoblast invasion (24).

Cnossen et al. (6), reviewing data regarding 79547 pregnant women with PE and 41131 fetuses with FGR, reported that UtA Doppler ultrasound showed a more accurate prediction when performed in the second than in the first trimester of pregnancy and that an increased PI with notching was the best predictor of PE. Moreover, an increased PI with notching was also the best predictor of overall and severe FGR among low-risk patients. The authors concluded that abnormal UtA waveforms are a better predictor of PE than FGR. PI and RI have been the most commonly used indices; however, large studies on UtA Doppler waveforms during pregnancy have uniformly used PI (13, 17, 18).

Cervical length measurements performed by transvaginal ultrasound at the time of routine second trimester are a recommended method of screening for increased risk of early preterm birth (<32 weeks of gestation). Thus, we evaluated the distribution of UtA Doppler PI measurements using transvaginal ultrasound rather than RI and/or transabdominal approach. PI showed better the velocity waveform, which includes the area below the curve in the formula. Thereat, PI gives detailed

Table 1. Demographic characteristics of study population

	Mean	Min–Max	Percentage
Age (years)	30.6	16.6–44.1	
Height (cm)	168	145–187	
Weight (kg)	76	43–146.2	
BMI (kg/m^2)	28.2	16.3–57.1	
Number of pregnancies	2	1–5	
Parity	1	0–4	
Gestational age (weeks)	22.9	20–24.9	
Ethnicity			82.6 (white)
Smoker			1.4
Alcohol consumption			2.8
BMI: body mass index			

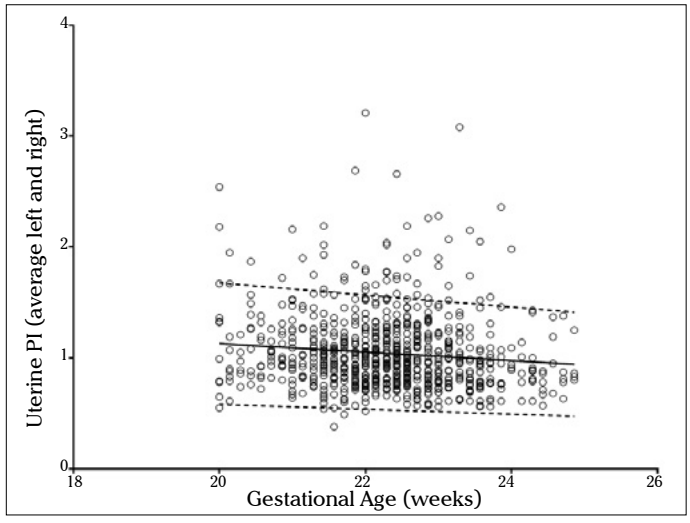

Figure 3. Scatterplot of the mean uterine artery Doppler as function of gestational age (weeks)

Table 2. Estimated 5th, 50th, and 95th percentiles for the mean uterine artery pulsatility index according to gestational age

GA (weeks)	5th	50th	95th
20	0.58	1.14	1.68
21	0.56	1.10	1.62
22	0.54	1.06	1.57
23	0.52	1.03	1.51
24	0.49	0.99	1.46
GA: gestational age			

information about the presence or absence of a protodiastolic notch (7).

Gómez et al. (7), using transvaginal and transabdominal ultrasound examinations from 11 to 41 weeks of gestation, demonstrated a significant decrease in the mean UtA Doppler PI between 11 and 34 weeks, whereas a stable plateau was seen until 41 weeks. Our observation of a significant decrease in the mean UtA Doppler PI from 20 to 24w6d of gestation is in agreement with that reported by Gómez et al. (7), although UtA Doppler PI was transabdominally evaluated in this study (1.10–0.89). The same findings (1.14–0.95) have been confirmed by Ferreira et al. (8) and by our results. Furthermore, our study also confirms previous reports (6, 25-27) indicating that the mean UtA Doppler PI has a significant and progressive decline with advancing GA.

Takahashi et al. (28) have established reference range of mean PI UtA Doppler PI between 16 and 23 weeks of gestation in 1266 singleton Japanese pregnant women. The best-fit curve was a logarithmic one that represented the relationship between mean UtA Doppler PI and GA: $\log_{10}$ mean PI=−0.0211×GA+0.438. Similarly, Bahlmann et al. (29) have determined reference values for blood flow velocity of UtA between 18 and 42 weeks of gestation in 921 singleton low-risk Germany pregnant women. In this study, the reference curve of the mean UtA Doppler PI was characterized by a linear pattern, showing a decrease from 0.89 to 0.65 from 18 through 42 weeks of gestation.

Reference ranges for UtA PI were established using the transabdominal route in other studies (6, 30, 31). Gómez et al. (7) assessed 620 pregnant women in a Spain population, Medina Castro et al. (31) assessed 2081 women in a Mexico population, and Jamal et al. (30) assessed 435 women in an Iran population. The means UtA PI of our study were similar to these studies performed in different ethnic population. Although our study assessed the UtA Doppler by transvaginal route, we believe that our results may be generalized for other ethnic populations.

Few limitations are underlined as follows: (1) this was a cross-sectional, retrospective study, and the postnatal outcomes were not available; pregnant women with BMI >35 kg/m^2 were not excluded, and two different apparatuses were used to perform the ultrasound scans. Nonetheless, cases with PE and FGR (known conditions with elevated midtrimester UtA Doppler PI) are potentially part of the studied population. Intra- and interobserver reproducibility tests were not performed because all ultrasound examinations were carried out only two examiners with full registration by the FMF. In a recent study assessing the intra- and interobserver reproducibility of transabdominal and transvaginal ultrasound in first and second trimesters, both techniques have demonstrated similar reproducibility in the assessing of UtA Doppler PI (8). In addition, when the mean UtA PI Doppler values was measured by transvaginal ultrasound at 20–24w6d of gestation, decrease in UtA Doppler PI values with advancing GA was observed. Moreover, evaluation of UtA Doppler PI can be achieved at the same time when cervical length assessment is carried out to estimate the risk of premature delivery during second trimester scan.

In summary, UtA Doppler PI decreases with advancing GA when the mean UtA Doppler PI is measured at 20–24w6d of gestation by means of transvaginal ultrasound. Finally, reference range for the mean UtA Doppler PI at 20–24w6d of gestation using the transvaginal ultrasound in a low-risk Brazilian population was established. We believe that this reference range may be of clinical value in daily obstetric practice.

Ethics Committee Approval: *Ethics committee approval was received for this study from the Local Ethics committee of University of Uberaba (UNIUBE) (CAAE: 50412115.0.0000.5145).*

Informed Consent: *Consent form was not necessary, because it was a retrospective study.*

Peer-review: *Externally peer-reviewed.*

Author Contributions*: Concept - E.A.J., W.P.M.; Design - A.B.P., W.P.M.; Supervision - E.A.J.; Materials - P.A.M., A.B.P.; Data Collection and/or Processing - A.B.J., T.M.R.C.C. P.A.M.; Analysis and/or Interpretation - W.P.M.; Literature Review - E.A.J., G.T.; Writer - E.A.J., G.T.; Critical Review - G.T.*

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Utility of preoperative neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios to distinguish malignant from benign ovarian masses

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Abstract

Objective: We aimed to investigate the utility of preoperative neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and lymphocyte count as biomarkers to distinguish malignant from benign ovarian masses.

Material and Methods: We retrospectively reviewed the histopathological results of 185 benign and 33 malignant cases following surgery for an initial diagnosis of adnexal mass and confirmed ovarian masses. Age, cancer antigen 125 (CA-125), white blood cell (WBC) count, hemoglobin (Hb), hematocrit (Hct), mean platelet volume (MPV), platelet distribution width (PDW), NLR, PLR, and lymphocyte counts were compared between groups.

Results: The significant diagnostic factors to distinguish malignant from benign disease were age (35.5±22 vs. 62±13 years; p<0.001) and CA-125 levels (16.6±21 vs. 98±366 U/mL; p<0.001). No significant difference was observed in WBC count, Hct, Hb, platelet count, PDW, and MPV between groups. To distinguish malignant from benign masses, lymphocyte count (1.29±0.91 vs. 1.80±0.67×10^3 cells/μL, p<0.001), NLR (4.95±5.36 vs. 3.32±2.72, p=0.024), and PLR (203.41±107.84 vs. 160.75±70.84, p<0.001) were identified as markers. The cutoff values were lymphocyte count of >1500 cells/μL (p<0.001), NLR of 3.4732 (p=0.033), PLR of 161.13 (p<0.001), CA-125 of >40 U/mL (p<0.001), and age of >53 years (p<0.001); their respective sensitivity and specificity were 66.7% and 77.8% [area under the curve (AUC), 0.723±0.055], 68.8% and 54.1% (AUC, 0.624±0.058), 81.8% and 50.8% (AUC, 0.683±0.052), 78.8% and 77.8% (AUC, 0.797±0.057), and 81.8% and 82.2% (AUC, 0.888±0.025). Multiple logistic regression analysis revealed cutoff explanatory and accuracy values of 68.2% and 94.9%, respectively, for lymphocyte count, NLR, PLR, CA-125, and age as independent parameters to distinguish malignant from benign ovarian masses.

Conclusion: In combination with age and CA-125 levels, NLR, PLR, and lymphocyte count may be helpful to preoperatively distinguish malignant from benign ovarian masses. (J Turk Ger Gynecol Assoc 2016; 17: 21-5)

Keywords: Neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, malignant ovarian mass, benign ovarian mass

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Introduction

Ovarian cancer is a gynecological malignancy with the highest cancer-related mortality rate observed among women worldwide (1). Because there are limited sensitive and specific markers for prognosis of ovarian cancer in the early stages of disease and many patients are asymptomatic before diagnosis, most cases are detected in the advanced stages when there are only few treatment options available (2).

When identifying potential adnexal masses, it is challenging to distinguish benign ovarian masses from ovarian cancer before surgery. However, prognosis is excellent in cases where the diagnosis is established in the early stages of the disease incidentally or because of the symptoms presented, and also, the 5-year survival rate can exceed up to 90% (3).

Numerous prognostic factors have been established to effectively estimate patient outcomes. No diagnostic marker superior to cancer antigen 125 (CA-125) has been recognized for ovarian cancer in the past 40 years. Therefore, along with the development of new technologies, identification of new biomarkers to increase the sensitivity of cancer antigen-125 (CA-125) in combination with hematological, inflammatory, or immunologic markers has become necessary (4).

Interactions between tumor cells and host immune system may promote tumor growth and progression. The immune response, which integrates both inflammatory and coagulation processes, plays a critical role in the development and progression of various cancers by upregulating various cytokines and inflammatory mediators, inhibiting apoptosis, inducing angiogenesis, stimulating DNA damage, mediating

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immunosuppression and remodeling the extracellular matrix (5, 6).

The neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) have been gaining attention as systemic inflammatory response markers. They have been successfully applied as predictive markers or prognostic factors in various gynecological cancers (4, 7-10). Although the pathophysiological mechanisms of interactions between inflammation and carcinogenesis are yet to be completely clarified, the identification of new biomarkers with a suspected predictive value for carcinogenesis continues to draw attention. Therefore, we assessed the utility of NLR and PLR as preoperative inflammatory markers, which are cost effective, to better distinguish malignant from benign ovarian masses in the preoperative period.

Here, we mainly aimed to compare patient age with CA-125 levels and with preoperatively defined white blood cell (WBC) count, lymphocyte count, NLR, hemoglobin (Hb), hematocrit (Hct), platelet count, platelet distribution width (PDW), mean platelet volume (MPV), and PLR to identify new biomarkers to distinguish between benign and malignant ovarian masses.

Material and Methods

This retrospective study included 221 patients who underwent surgery for a suspected adnexal mass and had an ovarian mass, as identified by the School of Medicine, Department of Obstetrics and Gynecology, Kahramanmaraş Sütçü İmam University, between April 2007 and April 2015. Histopathological examination revealed that 185 cases were benign (52 endometriomas, 67 mature cystic teratomas, and 66 simple ovarian cysts or serous and mucinous cystadenomas) and 33 were malignant (25 serous cystadenocarcinomas, 5 mucinous cystadenocarcinomas, 1 endometrioid carcinoma, 1 granulosa cell tumor, and 1 theca cell tumor). We excluded three patients in whom borderline ovarian tumors were detected. The remaining 218 patients were divided into two groups, benign and malignant, according to the outcomes of the histopathological examination.

The preoperative complete blood count (CBC) and CA-125 levels were obtained from hospital records. The two groups were compared in terms of age, CA-125 levels, WBC count, Hb, Hct, MPV, PDW, NLR, PLR, and lymphocyte count. Further statistical analyses were performed to identify significant differences between these parameters and determine whether they were effective to distinguish malignant from benign masses. The study protocol was approved by the Science Research Ethics Committee of Kahramanmaraş Sütçü İmam University.

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 22.0 statistical software (IBM-SPSS Inc.; Chicago, IL, USA). The Shapiro–Wilk test was used to define the compliance of data to normal distribution, and Levene's test was used to assess homogeneity of variance. The independent samples t-test with bootstrap results was used to compare two independent groups, whereas the Mann–Whitney U test was used with the Monte Carlo simulation technique. Correlations between classifications, which were separated by the cutoff values of the patient groups calculated according to the variables and the actual classification, were expressed as sensitivity and specificity using the receiver operating characteristic (ROC) curve analysis. Logistic regression analysis was used to identify cause–effect relationships between the categorical response variables with explanatory variables in binomial and multinomial categories. Quantitative data were expressed as means±standard deviations, whereas categorical data were expressed as numbers (n) and percentages (%). A p value of <0.05 was considered statistically significant.

Results

Patients with benign ovarian masses were significantly younger than those with malignant ovarian masses ($p<0.001$). Similarly, the CA-125 levels were significantly higher in the malignant group than in the benign group ($p<0.001$). No statistically significant difference was observed between groups with respect to WBC count, Hct, Hb, platelet count, PDW, and MPV ($p=0.122$, 0.338, 0.571, 0.327, 0.584, and 0.290, respectively). The mean lymphocyte count was significantly higher in the benign group than in the malignant group ($p<0.001$). The mean NLR was significantly higher in the malignant group than in the benign group ($p=0.024$). The mean PLR was significantly higher in the malignant group than in the benign group ($p<0.001$; Table 1).

For the ROC curve analysis, the cutoff values were calculated for lymphocyte count (1500 cells/μL, $p<0.001$), NLR (3.4732, $p=0.033$), PLR (161.13, $p<0.001$), CA-125 (40 U/mL, $p<0.001$), and age (53 years, $p<0.001$; Table 2). The lymphocyte count below the cutoff value had 66.7% sensitivity and 77.9% specificity for the diagnosis of a malignant ovarian mass. NLR above the cutoff value had a sensitivity of 68.8% and specificity of 54.1% for the diagnosis of a malignant ovarian mass. PLR showed 81.8%

Table 1. Comparison of blood components between groups

	Benign ovarian masses (n=185)	Malignant ovarian masses (n=33)	p
Age (years)	35.50±22.00	62.00±13.00	<0.001
Lymphocyte ($\times10^3$ cells/μL)	1.80±0.67	1.29±0.91	<0.001
NLR	3.32±2.72	4.95±5.36	0.024
Hb (g/dL)	12.00±1.80	11.40±1.70	0.571
Hct (%)	36.00±5.00	33.10±8.10	0.338
Plt ($\times$103/μL)	307.00±67.00	297.00±90.00	0.327
PDW	49.00±7.10	50.00±7.90	0.584
MPV (fL)	9.00±1.20	9.00±0.80	0.290
PLR	160.75±70.84	203.41±107.84	0.001
CA125 (U/mL)	16.60±21.00	98.00±366.00	<0.001
WBC ($\times10^3$ cells/μL)	8.44±1.96	9.00±3.42	0.122

WBC: white blood cells; NLR: neutrophil-to-lymphocyte ratio; Hb: hemoglobin; Plt: platelet; PDW: platelet distribution width; MPV: mean platelet volume; PLR: platelet-to-lymphocyte ratio; Hct: hematocrit; CA: cancer antigen
Mann–Whitney U test (Monte Carlo) and Independent t-test (Bootstrap). All data are expressed as means±standard deviations.

Table 2. Sensitivity and specificity of lymphocyte count, neutrophil-to-lymphocyte ratio, neutrophil-to-lymphocyte ratio, cancer antigen 125, and age parameters in benign–malignant distinction of ovarian masses at cutoff values defined by receiver operating characteristic curve analysis

	Benign ovarian masses (n=185)	Malignant ovarian masses (n=33)	AUC±SE	p
Lymphocyte >1500 cells/μL	144 (77.8)**	11 (33.3)	0.723±0.055	<0.001
Lymphocyte ≤1500 cells/μL	41 (22.2)	22 (66.7)*		
NLR ≤3.4732	100 (54.1)**	10 (31.3)	0.624±0.058	0.033
NLR >3.4732	85 (45.9)	22 (68.8)*		
PLR ≤161.13	94 (50.8)**	6 (18.2)	0.683±0.052	<0.001
PLR >161.13	91 (49.2)	27 (81.8)*		
CA-125 ≤40 U/mL	144 (77.8)**	7 (21.2)	0.797±0.057	<0.001
CA-125 >40 U/mL	41 (22.2)	26 (78.8)*		
Age ≤53 years	152 (82.2)**	6 (18.2)	0.888±0.025	<0.001
Age >53 years	33 (17.8)	27 (81.8)*		

ROC Analysis (Honley and McNell - Youden index J)
Sensitivity*, Specificity**
AUC: area under the ROC curve; NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio; SE: standard error; CA: cancer antigen

Table 3. Significance of lymphocyte count, neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, cancer antigen 125, and age as independent variables

Independent Variables	B±SE	p	Odds ratio (95% CI)
Lymphocyte >1500 cells/μL	-2.52±0.88	0.111	12.37 (2.20–69.64)
NLR >3.4732	-1.39±0.87	0.004	4.01 (0.73–22.06)
PLR (>161.13)	-0.54±0.84	0.519	1.72 (0.33–8.94)
CA-125 >40 U/mL	3.82±0.79	<0.001	45.51 (9.60–215.72)
Age >53 years	4.05±0.80	<0.001	57.30 (12.03–272.95)
Constant	-3.26±1.03	<0.001	
Dependent Variable Groups	Nagelkerke R^2=0.682	Predicted (%) =94.9	p <0.001

Multiple logistic regression
B: set of coefficients estimated for the model; CI: confidence interval; NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio; SE: standard error

sensitivity and 50.0% specificity at values above the cutoff for the diagnosis of a malignant ovarian mass. For CA-125, levels higher than the cutoff value had 78.8% sensitivity and 77.8% specificity for the diagnosis of a malignant ovarian mass. Finally, age greater than the optimal cutoff value (53 years) had 81.8% sensitivity and 82.2% specificity for the diagnosis of a malignant ovarian mass (Table 2). Lymphocyte count, NLR, PLR, CA-125 levels, and age were identified as independent variables and further assessed using multiple logistic regression analysis to distinguish malignant from benign ovarian masses (Table 3). Because the created model had an explanatory value of 68.2% and considering the cutoff values, the accuracy to distinguish malignant from benign ovarian masses using these markers was 94.9%.

Discussion

Because the majority of pelvic masses are benign and only ~20% are malignant, the identification of novel markers for use in the preoperative period to determine whether suspected adnexal masses are malignant or benign has become necessary (4, 11). In this study, we observed that age and CA-125 levels were higher in the malignant group. In addition, we observed that NLR, PLR, and lymphocyte count were significantly higher in the malignant group than in the benign group. The effectiveness of these five independent parameters to distinguish ovarian malignancy from benign masses reached 94.9%.

Inflammation contributes to the development and progression of various cancers. The wide intracellular array of signaling pathways is often deregulated during inflammation, thereby resulting in malignant transformation through genomic instability induction, DNA damage, and cell proliferation and angiogenesis promotion (12). Furthermore, inflammatory mediators located in the tumor microenvironment, including cytokines and interleukins, are associated with chemoresistance in various types of tumors, including ovarian cancer (13). Consequently, the interest in blood parameters such as NLR and PLR, which is based on neutrophil, lymphocyte, and platelet counts obtained from CBC in the peripheral blood, has increased. CBC is a basic preoperative laboratory test to evaluate the concentrations of blood components in epithelial ovarian cancers (8, 10, 14). In a study of 136 patients, Bishara et al. (15) studied whether pre-treatment WBC subtypes are prognostic markers in the follow-up course in epithelial ovarian cancers and observed a correlation between low lymphocyte fractions and mortality and also between high numbers of monocytes and recurrence. In a study by Yildirim et al. (16), the neutrophil and platelet counts were higher and lymphocyte counts were lower in patients with malignant tumors than in those with benign

tumors. In vivo and in vitro studies have suggested that various platelet mechanisms play important roles in the progression of ovarian cancers. In one such study, tumor-related increases in interleukin-6 levels induced hepatic thrombopoietin expression; thus, thrombocytosis may support tumor growth (17). In addition, the direct proliferative effect of platelet count on cancer cells, independent from direct contact, decreased, whereas proliferation indices were increased by anti-platelet TGF-β1 blockage in ovarian cancer cells following platelet infusion (18). NLR has been established as a prognostic marker of host inflammation. Cho et al. (4) reported that NLR was greater in patients with ovarian cancer than in those with benign gynecological growths or healthy controls. Nevertheless, the sensitivity and specificity of NLR for the identification of ovarian cancer were 55% and 81%, respectively (NLR cutoff, 3.35). NLR was identified as a marker of CA-125-negative cases and to be more sensitive than CA-125 for predicting survival (4). CA-125 levels are directly correlated with increased neutrophil count and decreased lymphocyte count. Therefore, NLR can be a useful pathogenic marker of disease status, and subsets of CA-125 levels and leucocyte counts may be correlated, which may impact the inflammatory response and alter CA-125 levels (7). Among the blood components, the potential mechanism underlying the prognostic value of NLR may be the correlation between high NLR and inflammation. Neutrophilia contributes to malignant progression by releasing both related host cells such as tumor cells and leukocytes and tumoral growth factors (e.g., vascular endothelial growth factor), thereby upregulating the production of inflammatory cytokines and chemokines (19). However, neutrophilia involving immune cells as an inflammatory response against cancer will suppress the cytotoxic activities of lymphocytes and natural killer cells, thereby hindering the immune response (20). NLR reflects these inflammatory changes and therefore may be a useful prognostic marker for cancers with no reliable marker (21). NLR can help predict the prognosis of patients with ovarian cancer. Williams et al. (7) retrospectively evaluated 519 ovarian cancer patients and demonstrated that a high NLR was correlated with disease progression and poor survival in addition to other known risk factors, including familial history. When patients with malignant ovarian masses were compared with those with benign ovarian masses, CA-125 appeared to be the most important screening marker, whereas NLR and neutrophil count could be used alone or in combination with CA-125 (16).

In the present study, we also evaluated PLR as an alternative marker to NLR. PLR and cancer stage and prognosis have been reported to be correlated. PLR of 200 has a good predictive value. In a study, Asher et al. (9) reported the usefulness of NLR and PLR as prognostic markers, as evaluated in 235 ovarian cancer patients. The authors found that age, disease stage, surgical outcome, disease grade, definite neutrophil count, platelet count, NLR (≥4), and PLR (≥300) were significantly correlated with poor survival. Only stage, residual disease, and PLR were found to be independent prognostic factors for survival. Furthermore, the predictive value of PLR in the diagnosis of ovarian neoplasms has been studied. In these studies, an increased PLR was found to be correlated with poor prognosis and decreased survival (9, 10, 22). In studies to identify alternative markers, a comparison between PLR and CA-125 revealed that a significant increase in PLR was correlated with CA-125 levels ($p<0.01$). In addition, among platelet indices, PLR was found to be the only factor useful to discern early from advanced stage disease (23). In a similar study, PLR was found to be more sensitive than CA-125 to distinguish early from advanced stage ovarian cancers (24). When PLR was compared with the other blood components, such as platelet count and NLR, PLR was found to be a better prognostic marker. In addition, PLR and poor survival of patients with advanced disease are markedly correlated (9, 22, 25).

A possible limitation to this study is that we could not compare parameters according to histological subtypes in benign and malignant masses. However, because the number of the cases in each subtype was relatively small, a statistical significance would have been questionable. Furthermore, we could not assess patients with borderline tumors as a separate group because there were only three such patients. Nonetheless, we excluded these patients from the study because of insufficient histological analyses. Another limitation could be that we analyzed only CA-125 among the other tumor markers. Furthermore, the study was of a retrospective design with a relatively small sample size.

It is important to distinguish malignant from benign masses at any age. Hence, the accuracy of diagnoses can be optimized by the inclusion of additional biomarkers to the imaging modalities. CA-125 alone is insufficient. NLR, PLR, and lymphocyte count in the preoperative period in combination with age and CA-125 may be helpful to distinguish malignant from benign masses.

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

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Changing trends in emergency peripartum hysterectomy in a tertiary obstetric center in Turkey during 2000–2013

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Abstract

Objective: To evaluate emergency peripartum hysterectomy (EPH) cases over a 14-year period in a tertiary center in İstanbul, Turkey.

Material and Methods: In this retrospective descriptive study, the records of all cases of EPH performed at the Zeynep Kamil Women and Children's Training and Research Hospital between January 2000 and January 2014 were analyzed. Results for 2000–2006 and 2007–2013 were compared to identify changing trends. Demographic and clinical factors associated with EPH were assessed.

Results: During the 14-year study period, a total of 161,836 births occurred, out of which 104,783 (64.8%) were vaginal deliveries and 57,053 (35.2%) were cesarean section (CS). EPH was performed in 81 patients with an overall incidence of 0.5 in 1000 deliveries. The EPH rate in 2007–2013 (0.07%) was significantly higher than in 2000–2006 (0.03%). The major difference in the EPH populations between the two periods was the higher number of previous CS in 2007–2013 compared with 2000–2006 (p=0.01). Indications for EPH did not differ between the two periods. There were 7 (8.6%) maternal deaths in 2000–2013, with significantly fewer maternal deaths in 2007–2013 than in 2000–2006 (19.2% vs. 3.6%).

Conclusion: Rate of EPH increased considerably from 2000 to 2013. This increase was mostly related to the increasing rate of CS. Indications for EPH did not change over the study period, and the number of maternal deaths markedly decreased. (J Turk Ger Gynecol Assoc 2016; 17: 26-34)

Keywords: Postpartum hemorrhage, placenta accreta, emergency peripartum hysterectomy, trend

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Introduction

Emergency peripartum hysterectomy (EPH) is performed as a life-saving procedure in cases of intractable obstetric hemorrhage secondary to uterine atony, uterine rupture, placental disorders, fibroids, and lacerations during cesarean section (CS) or vaginal parturition (1). Postpartum hemorrhage is a leading cause of maternal morbidity and mortality (2-4).

The incidence of EPH varies from 0.2–1.6 per 1000 deliveries per year in developed countries, with a higher incidence in developing countries (5-7). Indications for peripartum hysterectomy have changed throughout the years. In developing countries, uterine atony and uterine rupture are the most common indications for EPH, but in developed countries, abnormal placental invasion is the most common indication (1, 7). In particular, the increasing rate of cesarean delivery worldwide has been associated with an increasing rate of placenta previa and accreta (8).

The incidence of EPH reported in previous studies from Turkey varies between 0.25 and 5.3 per 1000 deliveries (9-12). This wide variation is probably a result of differences in population characteristics and the availability of health services among regions in Turkey. During the past decade, the practice of obstetrics has changed and the rate of cesarean delivery has increased nationwide.

The aim of the present study was to review EPHs performed over a 14-year period at a tertiary care center and to determine trends in EPH by comparing two different time periods.

Material and Methods

In this retrospective descriptive study, the records of all cases of EPH performed at Zeynep Kamil Women and Children's Training and Research Hospital, between January 2000 and January 2014, were analyzed. The study was approved by the Institutional Ethics Committee. All participants' rights were protected, and informed consents were obtained according to the Helsinki Declaration.

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Table 1. Maternal and delivery characteristics of cases of emergency peripartum hysterectomy during 2000–2006 and 2007–2013

Characteristics	2000–2006 (n=26)	2007–2013 (n=55)	p
Age (years)	32.8±5.1	33.4±5.6	0.55
18–25	2 (7.7%)	4 (7.3%)	
26–35	17 (65.4%)	35 (63.6%)	
36–40≥	7 (26.9%)	16 (29.1%)	
Gravidity	3.4±1.8	3.5±1.3	0.65
3≥	19 (73.1%)	46 (83.6%)	0.26
Parity	1.9±0.9	2.0±1.1	0.65
0	2 (7.7%)	3 (5.5%)	
1	7 (26.9%)	14 (25.5%)	
2≥	17 (65.4%)	38 (69.1%)	0.74
Prior dilatation and curettage	4 (15.4%)	7 (12.7%)	0.35
Prior abortion	4 (15.4%)	12 (21.8%)	0.29
Previous CS in EPH population	0.9±0.8	1.5±0.9	0.01
1	8 (30.8%)	16 (29.1%)	
2	8 (30.8%)	23 (41.8%)	
3≥	0	7 (8.6%)	
Gestational age (weeks)	33.9±4.7	35.3±4.3	0.37
24–32	9 (34.6%)	13 (23.6%)	
33–37	10 (38.5%)	25 (45.5%)	
38–42	7 (26.9%)	17 (30.9%)	
Birthweight (g)	2571±937	2616±957	0.83
<2500	11 (42.3%)	20 (36.4%)	
2500–3999	13 (50%)	34 (61.8%)	
4000≥	2 (7.7%)	1 (1.8%)	
Fetal position			
Vertex	20 (76.9%)	44 (80%)	
Breech	3 (11.5%)	9 (16.4%)	
Transverse	3 (11.5%)	2 (3.6%)	
Mode of delivery			
Vaginal delivery	3 (11.6 %)	6 (10.9%)	
Vaginal delivery with prior CS	0	1 (1.8%)	
Cesarean delivery	23 (88.4%)	49 (89.1%)	
Cesarean delivery with no prior CS	7 (26.9%)	4 (7.3%)	
Cesarean delivery with prior CS	16 (61.5%)	45 (81.8%)	
Cesarean delivery indications			
Placenta previa and prior CS	11 (47.8%)	33 (60%)	
Placenta previa and no prior CS	2 (8.7%)	0	
Previous CS alone	5 (21.7%)	12 (22%)	
Placental abruption with no previous CS	2 (8.7%)	1 (2%)	
Fetal distress	3 (13%)	1 (2%)	
Cephalopelvic disproportion	0	1 (2%)	
Primigravid breech position	0	1 (2%)	

Data are expressed as mean±SD or n (%).
EPH: emergency peripartum hysterectomy; CS: cesarean section

Table 2. Indications for emergency peripartum hysterectomy during 2000–2006 and 2007–2013

Hysterectomy indications	2000–2006 (n=26)	2007–2013 (n=55)	p
Placenta accreta with placenta previa	11 (42.3%)	27 (49.1%)	NS
Placenta accreta without placenta previa	3 (11.5%)	8 (14.5%)	NS
Uterine atony after CS	5 (19.2%)	3 (5.5%)	NS
Uterine atony after vaginal delivery	2 (7.7%)	5 (9.1%)	NS
Uterine atony due to placental abruption	2 (7.7%)	8 (14.5%)	NS
Uterine atony due to myoma utery	0	1 (1.8%)	NS
Ruptured uterus with placenta previa	2 (7.7%)	3 (5.5%)	NS
Uterine invertion with myoma utery	1 (3.8%)	0	NS

Data are expressed as n (%).
NS: not significant

Table 3. Indications for emergency peripartum hysterectomy during 2000–2006 and 2007–2013

Characteristics	2000–2006 (n=26)	2007–2013 (n=55)	p
Total Hysterectomy	15 (57.7%)	42 (76.4%)	0.04
Measures to prevent hysterectomy			0.67
Curetage and tamponade of the uterus	6 (23.1%)	6 (29.1%)	
Ligation hypogastric arteries	4 (15.4%)	5 (9.1%)	
Ligation uterine arteries	1 (3.8%)	4 (7.3%)	
B-Lynch procedure	0	2 (3.6%)	
Bacri ballon use	0	5 (5.9%)	
Timing of hysterectomy			0.48
Primary cesarean hysterectomy	16 (61.5%)	38 (69.1%)	
Re-laparatomy post CS	7 (26.9%)	11 (20%)	
Laparatomy post vaginal delivery	3 (10.5%)	6 (11%)	
Operating time (min)	114±43	108±39	0.56
Hospitalization (days)	10.4±5.1	8.4±5.1	0.11
Drainage of the abdominal cavity	24 (92.3%)	49 (89.1%)	0.65
Low midline incision	1 (3.8%)	8 (14.5%)	0.02
Need for blood transfusion (units)	6.8±3.7	5.9±2.8	0.21
Placenta pathology			0.24
Accreta	3 (11.5%)	6 (10.9%)	
Increata	7 (26.9%)	15 (27.3%)	
Percreata	4 (15.4%)	16 (29.1%)	0.18
Placenta previa	13 (50%)	33 (60%)	0.40
Totalis	10 (38.5%)	27 (49.1%)	

Data are expressed as mean±SD or n (%).

EPH was defined as an operation performed up to 24 h after delivery to treat hemorrhage that could not be controlled using conservative approaches. EPH was only performed when medical or minor surgical procedures (bimanual uterine compression, administration of oxytocin and prostaglandins, uterine packing, and compression sutures such as the B-Lynch brace suture, etc.) failed to control postpartum hemorrhage.

The records of the 81 women who underwent EPH during the study period were reviewed to determine the following: (i) maternal demographic data (age, gravidity, parity, gestational age at delivery, previous uterine surgery, and a history of previous abortions), (ii) clinical details (mode of delivery, indications and type of hysterectomy, additional procedures, operating time calculated from endotracheal intubation to last skin suture, pre- and postoperative hemoglobin and

Table 4. Indications for emergency peripartum hysterectomy during 2000–2006 and 2007–2013

	Placenta accreta (n=49)	Uterine atony (n=26)	p
Age (years)	33.5±5.6	32.7±5.5	0.55
Parity	1.9±0.9	1.8±1.2	0.30
2≥	36 (73.5%)	14 (53.8%)	0.07
Gestational age (weeks)	34.1±4.4	36±4.2	0.04
Birth weight (g)	2483±952	2796±906	0.17
Need for blood transfusion (units)	5.9±3.2	7.3±3.2	0.09
Operating time (min)	112±40	102±40	0.30
Hospitalization (days)	8.6±5.1	10.2±5.5	0.21
Adnexectomy	4 (8.2%)	1 (3.8%)	0.001
Low midline incision	7 (14.3%)	2 (7.7%)	0.001
Couvelaire uterus	1 (2%)	3 (5.3%)	0.08
Placenta previa			0.001
Totalis	30 (61.2%)	7 (26.9%)	
Partialis	8 (16.3%)	1 (3.8%)	
Uterine rupture	7 (14.3%)	1 (3.8%)	0.16
Prior dilatation and curettage	7 (14.3%)	3 (11.5%)	0.73
Prior abortion	9 (18.4%)	6 (23.1%)	0.63
Mean previous CS number	1.7±0.8	0.7±0.8	0.001
Previous CS in EPH population	47 (95.9%)	13 (50%)	0.001
2≥	32 (65.3%)	5 (19.2%)	0.001
Total hysterectomy	35 (71.4%)	18 (69.2%)	0.84
Maternal complications	23 (46.9%)	21 (80.8%)	0.04
Bladder injury	9 (18.4%)	2 (7.7%)	
Pelvic hematoma	1 (2%)	1 (3.8%)	
Febrile morbidity	6 (12.2%)	3 (11.5%)	
Wound infection	0	3 (11.5%)	
Disseminated intravascular coagulopathy	3 (6.1%)	6 (23.1%)	
Acute renal insufficiency	0	1 (3.8%)	
Partial ureteral obstruction	1 (2%)	0	
Re-exploration after intraabdominal bleeding	1 (2%)	4 (15.4%)	0.03
Pneumonia	1 (2%)	0	
Cardiac ischemia	1 (2%)	1 (3.8%)	
Measures to prevent hysterectomy	16 (32.7%)	16 (61.5%)	0.04
Curettage and tamponade of the uterus	3 (6.1%)	9 (34.6%)	
Ligation hypogastric arteries	6 (12.2%)	2 (7.7%)	
Ligation uterine arteries	3 (6.1%)	2 (7.7%)	
B-Lynch-procedure	2 (4.1%)	0	
Bacri balloon	2 (4.1%)	3 (11.5%)	
Timing of hysterectomy			
Primary cesarean hysterectomy	42 (85.7%)	8 (30.8%)	0.001
Re-laparatomy post CS	6 (12.2%)	12 (46.2%)	
Post vaginal delivery	1 (2%)	6 (23.1%)	
Neonatal death	3 (6.1%)	6 (23.1%)	0.03
Maternal death	5 (10.2%)	2 (7.7%)	0.70
Intensive care unit admission	6 (12.2%)	9 (34.6%)	0.02

Data are expressed as mean±SD or n (%).
EPH: emergency peripartum hysterectomy; CS: cesarean section

Table 5. Maternal and clinical characteristics of total and subtotal emergency peripartum hysterectomy

	Total (n=57)	Subtotal (n=24)	p
Age (years)	32.7±4.7	34.1±6.7	0.30
Gravidity (mean)	3.5±1.5	3.2±1.7	0.49
Parity (mean)	1.9±0.8	2.1±1.3	0.50
Primipar	1 (1.8%)	4 (16.7%)	
Gestational age (weeks)	34.9±4.6	34.7±4.3	0.85
Birth weight (g)	2576±954	2636±930	0.79
Placenta previa totalis	27 (47.4%)	10 (41.7%)	0.43
Previous cesarean delivery	45 (78.9%)	17 (70.8%)	0.11
Previous cesarean number (mean)	1.3±0.9	1.2±0.9	0.66
Cesarean delivery	48 (84.2%)	24 (100%)	0.04
Hysterectomy indication			
Placenta acreata	35 (61.5%)	14 (58.3%)	
Uterine atony	18 (31.6%)	8 (33.3%)	
Uterine rupture	3 (5.3%)	2 (8.3%)	
Uterine invertion	1 (1.8%)	0	
Anormal uterine insertion			
Placenta acreata	5 (8.8%)	4 (16.7%)	
Placenta increata	15 (26.3%)	7 (29.2%)	
Placenta percreta	16 (28.1%)	4 (16.7%)	0.28
Hemoglobin (g/dL)			
Preoperative	11.3±1.3	11.6±1.1	0.32
Postoperative	7.6±1.7	8.5±1.9	0.03
Haematocrit (%)			
Preoperative	33.4±3.6	34.5±3.1	0.17
Postoperative	23±5.1	25.4±5.2	0.05
Timing of hysterectomy			0.51
Primary cesarean hysterectomy	37 (64.9%)	17 (70.8%)	
Re-laparotomy post cesarean section	11 (19.3%)	7 (29.2%)	
Post vaginal delivery	9 (15.8%)	0	
Maternal complications			
Bladder injury	8 (14%)	3 (12.5%)	
Pelvic hematoma	2 (3.5%)	0	
Febrile morbidity	4 (7%)	5 (20.8%)	
Wound infection	2 (3.5%)	1 (4.2%)	
Disseminated intravascular coagulopathy	8 (14%)	2 (8.3%)	0.87
Acute renal insufficiency	1 (1.8%)	0	
Partial ureteral obstruction	2 (3.5%)	0	
Re-exploration after intraabdominal bleeding	4 (7%)	1 (4.2%)	0.56
Pneumonia	0	1 (4.2%)	
Cardiac ischemia	2 (3.5%)	0	
Need for blood transfusion (units)	6.4±3.3	6.1±3.3	0.71
Operating time (min)	111±38	105±46	0.54
Hospitalization (days)	8.7±5.1	9.9±5.2	0.36
Adnexectomy	3 (5.3%)	3 (12.5%)	0.26
Low midline incision	7 (12.3%)	2 (8.3%)	0.61
Drainage with drains of the abdominal cavity	50 (87.7%)	23 (95.8%)	0.26
Intensive care unit admission	11 (19.3%)	5 (20.8%)	0.87

Data are expressed as mean±SD or n (%).

Table 6. Maternal and fetal complications during 2000–2006 and 2007–2013

Complications	2000–2006 (n=26)	2007–2013 (n=55)	p
Maternal complications	15 (57.7%)	31 (56.3%)	0.79
Bladder injury	4 (15.4%)	7 (12.7%)	
Pelvic hematoma	1 (3.8%)	1 (1.8%)	
Febrile morbidity	3 (11.5%)	6 (10.9%)	
Wound infection	0	3 (5.5%)	
Disseminated intravascular coagulopathy	4 (10.9%)	6 (15.4%)	0.26
Adnexectomy	2 (7.7%)	4 (7.3%)	
Acute renal insufficiency	0	1 (1.8%)	
Partial ureteral obstruction	0	2 (3.6%)	
Re-exploration after intraabdominal bleeding	2 (7.7%)	3 (5.5%)	0.16
Pneumonia	1 (3.8%)	0	
Cardiac ischemia	0	2 (3.6%)	
Intensive care admission	7 (26.9%)	9 (16.4%)	0.26
Maternal death	5 (19.2%)	2 (3.6%)	0.02
Neonatal death	3 (11.5%)	7 (12.5%)	0.88

Data are expressed as n (%).

Table 7. Risk factors in patients who had EPH due to placenta accreta

	OR (95% CI)[a]	p
Previous cesarean delivery count	3.6 (1.6–8.2)	0.001
Gestational age (weeks)	0.8 (0.7-0.9)	0.03
Maternal age	1.0 (0.9-1.1)	0.72
Placenta previa	3.5 (1.0-12.1)	0.04
Parity	0.9 (0.4-1.9)	0.92

EPH: emergency peripartum hysterectomy; OR: odds ratio; CI: confidence interval
[a]Adjusted for parity, maternal age, gestational age, placenta previa, previous cesarean delivery count

hematocrit, need for transfusion, need for re-operation, postoperative complications, postoperative conditions, postoperative duration of hospitalization, and peripartum maternal and fetal morbidity and mortality), and (iii) associated risk factors (previous cesarean delivery, number of previous cesarean sections, current placenta previa, mode of delivery, and birth weight).

Primary indications for EPH were recorded under the following titles: uterine rupture, uterine atony, placenta accreta (isolated accreta or previa accreta), and uterine inversion. Operative notes and reports on the gross pathology of the uterus and placenta were used to determine the indication for hysterectomy. Women who delivered before 24 weeks of gestation and women who had a hysterectomy for other reasons, such as sterilization or cancer, were excluded.

To determine changing trends in EPH over the 14 years of the study period, records for 2000–2006 and 2007–2013 were compared. The records were also compared according to hysterectomy type (subtotal or total) and the indication for EPH (placenta accreta or uterine atony).

Statistical analyses were performed using SPSS version 17.0 (IBM; Armonk, NY, USA). Normal distribution of continuous variable was assessed using the Kolmogorov–Smirnov test; chi-square analysis was used for categorical variables. Student *t*-test was used for the analysis of normally distributed continuous variables. And for non-normally distributed variables, Mann–Whitney *U* test was used. A multivariate analysis was conducted for each outcome using binary logistic regression (backward likelihood ratio method). Adjusted odds ratios (ORs) with 95% confidence intervals (CIs) were calculated. A *p*-value of <0.05 was considered to indicate statistical significance.

Results

During the 14 years of the study period, a total of 161,836 births occurred, out of which 104,783 (64.8%) were vaginal deliveries and 57,053 (35.2%) were CS. EPH was performed in 81 patients, with an overall incidence of 0.5 per 1000 deliveries. Of the 81 EPHs, 9 were performed after vaginal deliveries (0.09 per 1000 deliveries), and 72 were performed after CS (1.3 per 1000 deliveries). The rate of EPH was significantly higher in 2007–2013 (0.07%) than in 2000–2006 (0.03%).

The maternal and delivery characteristics of women who underwent EPH during 2000–2006 and 2007–2013 are presented in Table 1. The mean maternal age was not statistically different between the earlier and later period (32.8±5.1 vs. 33.4±5.6 years). Gravidity (3.4±1.8 vs. 3.5±1.3), parity (1.9±0.9 vs. 2.0±1.1), gestational age (33.9±4.7 vs. 35.3±4.3), and birthweight (2571±937 vs. 2616±957 g) were not statistically different between the two time periods. For women who required EPH, the ratio of vaginal to cesarean delivery was similar in the earlier and later periods (11.6% to 88.4% vs. 10.9% to 89.1%). The major difference between the two time periods was the number of previous CS in the EPH population (0.9±0.8 vs. 1.5±0.9, p=0.01).

The indications for hysterectomy during 2000–2006 and 2007–2013 are presented in Table 2. Overall, the most common indications for EPH were placenta accreta (60.5%), uterine atony (32.1%), uterine rupture (6.2%), and uterine inversion (1.2%). The indications did not differ between the two time periods.

The procedural characteristics of EPH cases during 2000–2006 and 2007–2013 are presented in Table 3. The mean operating time (114±43 vs. 108±39 min) and the mean duration of hospitalization (10.4±5.1 vs. 8.4±5.1 days) were similar for the two 7-year periods. All women received blood transfusions. The median number of units of transfused blood was 6 (range, 1–20). Ten patients (12.3%) needed ≥10 units. Compared with 2000–2006, during 2007–2013, the rate of total hysterectomy (57.7% vs. 76.4%) and the rate of low middle incision (3.8% vs. 14.5%) increased.

Maternal and delivery characteristics for women who underwent EPH for placenta accreta and uterine atony are presented in Table 4. Of women who required EPH for placenta accreta, 98% had a cesarean delivery, compared with 76% of women who required EPH for uterine atony ($p<0.01$). Placenta accreta was significantly more likely to be the indication for EPH than uterine atony when the number of previous CS was high ($p<0.01$).

The maternal and clinical characteristics for cases of total and subtotal EPH are presented in Table 5. Postoperative hemoglobin levels were significantly lower after total hysterectomy than after subtotal hysterectomy ($p=0.03$).

Maternal and fetal complications during 2000–2006 and 2007–2013 are presented in Table 6. Operative and postoperative complications did not differ in two periods. Bladder injury was the most common operative complication in both periods (15.4% and 12.7%, respectively). There were 10 (12.3%) neonatal deaths during the 14-year study period. The neonatal mortality rate was similar for the two time periods. There were 7 (8.6%) maternal deaths during the 14-year study period. The number of maternal deaths was significantly lower during the second 7-year period (19.2% vs. 3.6%).

Associated risk factors in women who underwent EPH for placenta accreta are presented in Table 7. The number of previous cesarean deliveries was an important risk factor for EPH for placenta accreta (OR 3.6, 95% CI 1.6–8.2). Gestational age and placenta previa were other important risk factors (OR 0.8, 95% CI 0.7–0.9 and OR 3.5, 95% CI 1.0–12.1, respectively).

Discussion

There is considerable variability in the incidence of EPH among countries and institutions. The overall incidence of EPH at our hospital was 0.5 per 1000 deliveries. In the literature, reported incidence rates are 0.33 per 1000 deliveries in the Netherlands (1), 0.41 per 1000 in the USA (5), and 4 per 1000 in Pakistan (7). The incidence of EPH at our hospital was consistent with the incidence reported in developed countries. However, there is a wide variation in the incidence of EPH in different regions of Turkey and for different study periods (0.25–5.3 per 1000 deliveries) (9-12).

In the present study, the incidence of EPH was 0.09 per 1000 after vaginal deliveries and 1.3 per 1000 after CS. Women who have had a previous CS have an increased risk of EPH compared with women with previous vaginal deliveries (6, 8, 13, 14). An increase in the number of cesarean sections has given rise to an increase in abnormal placentation, placenta previa, and scarred uterus (1, 5, 8, 9, 13, 15). Our study showed that the overall rate of CS and the incidence of EPH were higher in our hospital during the second 7 years of the study period compared with the first 7 years. The findings of a previous study (14) at our hospital showed that, during 1990–2003, the incidence of EPH was 0.02% and overall CS rate was 17%. Compared with 1990–2003, the incidence of EPH during 2007–2013 was 3.5 times higher, and the overall rate of CS was 2.5 times higher. This observation is alarming and reflects the rapidly increasing rate of cesarean section in Turkey. However, the magnitude of this increase at our hospital could be exaggerated because it is a referral center that accepts many high-risk obstetric patients. Therefore, our hospital has a high overall rate of CS, repeat CS delivery, and a high rate of placenta percreta. In other countries, Parazzini et al. (16) and Bateman et al. (5) reported that EPH incidence had increased by the time, but Flood et al. (6) reported a decrease in EPH incidence due to their lower CS rates and higher vaginal birth after CS rate.

In our study, placenta accreta was the most common indication (60.5%) for EPH performed during the 14-year study period. The percentage did not differ between the earlier and later 7-year periods, although the overall rate of CS was higher in the later period. However, a previous study at our hospital found that, during 1990–2003, only 10% of EPH was performed for placenta accreta (14). Therefore, there was approximately a six-fold increase in EPH for placenta accreta from the years 1990–2003 to 2007–2013.

Uterine atony was the second most common indication (32.1%) for EPH in our study, and the rate was similar for the earlier and later 7-year periods (34.6% vs. 30.9%). However, compared with the findings of the 1990–2003 study (14), which reported uterine atony as the indication for EPH in 62.7% of cases, there was a marked decrease in the 14 years of the present study.

Uterine rupture is a complication of a prolonged obstructed labor. Although it is the most common indication for EPH in developing countries (7), in our study, it was the third most frequent indication (6.2%). This result agrees with recent reports by Kwee et al. (1) and Flood et al. (6), which showed a decreasing incidence of uterine rupture as an indication for EPH (8.3% and 9%, respectively). The incidence of EPH for uterine rupture was similar for the two 7-year periods of the present study (7.7% vs. 5.5%). Rates of EPH for uterine rupture varied widely among studies from different parts of Turkey, ranging from 9% to 35% (14, 15, 17, 18).

In the emergency postpartum setting, total hysterectomy was the most common (70.4%) surgical procedure in our study. This finding was consistent with the findings of other studies (6, 8, 15). In the previous study at our hospital, Ozden et al. (14) found that total hysterectomy was performed in 42% of EPH. In the present study, during 2007–2013, total hysterectomy was the preferred procedure for EPH, although subtotal hysterectomy is reported to be technically easier with shorter operating time, less blood loss, shorter duration of hospitalization, and lower

morbidity (9, 19). However, we did not observe any significant difference in operating time, the need for blood transfusion, operative complications, or duration of hospital stay between total and subtotal hysterectomy groups. Only postoperative hemoglobin levels were significantly different. In cases of placenta accreta, where there is a high risk of low segment bleeding from the cervical branch of uterine artery, total hysterectomy is preferable to subtotal hysterectomy. Total hysterectomy should be considered when there is active bleeding from the lower uterine segment or cervix. However, the skill and experience of the surgeon should determine the method of choice. In a study, the rate of surgical injury of the ureter was higher in total hysterectomy (17).

In the present study, the complication rate did not differ between the two 7-year periods. The incidence of bladder injury was 13.6%, which lies within the range of 4%–15% reported in other studies (9, 12, 14, 18). Vesicouterine scars caused by previous CS increase the risk of bladder injury (11). In our study, there was a higher rate of bladder injury in women who had EPH for placenta accreta than uterine atony but did not differ between total and subtotal hysterectomy cases, although some studies have reported a higher rate of bladder injury after total hysterectomy and placenta accreta (10, 18).

In the present study, 12.3% of patients developed disseminated intravascular coagulation (DIC). Although maternal care and intensive care unit facilities improved over the study period, the rate of DIC was similar for the earlier and later 7-year periods (10.9% vs. 15.4%). Because our hospital is a tertiary health center, women referred for emergency treatment are often hemodynamically instable on admission. The rate of DIC was higher in women who underwent EPH for uterine atony than for placenta accreta (23.1% vs. 6.1%).

Re-exploration after EPH and re-laparotomy EPH after cesarean section were associated with an increased bleeding rate. In nonobstetric hysterectomy, re-exploration is required in only 0.5% of cases (17). In our study, 6.2% women underwent re-exploration after EPH for persistent bleeding. In other studies, this rate varied from 4% to 25% (10, 12, 14, 18). In the present study, EPH for uterine atony had a higher rate than EPH for placenta accreta (15.4% vs. 2%). Re-exploration was more likely after total hysterectomies compared with subtotal hysterectomies, but the difference was not significant (7% vs.4.2%). Wright et al. (18) and Gungorduk et al. (9) reported a higher rate of re-exploration after subtotal hysterectomies, but Ozden et al. (14) reported a higher rate after total hysterectomies.

Re-laparotomy for bleeding and sepsis after CS is associated with a high mortality. In our study, 22.2% women required re-laparotomy after CS, but the rate decreased from the earlier to the later 7-year period. Women who had EPH for uterine atony had a higher rate of re-laparotomy compared with women who had EPH for placenta accreta. Compared with women who did not require re-laparotomy, women who had re-laparotomy after EPH had a higher rate of surgical complications (46% vs. 94%), DIC (4.8% vs. 38.7%), and admission to the intensive care unit (11% vs 50%). Seffah (20) reported a rate of re-laparotomy after CS 0.7%, and the most frequent indication was bleeding secondary to uterine atony.

The strength of our study is that it brings a comprehensive overview on EPH with 14-years following period and it compares between the earlier and later 7-year periods, which gives valuable information on trends in EPH in the largest tertiary referral obstetrics center in Istanbul. Major limitation of our study is its retrospective design, so the cause and effect relationship cannot be established.

In conclusion, our results demonstrated an increasing trend in the rate of EPH in parallel with an increasing rate of repeat CS, emphasizing the importance of the mode of delivery. Cesarean deliveries lead to repeat CS, which increases the incidence of abnormal placentation and the risk of EPH. Because EPH is associated with significant morbidity and mortality, to prevent repeat CS, vaginal delivery may be advised after CS deliveries. Although the indications for EPH did not change over the 14-year study period, the maternal death rate decreased as a result of improvements in health care.

Ethics Committee Approval: *Ethics committee approval was received for this study from the ethics committee of Zeynep Kamil Women and Children's Training and Research Hospital*

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

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In vitro chemosensitivity in ovarian carcinoma: Comparison of three leading assays

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Abstract

Objective: An alternative approach to the current therapy of ovarian carcinoma is the individualization of treatment by determining the sensitivity of tumoral tissue to chemotherapeutic agents before the initiation of chemotherapy. The objectives of the study are to determine the efficacy of *in vitro* chemosensitivity assays in ovarian carcinoma and to measure the correlation of three leading assays.

Material and Methods: Fresh tumoral tissue samples of 26 newly diagnosed primary ovarian cancer patients were studied with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolyum bromide (MTT) assay, adenosine triphosphate-tumor chemosensitivity assay (ATP-TCA) and differential staining cytotoxicity (DISC) assays. Chemosensitivity of tumors were studied for paclitaxel, carboplatin, docetaxel, topotecan, gemcitabine, and doxorubicin with each of the three assays. Subgroup analysis was performed for stage, grade, and histologic type.

Results: The *in vitro* chemosensitivity results of MTT, ATP, and DISC assays were found to be similar. The subgroups in which *in vitro* assays would be more useful were encountered for patients with advanced stage and serous histology ovarian carcinoma.

Conclusions: *In vitro* chemosensitivity can be determined in ovarian carcinoma with ATP, MTT, or DISC assays before the initiation of chemotherapy. These three assays correlate well with each other and are particularly useful for serous and advanced cancers. Large prospective studies comparing standard versus assay-directed therapy with an endpoint of overall survival are required before routine clinical utilization of these assays. (J Turk Ger Gynecol Assoc 2016; 17: 35-40)

Keywords: Ovarian cancer, *in vitro* chemosensitivity, ATP, MTT, DISC

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Introduction

Ovarian cancer is still one of the most difficult cancers to combat, and the mortality rate is high (1). Epithelial histologic type constitutes 90% of all ovarian cancer cases (2). Because there is no effective screening test for early detection, three-fourths of cases are still in the advanced stage at presentation. The standard therapy basically consists of surgical resection of the tumor mass followed by chemotherapy that targets residual disease. Except for early-stage and well-differentiated tumors, all patients receive a first-line adjuvant chemotherapy regimen including carboplatin and paclitaxel (3, 4). Surgery followed by combination chemotherapy, which is based on evidence from previous clinical trials, reveals a response rate of approximately 70–80% (5). However, most patients will have a relapse in the follow-up, and only-one third will survive after 5 years (6). As the disease recurs, there is no standard for therapy and no single treatment regimen has significant impact on overall survival (OS) (7).

All the patients with ovarian carcinoma may not have equal response to the standard chemotherapeutic regimen, although they have the same histologic type of tumor. The main factor causing this difference is thought to be the heterogeneity of the tumor tissue. Thus, histologic assessment yet does not provide data for the chemotherapeutic response. Furthermore, ploidy analyses, assessment of the proliferation rate of tumor cells, or determination of oncogens does not have a reliable correlation with the chemotherapeutic response. The sensitivity of a tumor to a chemotherapy regimen may only be determined after the completion of several cycles of cytotoxic drugs. An alternative approach to current therapy is the individualization of treatment by determination of the sensitivity of tumor tissue to chemotherapeutic agents before the initiation of chemotherapy (8). To date, chemosensitivity comes around by the need for selection of sensible

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agents to the tumor and for the application of most efficient chemotherapeutic drugs.

The individualized chemotherapy methods are the area of research since the pioneering studies of Black and Speer in 1950s (9). The chemosensitivity tests allow detecting the cytotoxic, cytostatic, and apoptotic effects of the chemotherapeutic agent outside the organism. Such an approach is particularly thought to prevent the harmful toxic effects of these agents. Many molecular and cellular assays have been developed to date for the *in vitro* detection of chemosensitivity. The molecular methods detect the chemosensitivity at the protein or gene level (10, 11). Although a single gene may be sufficient for the evaluation, a cytotoxic drug sometimes generates an excessive cellular response (12, 13). Cellular methods provide the efficacy results for multiple drugs simultaneously. Several tests based on cellular methods, including 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolyum bromide (MTT) chemosensitivity assay, adenosine triphosphate-tumor chemosensitivity assay (ATP-TCA), and differential staining cytotoxicity (DISC) assays, have been described in the literature.

Despite the logic that any method that predicts the chemosensitivity of a tumor for an individual patient would be helpful for better regimens, no chemosensitivity assay has achieved widespread clinical use to date. In a study from USA with 262 patients, all the patients were treated empirically, but a chemosensitivity assay was also performed concomitantly for all. In addition, in the subgroup of patients treated with assay-sensitive agents, progression-free survival (PFS) and OS are found to be improved, and further analysis has confirmed these results (14, 15). Recently, an observational study has also evaluated chemosensitivity profiles of type 1 and type 2 epithelial ovarian cancer (EOC) (16). There is a dearth of large randomized prospective trials for ovarian cancer evaluating the survival of patients treated by empirically decided therapy versus selected chemotherapeutic regimen based on the *in vitro* chemosensitivity assay results (assay-directed therapy).

Furthermore, no study has compared the *in vitro* chemosensitivity assays MTT, ATP-TCA, and DISC in the same fresh ovarian tumor samples. Thus, we aimed to determine the clinical consistency of three abovementioned leading *in vitro* chemosensitivity assays using a series of EOC tumors and discuss the feasibility of individualized chemotherapeutic treatment in the future.

Material and Methods

In total, 26 patients with EOC diagnosed from January 2011 to April 2012 at the Gynecologic Oncology Unit, Department of Obstetrics and Gynecology, Hacettepe University School of Medicine, Ankara, Turkey are included in this study. All patients were operated for pelvic mass and underwent to cytoreductive surgery after the frozen section confirmed epithelial ovarian carcinoma.

After frozen section evaluation of the tumor, approximately 2 cm^3 of fresh tumor tissue was obtained for *in vitro* chemosensitivity testing. The tumor sample was transported in a plastic bottle containing liquid composed of carbon dioxide-free medium, horse serum, calf serum, penicillin–streptomycin–glutamin combination, gentamycin, and amfotericin B. The *in vitro* chemosensitivity assays were performed by the co-author at Onkosel Biotechnology Company, Hacettepe Technopolis, Ankara, Turkey.

The chemosensitivity results were obtained for each patient with regard to three different assays (MTT, ATP, and DISC assays) and six different drugs (paclitaxel, carboplatin, docetaxel, topotecan, gemcitabine, and doxorubicin). Results were categorized according to the percentage of cell death after the administration of chemotherapeutic agent to the cell cultures. For the DISC assay, LC_{90}, which represents the dose which causes 90% loss of viability in cell population, constitutes the control. For ATP and MTT tests, two controls, including the 100% cell death after high-dose fluorouracil application group and no drug added, i.e., the 0% cell death group, were used. The remaining viable cell percentage in comparison with that in the control group without any drugs added (0% cell death) for each specimen was categorized as sensitive, moderately sensitive, resistant, and extremely resistant (<30%, 30%–49%, 50%–90%, and >90% remaining viable cells in comparison with those in the control group, respectively).

ATP assay

For the ATP assay, chemosensitivity was evaluated with the ATP-TCA kit (TCA-100; Innovative Diagnostik Systeme (DCS), Hamburg, Germany). Tumor cells acquired from tumoral tissue disintegrated first mechanically and then by enzymatic dissociation (Collagenase Worthington Type CLS III, Biochrom; Berlin, Germany). A single cell suspension was then acquired, and dissociated cells were counted to have at least 7500 viable cells. After seeding in a 96-well microplate, cells were incubated for 5 days at 37°C and 5% carbon dioxide and then treated with different drugs to be tested. In each plate, there were two types of control: a control without any drug added and a control of maximum inhibitor, which kills all the cells. After the incubation period, lysis of the surviving cells was performed by adding tumor cell extraction reagent (DCS Innovative Diagnostik Systeme). A sample of the suspension from each well was added to corresponding wells in another 96-well microplate. A luciferin–luciferase reagent was added, and the illumination level corresponding to ATP level was measured using a luminometer (Berthold; Hamburg, Germany) and analyzed. Luminescence is correlated with ATP levels, and this reflects the inhibition in comparison with that of untreated controls included in each plate and reported as “percent inhibition.”

MTT assay

For the MTT assay, tumor samples were cut into small pieces of 5×5 mm. The tumor in the transport medium was further lysed mechanically and was put over 5 mL of solution (Ficoll 400, Telebrix 35, and distilled water) and centrifuged for 30 min at 2200 rpm. Tumor cells were collected, washed, and centrifuged again for 5 min at 1600 rpm. If there were red blood cells (RBCs) in pellets, RBCs were lysed by adding 5 mL of aqua pro. Then, washing medium was added and centrifuged for 5 min at 1200 rpm. Cells were resuspended at concentrations of 1×106 viable cells/mL in cultivation medium and seeded in

96-well microplates, with approximately 80 μL of cell suspension per well. Following this, tumor cells were exposed to chemotherapeutics for 72 h. Two columns were controls. After the cultivation occurred, 10 μL MTT (5 mg/mL) was added to each well. Formazan crystals have emanated, and 100 μL of 10% sodium dodecyl sulfate (SDS) solution was added. Next, the plates were assessed for absorbance at 560 nm using a spectrophotometer (Tecan Spectra Fluor-Plus; Salzburg, Austria).

DISC assay

For the DISC assay, a cell culture was prepared from fresh tumor tissue by enzymatic digestion. Tumor cell clusters were incubated for 3 days in 96-well microplates. Differential staining was performed with fast green dye. Following centrifugation, normal and neoplastic cells were fixed on slides. The control group without administration of any chemotherapeutic agent and complete cell death group with administration of overdose fluorouracil were compared with the group of cells exposed to a specific chemotherapeutic agent. Viable cells were detected with red or pink color, and dead cells were blue or purple (Figure 1).

Statistical analysis

Comparison of MTT, ATP, and DISC assays for chemosensitivity was performed using matched t test and Pearson correlation analysis to detect the power and tendency of the relation among them. For chemosensitivity analysis of agents for each test, variance analyses for recurrent measurements were used. The difference in chemosensitivity between drugs was investigated by variance analyses of recurrent measurements, and Bonferroni related t test was used. The relationship between categorical values, including chemosensitivity discrimination, in age groups was analyzed by Pearson chi-square test and Fisher's Chi-square test. The power (p) was also reported for the recurrent measurements of variance analyses. Statistical analysis was conducted by SPSS 16.0 (SPSS Inc.; Chicago, IL, USA), and $p<0.05$ was determined to be significant.

This study was approved by the Hacettepe University Ethics Committee. A written informed consent was obtained from all the patients before the operation for enrollment to the study. The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 and 2008.

Results

The mean age of the patients were 56.0 years (range 41–72 years). Of the patients, 19 (73.0%) had serous, four had endometrioid, two had mucinous, and one had undifferentiated type of tumor. Fourteen patients had grade 3 (53.8%), six had grade 2, and six had grade 1 tumor. Sixteen patients (61.5%) had stage III disease, four had stage II disease, and six had stage Ic disease.

The *in vitro* chemosensitivity of tumor cells to chemotherapeutic agents, including paclitaxel, carboplatin, docetaxel, topotecan, gemcitabine, and doxorubicin was measured by MTT, ATP, and DISC assays (Table 1).

The spectrophotometric quantitative value of ATP and MTT tests correlated with that of the DISC test for each chemotherapeutic agent. The correlation of results of MTT and DISC tests for paclitaxel ($r=0.95$, $p<0.01$), carboplatin ($r=0.94$, $p<0.01$), docetaxel ($r=0.92$, $p<0.01$), topotecan ($r=0.84$, $p<0.01$), gemcitabine ($r=0.86$, $p<0.01$), and doxorubicin ($r=0.40$, $p<0.046$) was found to be significant. The correlation of results of ATP and DISC tests for carboplatin ($r=0.87$, $p<0.01$), docetaxel ($r=0.86$, $p<0.01$), paclitaxel ($r=0.84$, $p<0.01$), topotecan ($r=0.95$, $p<0.01$), gemcitabine ($r=0.95$, $p<0.01$), and doxorubicin ($r=0.70$, $p<0.01$) was also significant. No difference in chemosensitivity profiles of MTT, ATP, and DISC tests for carboplatin ($p=0.68$), docetaxel ($p=0.82$), paclitaxel ($p=0.89$), topotecan ($p=0.32$), and doxorubicin ($p=0.34$) was found. However, for gemcitabine, the difference was significant ($p<0.05$).

The *in vitro* chemosensitivity results of chemotherapeutic agents was also studied in terms of prognostic parameters, including stage, grade, and histologic type. When results of stage I patients were compared with those of stage II and III patients, no significant difference was found between agents at

Table 1. The *in vitro* chemosensitivity test results (n=26)

Drug	Sensitive	Moderately sensitive	Resistant	Extremely resistant
MTT				
Carboplatin	15 (57.7%)	3 (11.5%)	5 (19.3%)	3 (11.5%)
Docetaxel	11 (42.3%)	5 (19.3%)	9 (34.6%)	1 (3.8%)
Paclitaxel	12 (46.1%)	4 (15.4%)	8 (30.8%)	2 (7.7%)
Topotecan	9 (34.6%)	6 (23.1%)	9 (34.6%)	2 (7.7%)
Gemcitabine	8 (30.8%)	3 (11.5%)	11 (42.3%)	4 (15.4%)
Doxorubucine	6 (23.0%)	2 (7.7%)	13 (50.0%)	5 (19.3%)
ATP-TCA				
Carboplatin	15 (57.7%)	3 (11.5%)	8 (30.8%)	0
Docetaxel	11 (42.3%)	5 (19.3%)	7 (26.9%)	3 (11.5%)
Paclitaxel	10 (38.5%)	8 (30.8%)	7 (26.9%)	1 (3.8%)
Topotecan	10 (38.5%)	6 (23.0%)	10 (38.5%)	0
Gemcitabine	4 (15.4%)	11 (42.3%)	10 (38.5%)	1 (3.8%)
Doxorubucine	5 (19.3%)	8 (30.8%)	12 (46.1%)	1 (3.8%)
DISC				
Carboplatin	16 (61.5%)	4 (15.4%)	5 (19.3%)	1 (3.8%)
Docetaxel	10 (38.5%)	6 (23.0%)	8 (30.8%)	2 (7.7%)
Paclitaxel	15 (57.7%)	2 (7.7%)	7 (26.9%)	2 (7.7%)
Topotecan	9 (34.6%)	6 (23.1%)	9 (34.6%)	2 (7.7%)
Gemcitabine	4 (15.4%)	5 (19.3%)	12 (46.0%)	5 (19.3%)
Doxorubucine	5 (19.3%)	4 (15.4%)	16 (61.5%)	1 (3.8%)

MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolyum bromide; ATP-TCA: adenosine triphosphate tumor chemosensitivity assay; DISC: differential staining cytotoxicity

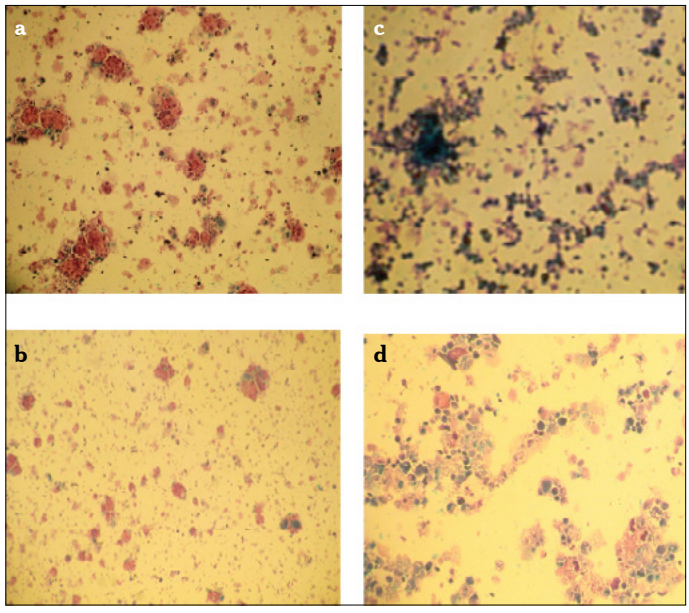

Figure 1. a-d. DISC assay results; control without drug (a), paclitaxel (sensitive) (b), control (extreme dose flourouracil) (c), Gemcitabine (resistant) (d)

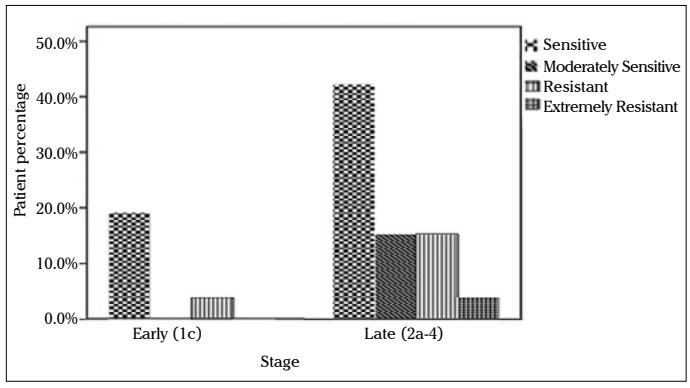

Figure 2. *In vitro* chemosensitivity of carboplatin according to DISC assay versus stage

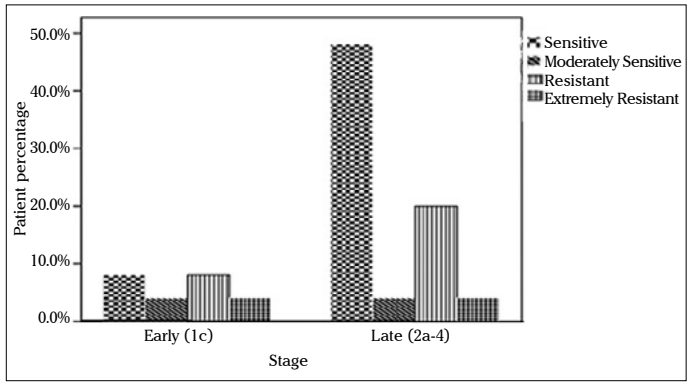

Figure 3. *In vitro* chemosensitivity of paclitaxel according to DISC assay versus stage

early stage (p=0.718); however, there was a significant difference for advanced stage (stage II and III) (p=0.028). The difference between agents in the advanced stage was significant for doxorubicin and paclitaxel (p=0.048).

When grade 1 and 2 tumors together were compared with grade 3 tumor, no significant difference was found in grade 1 and 2 (p=0.221) and grade 3 groups with regard to chemotherapeutic agents (p=0.093).

In terms of histology, the series was categorized as serous and nonserous tumors. There were significant differences between agents for serous carcinoma (p=0.004), but the difference was not significant in other histologic types (p=0.573).

Since the standard first-line chemotherapy of ovarian carcinoma consists of paclitaxel and carboplatin, subgroup analysis was also performed for these drugs with respect to stage, grade, and histology. The *in vitro* efficacy of carboplatin and paclitaxel with the DISC assay according to the groups based on the stage of patients is presented in Figures 2 and 3. No statistically significant difference was found between stage Ic and stage II and III groups. When grade 1 and 2 tumors were grouped together and compared with grade 3 tumors, grade 1 and 2 patients were more sensitive to carboplatin and paclitaxel than grade 3 patients (p=0.009). The efficacy of paclitaxel and carboplatin was studied in histologic type groups for serous and nonserous tumors; no statistical difference was found (Figures 4 and 5, p>0.05).

Discussion

The main steps in the treatment of ovarian carcinoma are surgical resection of tumor mass, followed by employment of combination chemotherapy. The selection of chemotherapeutic agents for ovarian carcinoma is based on the results of previous clinical trials. Despite advances in therapy, progression of the disease and even mortality are still problems originating from drug resistance. For an attempt to ameliorate the response to current treatment modalities, *in vitro* chemosensitivity assays have been developed for predetermining the possible effectivity (or ineffectivity) of chemotherapeutic agents prior to their administration to a patient. Several methods have been invented to foresee the effectivity of the treatment; however, debate still continues for the optimal application of these technologies. Sensitivity testing in primary ovarian cancer to individualize treatment remains an active area of interest. Furthermore, in recurrent ovarian cancer, there is lack of large prospective studies in which patients are randomized between standard therapy and assay directed therapy to show whether the directed therapy improves OS. However, some prospective and retrospective studies are promising that assay-directed therapy may offer a survival benefit (17, 18). In addition to the selection of effective drug for therapy, *in vitro* chemosensitivity assays are used for the prevention of unintended toxicity by the use of an inefficient chemotherapeutic agent.

Prognostic parameters, including stage, grade, and histologic type, were also studied with the scope of identifying subgroups in which *in vitro* chemosensitivity assays would be more useful. In addition, it particularly seems to be useful according to our study in advanced serous tumors. Furthermore, standard

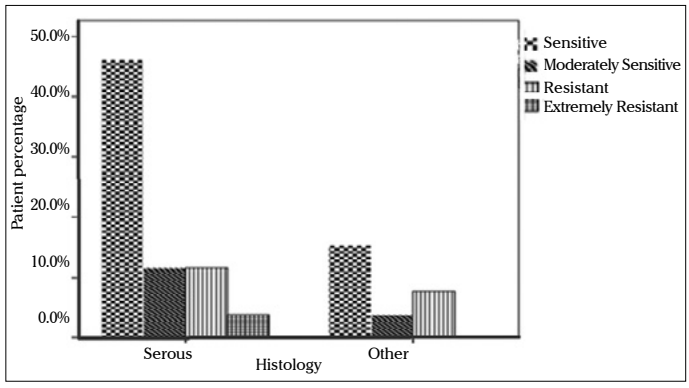

Figure 4. ***In vitro*** **chemosensitivity of carboplatin according to DISC assay versus histologic type**

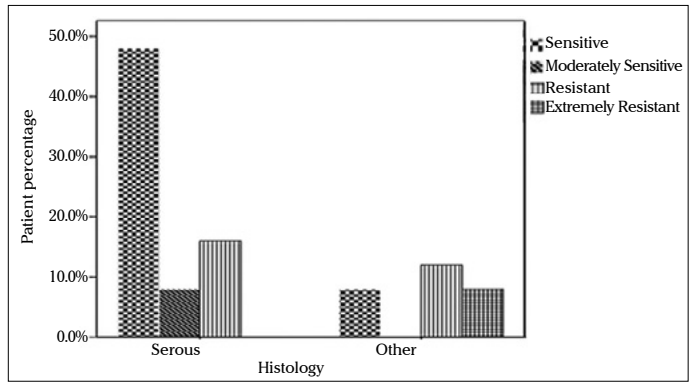

Figure 5. ***In vitro*** **chemosensitivity of paclitaxel according to DISC assay versus histologic type**

chemotherapy seems to be more effective in low-grade tumors, as an expected result.

The present study is based on three different assays (MTT, ATP, and DISC assays) for the determination of *in vitro* chemosensitivity. ATP and MTT assays have been used to predict chemosensitivity in the literature with good clinical correlation (19, 20). However, they are unable to discriminate the tumor cells with other cells in the specimen and have difficulty in the determination of the level of cytotoxicity. The ratio of dead cells to all cells in the DISC assay gives information about the sensitivity and can distinguish contaminating cells, thereby avoiding false results. In addition, metabolically inactive cells can be seen as alive in the DISC assay, whereas in other tests with metabolic end-points, they may be misleadingly counted as dead. However, the DISC assay is a time-consuming and somewhat subjective method.

The correlation of the quantitative values of the ATP and MTT assay spectrophotometrically with those of the DISC assay for chemotherapeutic assay was studied in a series of 26 primary ovarian cancer cases. It has been found that results of these three different tests were not different from each other for the drugs paclitaxel, carboplatin, docetaxel, topotecan, and doxorubicin. However, gemcitabine represents an exception to this finding, and no explanation could be found for that difference. One of the important findings of this study is that *in vitro* chemosensitivity testing can be performed successfully with one of these three tests because the results were found to be similar.

The clinical utility of *in vitro* chemosensitivity assays has been evaluated in clinical trials that studied the relationship between *in vitro* test results and the patient's actual clinical response to that chemotherapeutic agent. For ovarian cancer, the negative predictive value ranged from 62% to 100%, whereas the positive predictive value was between 58% and 91%. Negative predictive values are generally higher than positive predictive values, which suggests that *in vitro* assays are better in identifying ineffective drugs (20).

In conclusion, *in vitro* chemosensitivity can be determined in ovarian carcinoma with one of ATP, MMT, and DISC assays before the initiation of chemotherapy. *In vitro* assays seem to be more useful in subgroups with advanced stage and serous histology. Larger prospective randomized studies are required to support the routine clinical use of in vitro chemosensitivity assays.

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Hypodontia and ovarian cancer: A systematic review

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Abstract

Hypodontia can be defined as the non-formation of one or more teeth during the developmental period. Mutation in several genes related to tooth formation has previously been correlated with cancer. Regarding the ovarian cancer, there are few studies that associate the presence of hypodontia with ovarian cancer. A systematic literature search was performed in PubMed and Scopus. In total, 385 patients were included in this study. Control group was present in 3 out of 4 studies (340 patients). Hypodontia was present in 56 out of 290 patients (incidence of 19.3%). Only in 2 out of 4 studies, the number of missing teeth was mentioned (47 teeth), while the majority of them were either maxillary second premolars or maxillary lateral incisors. Unilateral distribution of the missing teeth was present in 28 out of 46 patients, while bilateral distribution of the missing teeth was present in 18 out of 46 patients. The presence of ovarian cancer in the family medical history occurred in 12 out of 33 patients. Only 1 out of 4 studies examined the presence of genes with mutations in the included patients. Based on our findings, the lack of clinical studies was the principal obstacle to clarify the possible predictive value of hypodontia in the early prediction of patients with higher risk of ovarian cancer. (J Turk Ger Gynecol Assoc 2016; 17: 41-4)

Keywords: Hypodontia, ovarian cancer, EDA gene, AXIN2 gene, WNT10A gene, BRCA gene

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Introduction

Hypodontia or selective tooth agenesis can be defined the non-formation of one or more teeth (<6 teeth) during the developmental period combined with variations in size, shape, and eruption time (1). The worldwide prevalence ranges between 2.6% and 11.3% (2, 3). Women are affected more than males at a ratio of 3:2. Hypodontia may be presented either as part of a clinical syndrome or as a non-syndromic form, the latter being more frequent. Both genetic and environmental explanations for hypodontia have been reported (1). More than 300 genes are involved in odontogenesis (4). Mutation in several genes related to tooth formation has previously been correlated with cancer (5-7). Regarding the ovarian cancer, there are few studies that link the presence of hypodontia with ovarian cancer.

The purpose of our study is to review the available literature on the correlation of hypodontia and ovarian cancer.

Methods

Data sources

A systematic search was performed in PubMed (May 1, 2015) and Scopus (May 1, 2015) to retrieve the included studies. The applied search strategy, in all the searched databases, included the combination of the key words: hypodontia and ovarian cancer. For additional studies, the references of the included articles have also been hand-searched.

Study selection criteria

All the studies that reported data on the presence of hypodontia and ovarian cancer were included in this review. Abstracts in scientific conferences, animal studies, editorials, letters to the editor, review articles, and studies published in languages other than English, German, Greek, French, Italian, and Spanish were excluded from this review. The retrieved data from each of the included studies were focused on the publication type, the number of patients included in each study, the number of patients included in the control group, the incidence of hypodontia, the number of missing teeth, the type of missing teeth, the distribution of hypodontia, the presence of ovarian cancer in family medical history, and the presence of isolated genes correlated to ovarian cancer.

Results

The performed search in PubMed and Scopus revealed a total of 43 and 39 search results, respectively, among which 4 studies (4 case series) were identified as eligible for inclusion in this review, according to the inclusion criteria (8-11). No additional studies were identified by searching the references of the included studies. The selected studies for inclusion are presented in detail in Figure 1 (flow diagram).

The principal characteristics of the included studies in our review (publication type, number of patients, control group, incidence of hypodontia, number of missing teeth, type of miss-

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Table 1. Studies reporting data regarding the correlation of hypodontia with ovarian cancer

First author, year, country (Ref)	Publication type	No. of patients	Control group	Incidence of hypodontia (%)	No. of missing teeth	Type of missing teeth (%)	Distribution of hypodontia (%)	Presence of ovarian cancer in family medical history (%)	Isolated genes (%)
Fekonja et al. (8), 2015, Slovenia	retrospective study	120	120	23/120 (19.2)	NM	NM	Unilateral: 10/23 (43.5) Bilateral: 13/23 (56.5)	9/23 (39)	NM
Bonds et al. (9)*, 2014, USA	retrospective study	95	-	NM	NM	NM	NM	NM	BRCA1: 7/50 (14) EDA: 1/50 (2) WNT10A: 6/50 (12) AXIN2: 1/50 (2)
Fekonja et al. (10), 2014, Slovenia	NRCT	120	120	23/120 (19.2)	31	maxillary SP: 14/31 (45.1) maxillary LI: 10/31 (32.3) mandibular SP: 5/31 (16.1) mandibular CI: 2/31 (6.5)	Unilateral: 18/23 (78.3) Bilateral: 5/23 (21.7)	NM	NM
Chalothorn et al. (11), 2008, USA	NRCT	50	100	10/50 (20)	16	maxillary LI, maxillary SP	NM	3/10 (30)	NM

USA: United States of America; y.o.: years old; NM: not mentioned; NRCT: non-randomized controlled trial; SP: second premolars; LI: lateral incisors; CI: central incisors; BRCA1: breast cancer 1; EDA: ectodysplasin A; WNT10A: wingless-type MMTV integration site family member 10A; AXIN2: axis inhibition protein 2
*Refers to patients included in the Chalothorn et al. (11) study

ing teeth, distribution of hypodontia, presence of ovarian cancer in family medical history, and isolated genes) are presented in Table 1. In total, 385 patients were included in this study. Control group was present in 3 out of 4 studies (340 patients). Hypodontia was present in 56 out of 290 patients (19.3%). Only in 2 out of 4 studies, the number of missing teeth was mentioned (47 teeth), and the majority of them were either maxillary second premolars or maxillary lateral incisors. Unilateral distribution of the missing teeth was present in 28 out of 46 patients, while bilateral distribution of the missing teeth was present in 18 out of 46 patients. The presence of ovarian cancer in the family medical history occurred in 12 out of 33 patients. Only 1 out of 4 studies examined the presence of genes with mutations in the included patients. Specifically, breast cancer 1 (BRCA1) gene and wingless-type MMTV integration site family member 10A (WNT10A) gene was present in 7 and in 6 out of 50 patients, respectively.

Discussion

Tooth embryogenesis is controlled by migration of neural crest cells that specialize in formulating specific type teeth. Genetic control during embryogenesis could be related to teeth shape, type, affiliation, and prolapse with the help of homeobox genes such as msh homeobox (MSX) 1 and MSX2 (12). Hypodontia is the most common congenital anomaly of the teeth (13). The data so far suggested that the prevalence is around 3.5-6.5% of the general population; however, it is higher in Africa, where it reaches 13.4% (1). Our review of the current studies revealed an incidence of 19.3% in patients with ovarian cancer.

It is thought to be a multifactorial condition related both to genes as well as environmental effect. In recent studies, it was shown that genes related to hypodontia could also be related to ovarian cancer as women with hypodontia have 8 times higher risk of ovarian cancer (11). Some of the genes that are related to hypodontia are paired box 9 (PAX9), MSX1, and axis inhibition protein 2 (AXIN2) (14, 15). WNT10A is the most commonly mutated gene in hypodontia (16). These genes are also expressed in tumor cells of the female reproductive system. Hypodontia was also associated with positive self-reported family history of cancer and with variants in genes fibroblast growth factor 3 (FGF3), FGF10, and FGFR2 (17). However, BRCA genes show no evidence of involvement in

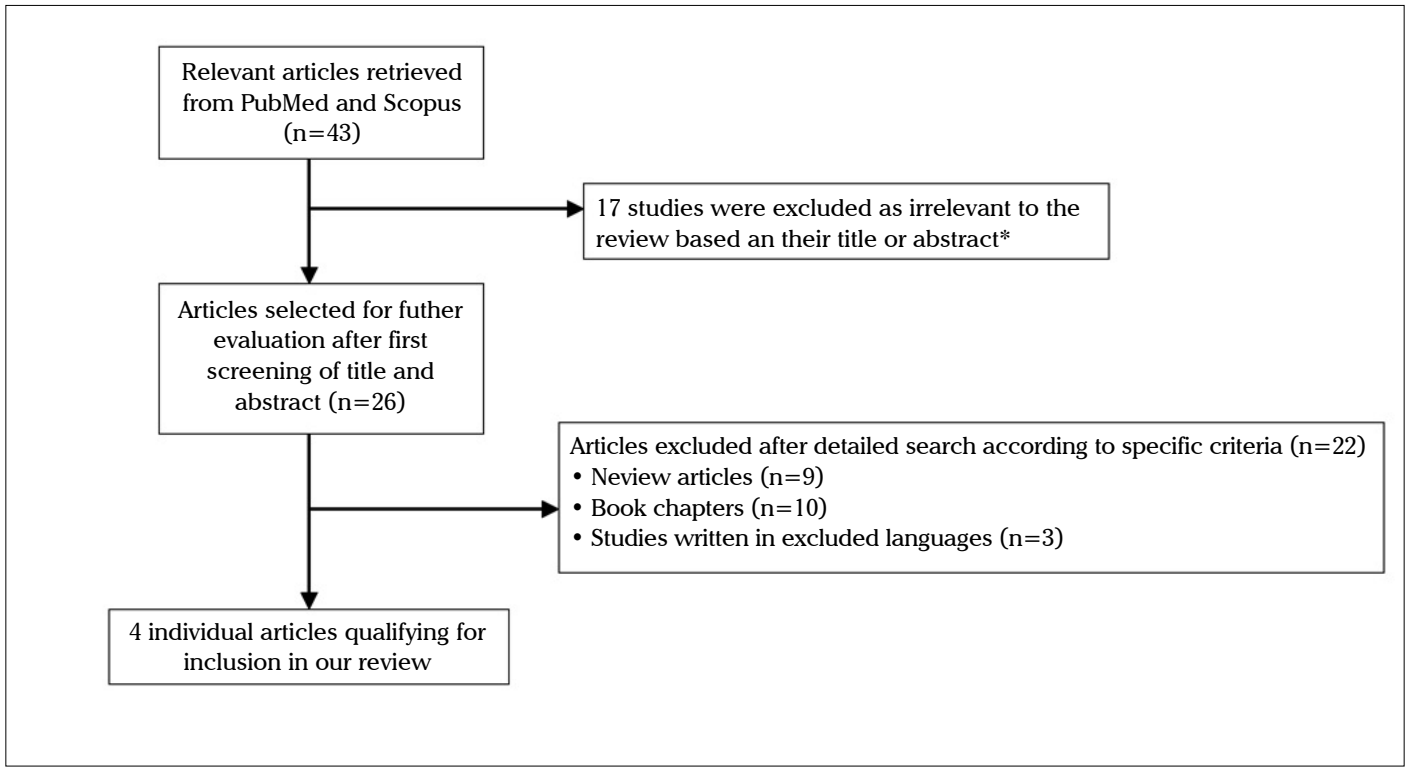

Figure 1. Flow diagram of the selection process of the included articles in the review
***The majority of studies were found in both databases**

hypodontia, although they are highly associated with ovarian cancer (9). Regarding environmental effect, it is related to early radiation of dental germ, thalidomide, osteomyelitis, or moving the dental germ of the permanent tooth during extraction of milk teeth (18). A family history could be related to hypodontia; however, there is no correlation with maternal health problems during pregnancy (13). Hypodontia is also related to extradermal dysplasia, cleft lip and palate, van der Woude syndrome, and Down syndrome (18).

The teeth that are mainly related to hypodontia are maxillary second premolars or maxillary lateral incisors (10). The lower premolars are usually missing in the Caucasians, while the lower lateral incisors in the Chinese population (10). Our review confirmed that the majority of the teeth are either maxillary second premolars or maxillary lateral incisors. Unilateral distribution of the missing teeth was present in 60.8% of the patients included in the review.

Different parameters are correlated with ovarian cancer such as history of diabetes mellitus or endometriosis (19, 20). The review of the current literature reveals strong correlation between hypodontia and ovarian cancer. Recently, Fekonja et al. (8) performed a subanalysis of their data showing that bilateral ovarian cancers are more common than unilateral in patients with hypodontia, and these patients have a higher incidence of other malignant tumors as well compared to controls. However, Lindor et al. (21) did not find any correlation of colorectal cancer with hypodontia. On the other hand, it was also revealed that there is no correlation of hypodontia with histological ovarian cancer subtype, but a statistically significant difference was found in regard to stage and grade compared to the control group (8).

Our review supports a possible correlation between hypodontia and ovarian cancer, and for this reason, hypodontia could potentially become a risk marker for future ovarian cancer development. A future step could be the earlier referral of such patients for ovarian cancer screening. According to van Nagell et al. (22), if a correlation was found between hypodontia and ovarian cancer in more prospective studies, then semiannual screening should become the standard of care for those women in order to achieve earlier detection.

Before reaching any conclusion, there are several limitations that should be taken into consideration. The limited number of the existing studies and, as a consequence, the small number of the included patients cannot allow us to draw any safe conclusion. However, our review reveals quite a strong correlation between hypodontia and ovarian cancer. Regarding the adopted search strategy, this could be considered as restricted due to the exclusion of abstracts in scientific conferences, review articles, conference papers, letters to the editor, animal studies, and editorials, while the limitation based on the language of the excluded articles could also be considered another weakness of this study.

Conclusion

Based on our findings, we believe that more prospective or retrospective studies should be organized in order to clarify the possible predictive value of hypodontia in the early prediction of patients with higher risk of ovarian cancer.

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Inherited thrombophilia and reproductive disorders

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Abstract

Apart from its established role in the pathogenesis of venous thromboembolism (VTE), inherited thrombophilia has been proposed as a possible cause of pregnancy loss and vascular gestational complications. There is a lot of controversy in the literature on the relationship between inherited prothrombotic defects and these obstetric complications. This is a review of the literature on inherited thrombophilia and reproductive disorders. Factor V Leiden, prothrombin G20210A mutation, and protein S deficiency seem to be associated with late and recurrent early pregnancy loss, while their impact on other pregnancy complications is conflicting. No definite association has been established between protein C and antithrombin deficiency and adverse pregnancy outcome, primarily due to their low prevalence. Screening is suggested only for women with early recurrent loss or late pregnancy loss. Anticoagulant treatment during pregnancy should be considered for women with complications who were tested positive for thrombophilia. (J Turk Ger Gynecol Assoc 2016; 17: 45-50)

Keywords: Inherited thrombophilia, reproductive disorders, recurrent pregnancy loss

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Introduction

Thrombophilia is a disorder caused by inherited and acquired defects and is defined as a predisposition to thrombosis (1). The most common cause of acquired thrombophilia is the antiphospholipid syndrome (APS). Inherited thrombophilia constitutes a group of abnormalities of blood coagulation, including the factor V Leiden mutation (FVL) (homozygous or heterozygous), the prothrombin (FII) G20210A mutation (Pm) (homozygous or heterozygous), and deficiencies of the endogenous anticoagulants, antithrombin (AT), protein C, and protein S. Among these conditions, FVL and Pm are relatively common, while the others are rare. FVL is a point mutation (G1691A), resulting in an altered factor resistant to inactivation by protein C. The Pm leads to a 20%–50% increase in plasma prothrombin levels (2, 3).

The prevalence of inherited thrombophilia in the general western population is estimated to be approximately 15% (4). However, it seems that there is a significant variation in the prevalence of these conditions among different geographical and tribal populations. FVL varies from 0.6% to 7.0%, with the lowest frequency observed in Africa (0%–0.6%) and the highest in Southern Europe (7%). The mean prevalence in Northern Europe is 4%. The prevalence of Pm varies from 0.2% to 3%, being lowest in Africa (0%–0.3%) and highest in Southern Europe (3%). The mean value in Northern Europe is 2%. Protein C, protein S, and AT deficiencies are extremely rare (0.2%–0.4%, 0.03%–0.1%, and 0.02%–0.2%, respectively) (5).

The role of inherited thrombophilia in pregnancy loss and vascular gestational disorders has been investigated in several studies, and the results seem to be contradictory. The aim of this review was to elucidate the association of inherited thrombophilia and reproductive disorders. The value of screening women for inherited thrombophilia and the treatment options during pregnancy are also discussed.

The association between inherited thrombophilia and reproductive disorders

1. Inherited thrombophilia and infertility

Coulam et al. (6) reported that a prothrombotic tendency is associated with unexplained infertility, but this finding was not in agreement with data from Casadei et al. (7). To date, there has been no conclusive evidence in the literature to suggest an association of inherited thrombophilia and infertility other than recurrent implantation failure (8). Di Nisio et al. (9) conducted a systematic review and meta-analysis of all the available studies on the role of inherited thrombophilia in implantation failure. A significant association was found only for FVL [Odds ration (OR) 3.08; 95% confidence interval (CI) 1.77–5.36].

2. Inherited thrombophilia and recurrent pregnancy loss

It is estimated that approximately 25% of conceptions and 15% of all clinically recognized pregnancies end in a miscarriage (10, 11). Moreover, three or more successive losses affect 1%–2% of women of reproductive age, and two or more successive losses affect approximately 5% (12). Although several

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causes of recurrent pregnancy loss have been identified, 38% of cases remain unexplained (13, 14). Thrombophilias have been suggested as a possible cause of recurrent miscarriage (RM) (15). The hypothesis that inherited thrombophilia may be associated with miscarriage was first investigated by the European prospective cohort of thrombophilia (EPCOT) (16). The authors observed an increased risk of pregnancy loss in 571 women with inherited thrombophilia (OR 1.35; 95% CI 1.01–1.82).

In 2003, Rey et al. (16) conducted a meta-analysis of the data on the association of inherited thrombophilia and RM. They found a significant variability among studies in the definition of RM and the gestational period that miscarriage occurred. A significant association was identified for FVL, Pm, and protein S deficiency with non-recurrent and recurrent fetal loss. Protein C and AT deficiencies were not associated with RM; however, this result should not be considered conclusive because of their low prevalence in the general population.

A year later, Kovalevsky et al. (17) published the results of another meta-analysis, investigating the role of the two most common forms of inherited thrombophilia (FVL and Pm) in RM. The analysis reported significant among-study heterogeneity for FVL, but not for Pm. However, they did establish an association between FVL, Pm, and RM, with the carriers having a double risk for RM compared with women without thrombophilia. Finally, limiting the data to women with first-trimester recurrent pregnancy loss (RPL) appeared to weaken the association in the FVL analysis. Such an effect was not observed in the G20210A analysis.

The most recent and well-designed review on the association of inherited thrombophilia and RM was conducted by Rodger et al. (18) who reported an increased risk for miscarriage for women with FVL (OR 1.52; 95% CI 1.06–2.19), but no association was found for Pm.

Although it appears that the results of the published studies are conflicting, most reviews suggest an association of RM with FVL and Pm.

3. Inherited thrombophilia and late pregnancy loss

Kovalevsky et al. (17) also analyzed the association of inherited thrombophilia with loss at different stages of the pregnancy. Late loss was defined as a pregnancy being lost after its 13th week. They found that FVL was associated with early and late loss. Pm was associated with late loss and recurrent early loss but not isolated early loss. Protein S deficiency was also associated with late loss and early recurrent loss. AT and protein C deficiencies were not associated with pregnancy loss.

In a systematic meta-analysis (19) where late loss was defined as pregnancy loss after the 24th week, there was a significant risk for early loss in homozygous FVL but a lower, non-significant risk in heterozygous FVL or Pm. With respect to late loss (3rd trimester), there was a significant risk in heterozygous FVL and a lower, non-significant risk in heterozygous Pm. Although there was a higher risk for late loss in women with protein S deficiency, no such increase in risk was found for protein C and AT deficiency for early or late loss. Inherited AT deficiency which occurs in two forms (Type I: low antigen concentrations and activity; Type II: normal antigen concentrations, low activity) is associated with an increased risk of VTE and adverse pregnancy outcome as well (20). Type II AT deficiency associated with a defect at the heparin binding site (HBS) due to Leu99Phe mutation is a subtype that in its heterozygous form represents a minor risk for thrombosis, while homozygous carriers are prone to a high risk for early onset of arterial and venous thrombosis and pregnancy loss, despite anticoagulation with heparin (20).

Overall, it seems that FVL and Pm are more strongly associated with late loss (2nd and 3rd trimester) than early loss (1st trimester), whereas protein S deficiency seems to be significantly associated only with late loss.

4. Inherited thrombophilia and pre-eclampsia

A number of small studies have investigated the contribution of thrombophilia in the pathogenesis of pre-eclampsia (2, 21, 22). Mello et al. (23) conducted a large multicenter case-control study to assess the prevalence of thrombophilic defects in women with severe and mild pre-eclampsia. There was a significant association between Pm with mild or severe pre-eclampsia and FVL with severe pre-eclampsia. There was no relationship between protein C, protein S, and AT deficiency with pre-eclampsia, but the number of the subjects with such defects was too small to allow for definite conclusions. Moreover, a significant increase was reported in the incidence of early onset of pre-eclampsia (<28 weeks gestation) and disseminated intravascular coagulation [but not of hemolysis elevated liver enzymes low platelets (HELLP) syndrome, eclampsia, and pulmonary edema] in women with thrombophilia compared with the controls (23).

However, the most recent meta-analysis of Rodger et al. (18) did not observe any significant association between FVL and Pm with pre-eclampsia. Facchinetti et al. (24) studied the risk of recurrence of pre-eclampsia as well as the perinatal outcome following pre-eclampsia according to the presence or absence of thrombophilia in the mother. The rate of recurrence was 51.9% in women with inherited thrombophilia compared with only 25.9% in women without thrombophilia.

5. Inherited thrombophilia and intrauterine growth retardation (IUGR)

Wu et al. (19) found that embryos from homozygous FVL and heterozygous Pm mothers had an increased risk for IUGR. None of the other inherited prothrombotic defects were associated with IUGR in that study. Rodger et al. (18) failed to confirm the results by Wu et al. (19) because they reported no significant risk for IUGR in women tested positive for FVL or Pm.

6. Inherited thrombophilia and placental abruption

Wu et al. (19) reported a significant risk for placental abruption in pregnant women heterozygous for FVL (OR 4.70; 95% CI 1.13–19.59) or Pm (OR 7.71; 95% CI 3.01–19.76). This result however was questioned in the meta-analysis by Rodger et al. (18) who found no significant risk for placental abruption in women with FVL or Pm.

7. Inherited thrombophilia and VTE

It is well known that pregnancy is a thrombogenic condition, and the risk for VTE (deep venous thrombosis or pulmonary embolism) is significantly increased when additional prothrombotic factors coexist. The prevalence for VTE is gradually

Table 1. Association between different types of inherited thrombophilia with pregnancy loss and a variety of pregnancy complications

	FVL	Pm	Prot C def.	Prot S def.	AT def.
Recurrent implantation failure	−/+	−	0	0	0
Recurrent 1st trimester loss (≥3)	+	+	0	0	0
2nd or 3rd trimester loss	+	+	0	+	0
Pre-eclampsia	−/+	−/+	0	0	0
IUGR	−/+	−/+	0	0	0
Placental abruption	−/+	−/+	0	0	0
VTE	++	++	+	+	++
	0 Insufficient published data − No association −/+ Published data contradictory for an association + Weak association ++ Strong association				

FVL: factor V Leiden mutation; Pm: G20210A mutation; Prot C def.: protein C deficiency; Prot S def.: protein S deficiency; AT def.: antithrombin deficiency; IUGR: intrauterine growth retardation; VTE: venous thromboembolism

increased from 1/1000 pregnancies (when no other prothrombotic conditions exist) to 1/500 for heterozygous FVL women, 1/200 for heterozygous Pm, 4.6/100 for double heterozygous FVL/Pm, 1/113 for protein C deficiency, and 1/2.8 for AT deficiency (5). According to the findings of Wu et al. (19), all inherited prothrombotic conditions significantly increased the risk for VTE.

In summary, there is preliminary evidence to suggest that FVL and Pm are associated with second or third trimester loss, with insufficient evidence for the association with pre-eclampsia and IUGR. There is a need for larger, well-designed prospective trials. The association of different types of inherited thrombophilia and reproductive disorders is summarized in Table 1.

Is screening women for inherited thrombophilia prior to pregnancy necessary?

At present, there is no evidence to support universal screening for inherited thrombophilia prior to conception. Selective screening should be considered on the basis of family history of thrombosis or adverse reproductive history.

The National Society of Genetic Counselors in its guidelines for the genetic evaluation and counseling of couples with recurrent miscarriage adopted a more selective attitude for thrombophilia screening, including a personal or family history of VTE in its criteria. They suggested testing for FVL and Pm for all women with recurrent pregnancy loss. Until the results of further studies are published, testing for protein C, protein S, and AT deficiencies should be offered only to women with a personal/family history of VTE. Finally, full thrombophilia screening [AT, protein C, protein S, FVL mutation and APC Resistance Assay (APCR), Prothrombin Gene Mutation (G-20210-A, Lupus Anticoagulant (LA) and Anti-Cardiolipin antibodies (ACLA)] should be offered to women with RM and a common thrombophilic defect (FVL or Pm) diagnosed previously because the co-existence of another defect dramatically increases the risk for VTE and pregnancy complications (25, 26).

The American College of Obstetricians and Gynecologists (ACOG) did not recommend screening for inherited thrombophilia for women with a history of recurrent fetal loss or placental abruption because it is unclear whether anticoagulation treatment reduces the risk of recurrence. There is also insufficient evidence to support screening for thrombophilias in women with a history of IUGR or pre-eclampsia (27).

The Royal College of Obstetricians and Gynaecologists (RCOG) however recommended screening for inherited thrombophilia for women with second-trimester miscarriage. Testing should include FVL, Pm, and protein S (28).

Although there is no universal agreement on screening for inherited thrombophilia in reproductive disorders, a proposed screening strategy is outlined in Table 2.

Treatment

Antithrombotic agents could potentially increase the live-birth rate of subsequent pregnancies in women with inherited thrombophilia and RM or late loss, but the results of the studies investigating their role in the management of these conditions are controversial.

The treatment of inherited thrombophilia associated with reproductive disorders is governed by two considerations. The first is the prevention of venous thrombosis during pregnancy, which itself is a hypercoagulable state. Some practitioners would offer heparin treatment shortly after a pregnancy has been confirmed, whereas others offer heparin treatment only in the third trimester or just post-partum. The second consideration relates to the beneficial effect of treatment on pregnancy outcome. In this situation, the treatment will need to be started at a specific time of the pregnancy to improve the desired outcome. For example, if the intention of treatment is to reduce

Table 2. Recommendations on screening for inherited thrombophilia in women with a history of pregnancy loss and a variety of pregnancy complications

	FVL	Pm	Prot C def.	Prot S def.	AT def.
Recurrent implantation failure	–	–	–	–	–
Recurrent 1st trimester loss (≥3)	+	+	+	+	+
2nd or 3rd trimester loss	+	+	+	+	+
Pre-eclampsia	+ (1)	+ (1)	–	–	–
IUGR	+ (2)	+ (2)	–	–	–
Placental abruption	+ (2)	+ (2)	–	–	–
VTE	+	+	+	+	+
	– Screening not recommended + Screening recommended (1) In recurrence or early-onset (2) After the exclusion of common causes				

FVL: factor V Leiden mutation; Pm: G20210A mutation; Prot C def.: protein C deficiency; Prot S def.: protein S deficiency; AT def.: antithrombin deficiency; IUGR: intrauterine growth retardation; VTE: venous thromboembolism

the risk of recurrent first trimester loss, the treatments need to be started as soon as a pregnancy has been confirmed. If the intention of treatment is to prevent late loss or to reduce late pregnancy complications such as pre-eclampsia or IUGR, then the treatment should be started in the second trimester. There is much debate however about the likely benefit of heparin treatment on the outcome. Based on data extrapolated from the observation of the association (described earlier), it seems reasonable to offer treatment with heparin to women with FVL and Pm with recurrent or late pregnancy loss.

According to the ACOG and RCOG guidelines, there was insufficient clinical evidence that prophylaxis with unfractioned heparin or low molecular weight heparin (LMWH) prevents recurrence in women who had experienced miscarriage or placental abruption or IUGR. Although ACOG strongly discouraged the administration of any anticoagulation treatment in such cases, RCOG suggested that heparin therapy during pregnancy improves the outcome of women with mid-trimester miscarriage associated with inherited thrombophilias (27, 28).

The use of antithrombotics in pregnancy has to be monitored and evaluated for safety. The possibility of these agents causing more harm than good in these patients cannot be excluded. In contrast to coumarin derivatives, unfractionated heparin and LMWH do not cross the placenta; therefore, they do not have the potential to cause fetal bleeding or teratogenicity (29). Potentially serious maternal risks associated with heparin include bleeding, heparin-induced osteopenia, and heparin-induced thrombocytopenia. These risks are greater for unfractionated than LMWH.

Kaandorp et al. (30) compared the risk for maternal and neonatal adverse events between pregnant women who did not receive any anticoagulation treatment with those who received aspirin only or aspirin plus nadroparin. No significant increase in the risk of serious maternal complications or neonatal problems was observed. However, minor side effects, such as bruising and swelling or itching at the injection site were more common in women who received treatment. Similar results were reported in the Scottish Pregnancy Intervention (SPIN) trial (31). LMWH is as effective and safe as unfractionated heparin, with potential advantages in pregnancy, because it is less associated with thrombocytopenia and osteoporosis and it can be administered once daily (longer half-life). Low dose aspirin (less than 150 mg/day) also appears to be safe, while the safety of higher doses of aspirin during the first trimester is uncertain (29, 32).

In a multicenter randomized control trial (RCT), 139 pregnant women with inherited thrombophilia without antiphospholipid antibodies at <12 weeks of gestation were recruited from all university hospitals in the Netherlands, two university hospitals in Australia, and one university hospital in Sweden as well as from six non university/teaching hospitals in the Netherlands between December 2000 and 2009. Either daily LMWH (dalteparin, 5000 IU weight adjusted dosage) with aspirin 80 mg or aspirin 80 mg alone were administrated. According to the results of this trial, named FRUIT, adding LMWH to aspirin before 12 weeks gestation seems to reduce recurrent hypertensive disorders (HD) in women with previous early-onset HD and/or small for gestational age (SGA) in the context of an inheritable thrombophilia without antiphospholipid antibodies (33).

Between February 2000 and September 2012, the Thrombophilia in Pregnancy Prophylaxis Study (TIPPS) was conducted in 36 tertiary care centers in Canada, Australia, the USA, the UK, and France. In this open label randomized trial, 289 pregnant women with thrombophilia who were at an increased risk of placenta-mediated pregnancy complications, venous thromboembolism, or both were included. Women received either antepartum dalteparin 5000 international units (IU) once daily by subcutaneous self-injection from the day of randomization until 20 weeks of gestation followed by 5000 IU twice daily from 20 weeks until at least 37 weeks gestational age or no antepartum dalteparin. Antepartum dalteparin was not found to reduce the risk of either pregnancy loss, venous thromboembolism, or placenta-

Table 3. Recommendations on the treatment of pregnant women with inherited thrombophilia and a history of pregnancy loss or pregnancy complications

		High-risk thrombophilia (FVL +/+, Pm +/+, FVL/Pm, AT def.)	Low-risk thrombophilia FVL +/−	Pm +/−	Pr. C def.	Pr. S def.
Recurrent implantation failure		3	1	1	1	1
Recurrent 1st trimester loss (≥3)		3	2	2	2	2
2nd or 3rd trimester loss		3	2	2	2	2
Pre-eclampsia		3	2	2	1	1
IUGR		3	2	2	1	1
Placental abruption		3	2	2	1	1
VTE:	Once	4	3	3	3	3
	Recurrent (≥2)	4	4	4	4	4
		1: Intense surveillance during pregnancy, graduated compression stockings 2: Prophylactic dose LMWH (empirical treatment until further data available) 3: Prophylactic dose LMWH. expert advice, referral to a specialist hematologist or joint antenatal-hematology clinic 4: Prophylactic or therapeutic dose LMWH. Referral to a specialist hematologist or joint antenatal-hematology clinic				

FVL: factor V Leiden mutation; Pm: G20210A mutation; Prot C def.: protein C deficiency; Prot S def.: protein S deficiency; AT def.: antithrombin deficiency; IUGR: intrauterine growth retardation ; VTE: venous thromboembolism; LMWH: low molecular weight heparin

mediated pregnancy complications in pregnant women with thrombophilia [dalteparin 25/146 (17.1%; 95% CI 11.4%–24.2%) vs no dalteparin 27/143 (18.9%; 95% CI 12.8%–26.3%); risk difference −1.8% (95% CI 10.6%–7.1%)]. This was the first large trial to show no benefit of LMWH administration in this high risk group of pregnant women. Moreover, researchers found that delteparin administration was associated with an increased risk of minor bleeding and noted LMWH administration complications. Lastly, a meta-analysis was conducted showing low evidence to support that LMWH might prevent recurrent severe placenta-mediated pregnancy complications (34).

As a conclusion, the use of LMWH and aspirin during pregnancy is considered safe, with only minor side effects.

Should women with inherited thrombophilia be referred to a hematologist?

Most general obstetricians and gynecologists today are familiar with common prothrombotic disorders, and they are capable of investigating and diagnosing common forms of thrombophilia. In addition, specialists in RM and high-risk pregnancies often have the skills to undertake the management of pregnant women with thrombophilia.

RCOG advises that the opinion of a local expert should be sought for women with AT deficiency, those with more than one thrombophilic defect, or those with additional risk factors. Women with AT deficiencies (particularly type 1 with reductions in both activity and antigen) have a high risk of recurrence and may require higher doses of LMWH or AT concentrate during pregnancy. They are also likely to be on long-term anticoagulation treatment with warfarin. Such conditions should be managed in collaboration with a hematologist expert in thrombosis (35).

The recommendations on the treatment of pregnant women diagnosed with sole or multiple thrombophilic defects in conjunction with a history of reproductive disorders are summarized in Table 3.

Ongoing trials

There are two ongoing RCTs investigating the efficacy and safe dose of LMWH in pregnancy (36).

The first one is The Highlow study (NCT Clinicaltrials.gov 01828697). It is an investigator-initiated, randomized-controlled, open-label trial that aims to provide high-quality evidence on the optimal prophylactic dose of LMWH in pregnancy in women with a history of VTE, comparing two different doses of LMWH (36).

The second one, the ALIFE2 study (NTR 3361) is an open-label trial, including women with inherited thrombophilia (FVL, Pm, AT deficiency, protein C deficiency, protein S deficiency, or a combination) and two or more miscarriages. The effect of LMWH (enoxaparin 40 mg) on live birth and on adverse pregnancy outcomes (e.g., pre-eclampsia, HELLP syndrome, intra-uterine growth restriction, placental abruption, premature delivery, and congenital malformations) and adverse effects of treatment (hemorrhagic episodes, thrombocytopenia, and allergic skin reactions to LMWH) are being studied (36).

Conclusion

FVL is associated with repeated implantation failure, RM, and late loss. Pm is associated with RM and late loss. Protein S defi-

ciency is associated with late loss. There is insufficient evidence to suggest an association of other forms of inherited thrombophilia and reproductive disorders. Screening and treatment strategies based on the observed association seem reasonable, although there is as yet no firm evidence (such as RCTs) to confirm the benefits of treatment.

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Pelvic recurrence of stage 1a well-differentiated endometrial carcinoma after 13 years: A case report

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Abstract

A great majority of endometrial carcinoma recurrences are observed in high-risk patients and within the first 3 years of treatment. The relapse of endometrial carcinoma occurring more than 10 years after initial treatment has rarely been described. Initially diagnosed and treated for International Federation of Gynecology and Obstetrics (FIGO) stage 1a, grade 1 adenocarcinoma, our patient presented 13 years later with an isolated pelvic recurrence, demonstrating, to our knowledge, the longest disease-free interval with recurrence in the pelvis reported in literature. After surgical resection, the patient is being considered for enrollment in a clinical trial.

Despite favorable prognostic features, it is possible to observe the recurrence of endometrial carcinoma even 5 years after surveillance and remission. Successful salvage therapies are available but may depend upon early diagnosis. (J Turk Ger Gynecol Assoc 2016; 17: 51-4)

Keywords: Endometrial carcinoma, recurrence, disease-free interval

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Introduction

Endometrial carcinoma is the sixth most common cancer in women, with 320,000 new cases diagnosed worldwide in 2012 (1). In the United States and many developed countries, endometrial carcinoma is the most common cancer of female reproductive organs. The American Cancer Society estimated 52,630 newly diagnosed cases and 8,590 deaths due to uterine cancer in the United States in 2014 (2). Most women are diagnosed at stage 1 disease when surgery is curative and post-staging surveillance is appropriate. Patients undergoing surgical staging with or without adjuvant radiation therapy have reported recurrence rates between 3% and 15% for early-stage disease (International Federation of Gynecology and Obstetrics, FIGO, stages 1-2). Of the cases that recur, 76–87% is evident within the first 3 years of treatment initiation (3). This report describes the case of a patient who had a primary recurrence of endometrioid endometrial adenocarcinoma 13 years after initial treatment.

Case Presentation

In April 2001, a postmenopausal 57-year-old female was diagnosed with FIGO stage 1a, grade 1 endometrial carcinoma and subsequently underwent laparoscopic-assisted vaginal hysterectomy and bilateral salpingo-oophorectomy without adjuvant therapy. The carcinoma was limited to the endometrium without myometrial or lymphovascular invasion. The patient remained under close follow-up with a non-contributory interim medical history and without any evidence of recurrent disease until April 2014. At that time, 13 years after the initial diagnosis and treatment, a routine office visit revealed microscopic hematuria on urine analysis. The results of the initial urine analysis were found to be false after a follow-up urine analysis, and the cytoscopic evaluation was negative. The patient had no family history of cancer or personal history of endometriosis. She was asymptomatic with a body mass index of 19.6 kg/m^2 and denied vaginal bleeding, abdominal pain, changes in bowel habits, or weight loss. A pelvic examination revealed a right posterior mass approximately 7–8 cm. A magnetic resonance imaging scan of the abdomen revealed a 6×5.2×7.8 cm enhancing lobular heterogeneous mass in the right posterior pelvis. A computed tomography-guided biopsy of the mass was performed in June 2014 (Figure 1); however, the results were inconclusive due to inadequate tissue sampling.

Surgery was performed in July 2014 and included exploratory laparotomy, omentectomy, appendectomy, and pelvic mass resection. Intraoperative findings showed a 5 cm mass attached to the sigmoid colon. No residual disease remained at the conclusion of surgery with an estimated blood loss of

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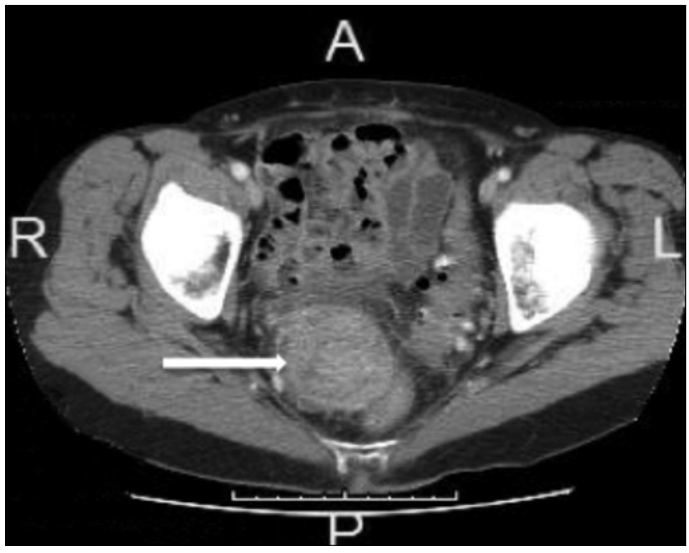

Figure 1. CT abd/pelvis with contrast. There is a heterogeneous right pelvis mass measuring 5.2×4.6 cm to the right of the rectum (mass indicated by white arrow)

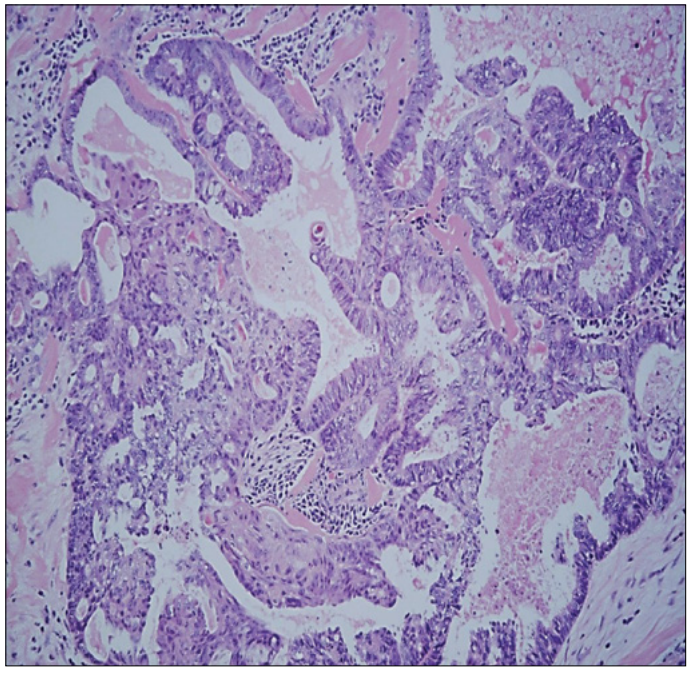

Figure 2. Histological examination. 100× microscopic view of an area of tumor showing cribriformed glands with associated squamous metaplasia, characteristic of endometrioid adenocarcinoma

150 mL. Surgery was well tolerated, and the patient was discharged on postoperative day 4.

The final pathology revealed moderately differentiated adenocarcinoma in the pelvic mass compatible with recurrent endometrioid endometrial adenocarcinoma. Omental and peritoneal biopsies were negative for tumor. Special stains were positive for estrogen and progesterone receptors and negative for p53 (Figure 2). Informed consent was obtained from the patient for reporting of her case.

Discussion

The Gynecologic Oncology Group (GOG) 99 trial showed that most initial relapses of endometrial carcinoma occur within 18 months, with the Postoperative Radiation Therapy in Endometrial Carcinoma (PORTEC) study reporting a median time to recurrence of 21 months (3). Those with low-risk carcinoma (G1 or G2 histology, myometrial invasion <50%, no cervical disease, and no lymphovascular invasion) have a very low probability of recurrence, 1–3%, following surgical treatment alone. Bell et al. (4) found that the omission of lymphadenectomy is a reasonable option in patients with low-risk disease diagnosed by intraoperative factors. In this study, 1.7% of the patients experienced recurrent disease with a mean time to recurrence of 43.7 months and 5-year overall survival of 95.8%, consistent with the excellent outcomes of stage 1 disease reported in other studies. Carcinoma recurring after 13 years in our patient represents a significantly longer time to relapse than what has previously been reported and, to our knowledge, is the longest disease-free interval with pelvic recurrence found in literature.

Although rare, recurrences of endometrial carcinoma occurring more than 10 years after initial treatment have been reported (Table 1). Only four cases of relapse more than 10 years after surgical management with or without adjuvant therapy have been described. The first reported case of stage 1a disease treated with an intracavitary radium implant followed by radical hysterectomy recurred 26.5 years later in an isolated lower vaginal recurrence, which was histologically identical to the original tumor (5). Another study reported the case of a 61-year-old woman who had a vaginal cuff recurrence 17 years after total abdominal hysterectomy and bilateral salpingo-oophorectomy with adjuvant pelvic external beam radiation therapy and who was successfully treated by vaginal brachytherapy (6). After salvage high-dose-rate brachytherapy to the cuff, the patient remained disease free until her death 7 years later from unrelated causes. Other reported studies discussed the possibility of the implantation of cancer cells to another site caused by a diagnostic or surgical procedure as the pathogenesis behind late recurrence. There is a reported case of the recurrence of endometrioid carcinoma at an abdominal scar 14 years after hysterectomy, most likely due to direct tumor cell seeding at the time of operation (7). This recurrence was resected without adjuvant therapy. Another patient with unusual recurrence in the upper urinary tract 11 years after hysterectomy that was likely due to implantation during partial ureterectomy during pelvic lymph node dissection was successfully treated with surgical resection and external beam radiation therapy (8).

In the setting of recurrent disease, certain clinical and pathological factors are associated with a good prognosis, including a longer disease free-interval, low-grade and endometrioid histology, and isolated recurrence at the vaginal cuff (9). Recurrent endometrial carcinoma presents with different patterns, including disease localized to the vagina, limited to the pelvis, or metastatic disease. The most common site for endometrioid carcinoma recurrence is local, involving the vaginal vault or cuff. The PORTEC trial showed a decreased incidence of an

Table 1. Reported recurrences with a disease-free interval longer than 10 years

Author (Cases)	FIGO stage, grade	Initial treatment	Disease-free Interval	Site of recurrence	Management of recurrence	Outcome
Lederman et al. (5)	FIGO stage 1a, grade 2 endometrioid	Intracavitary radium implant followed by radical hysterectomy	26.5 years	Lower vagina	Retreated with radiation therapy	No evidence of disease 1.5 years after diagnosis of recurrence
Yechieli et al. (6)	FIGO stage 1c, grade 2 endometrioid	Total abdominal hysterectomy and bilateral salpingo-oophorectomy followed by adjuvant external beam radiation to the whole pelvis	17 years	Vaginal cuff	Salvage high-dose rate intracavitary vaginal brachytherapy	No evidence of the disease for more than 7 years after salvage treatment; death from unrelated causes at age 88
Lorenz et al. (7)	FIGO stage 1a, grade 2 endometrioid	Total abdominal hysterectomy and bilateral salpingo-oophorectomy followed by radiation therapy of the vagina	14 years	Abdominal wall scar	Surgical excision of mass and portion of anterior abdominal wall	No evidence of disease
Tsurumaki et al. (8)	FIGO stage 2, grade 2 endometrioid	Radical hysterectomy	11 years	Upper urinary tract (left pelvic ureter to renal pelvis)	Nephroureterectomy with excision of bladder cuff followed by radiation (50 gray)	No evidence of disease

FIGO: International Federation of Gynecology and Obstetrics

isolated vaginal recurrence but worse overall survival if prior adjuvant radiation therapy was administered. The lack of survival benefit led the authors to conclude that the use of radiation therapy should be limited to those patients at a sufficiently high risk of recurrence (3).

The approach to women with recurrent locoregional disease limited to the pelvis is similar to the management for those with metastatic disease. Particularly for the patient with no prior radiation treatment, there is a role for radiation therapy in the treatment of a local or regional recurrence. Hormonal therapy with progestational agents versus tamoxifen is an alternative option for some women with recurrence. Response to endocrine therapy can be expected to be more favorable in patients with a well-differentiated histology (grade 1 or 2 endometrioid), positive estrogen or progesterone receptor expression, and long disease-free interval. The patient presented in the current case would be expected to have a favorable response to hormonal therapy, given the presence of all three factors in her disease. Carboplatin and paclitaxel regimen is usually recommended as first-line chemotherapy for recurrent endometrial carcinoma (9). Recent research has also shown metformin to be a potent inhibitor of cell proliferation in endometrial carcinoma cell lines. Considering the strong risk factors, obesity and diabetes, that drive the development of type I endometrial carcinomas, Cantrell et al. (10) theorized that metformin potently inhibits growth in a dose-dependent manner via multiple signaling pathways. Future investigations are underway, utilizing metformin as a strategy for endometrial carcinoma prevention and treatment. Finally, the recombinant monoclonal antibody against endothelial growth factor, bevacizumab, appears to be active against recurrent or persistent endometrial carcinoma (objective response rate of 15%) in recent studies. It is currently being evaluated with other agents in a recently closed phase II GOG study (9).

The treatment of patients with recurrent endometrial carcinoma is guided by a number of factors such as disease-free interval, the history of prior therapy, the site of recurrence, and grade and histology. The paucity of prospective randomized data with no treatment regimen demonstrating superiority over another suggests that clinical trials are beneficial for this patient population. The patient presented in this case is being considered for enrollment in a GOG clinical trial for recurrent endometrial carcinoma and will receive 6 cycles of carboplatin and paclitaxel with or without metformin hydrochloride if enrolled.

It can be posited that the etiology of the current disease, which we have considered to be a “recurrence,” may have been due to the malignant transformation of endometriosis and hence represent a metachronous primary disease. With no history of endometriosis and no evidence of ectopic uterine tissue seen on initial laparoscopy or original pathology, the de novo development of endometrioid carcinoma from ectopic endometriosis is unlikely (11). Another possibility to consider is the senescence of tumor cells remaining after initial surgery. The theory of dormant cancer cells postulates that malignant cells can persist for many years in dormancy, neither dividing nor undergoing apoptosis (12).

Sartori et al. (13) demonstrated a significant impact of disease-free interval on the overall survival of patients. Patients with later relapse (>24 months versus <24 months) were shown to have improved 5- and 10-year survival rates along with overall survival. The prognosis for the vast majority of recurrent

endometrial carcinoma patients is generally poor. However, a literature review shows successful salvage therapy for the very few cases reported for recurrences occurring more than 10 years later. Moreover, our patient demonstrates the first case of low risk endometrioid carcinoma with recurrence in the pelvis occurring more than 10 years later that shared a favorable outcome as the studies described previously. This report and review of prior cases, as listed in Table 1, emphasize the possibility of a very late recurrence of endometrial carcinoma despite favorable initial prognostic features. Though these recurrences are unexpected and startling, the good prognosis as previously described can be reassuring to patients and healthcare providers.

Ethics Committee Approval: *N/A.*

Informed Consent: *Written informed consent was obtained from patient who participated in this case.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Concept - A.K., L.C.; Design - A.K.; Supervision - L.C., L.N., T.K.; Resource - L.C., T.K.; Materials - L.C., T.K.; Data Collection and/or Processing - L.C., T.K.; Analysis and/or Interpretation - L.N., L.C., T.K.; Literature Search - A.K., L.N., L.C., T.K.; Writing - A.K., L.N., L.C., T.K.; Critical Reviews - A.K., L.N., L.C., T.K.*

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A novel mutated sequence in the *T-box* transcription factor-5 (*TBX-5*) gene (c.241A>T) in Holt–Oram syndrome

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Abstract

We report a case of a 31-year-old pregnant woman who was admitted to our perinatology outpatient clinic because of a fetal ventricular septal defect and limb reduction in the upper extremities of fetus revealed by ultrasonographic investigation diagnosed in the 16th week of gestation. First child of the family was diagnosed with Holt–Oram syndrome who had atrial septal defect and upper limb anomalies, whereas the father was documented to have arrhythmia and shortening of upper limbs. The pregnancy was terminated in the 16th week of gestation with the consent of the family. We performed mutation analysis in *T-box transcription factor-5 (TBX5)* gene coding exons, including exon/intron boundaries from peripheral blood or skin fibroblasts. The sequence analysis revealed c.241 adenine (A)>thymine (T) [p. arginine (Arg) 81 Tryptophan (Trp)] alteration in exon-3 of the *TBX5* gene in affected family members and fetus. This is a novel mutation causing Holt–Oram syndrome.

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Keywords: Holt–Oram syndrome; novel mutation; TBX5 gene; preimplantation genetic diagnosis

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Introduction

Holt–Oram syndrome (HOS) also called as the heart and hand syndrome or atriodigital dysplasia is an autosomal-dominant, inherited genetic condition that manifests itself as various cardiac malformations and skeletal deformities in the upper extremities (1). Cardiac defects occur in three quarters of the patients with HOS, and they may be structural, such as septal defects or functional, such as arrhythmias (2). Structural cardiac defects are encountered more frequently than functional defects. The most frequent cardiac structural defect is atrial septal defect (ASD) (3). Skeletal defects may be unilateral or bilateral and mostly affects the radial ray of the forearm with its phalanges and the thumb. Hypoplasia or aplasia of the radial, ulnar, or humeral bone, hypoplastic thumb, and absence of the thumb are the most commonly seen characteristic skeletal deformities. The incidence of HOS is 1 in 100,000 live-born babies with a very high penetrance (2). To date, a vast number of mutations have been defined in affected individuals. Approximately 70%–85% of the cases have been attributed to a novel mutation of the *T-box transcription factor-5* (*TBX5*) gene on the long arm of the chromosome 12 (q24.21) (4). *TBX5* is a member of the large T-box transcription factor family, which is present in a wide range of species from worms to humans and known to exert crucial functions in cardiogenesis and skeletal development. In the literature, missense, nonsense, frameshift mutations, splice mutations, and chromosomal rearrangements are defined, which are postulated to cause a disease through *TBX5* haploinsufficiency. Upper extremity anomalies are almost completely penetrant, whereas cardiac anomalies have approximately 75% penetrance (5).

Case Presentation

A 31-year-old Caucasian pregnant woman was referred to our perinatology unit in the 16th week of gestation for advanced fetal investigation for presenting with fetal cardiac and skeletal malformations. No increased risk of chromosomal abnormalities was revealed in the first-trimester screening. An ultrasonographic examination by a senior ultrasonographer revealed a single live fetus compatible with 16 weeks. A ventricular septal defect was located in the membranous septum with a mean diameter of 2.1 mm along with humeral, radial, ulnar aplasia in the left-upper extremity and right radial aplasia seen via ultrasonography. The woman was in a non-consanguineous marriage and had no significant medical or gestational history. Her first child, a 6-year-old girl, had ASD (secundum type, which was recently corrected with an angiographic operation), bilateral radial aplasia, and agenesis

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of thumbs that had been alleviated with two sequential operations known as "index finger pollicization" in the last 2 years. The patient's husband had bradyarrhythmia and needed a pacemaker, and his forearms were short. We learned that his sister also had the same defects. The family was seeking aid to deliver healthy offsprings in the future. Pedigree analysis suggested autosomal dominant inheritance (Figure 1).

The family was informed about fetal anomalies and recurrence risk of future pregnancies. They wanted the pregnancy to be terminated and to receive further genetic evaluation. The postmortem examination of the fetus was in agreement with prenatal ultrasonographic findings, including aplastic radius, aplastic thumb, hypoplastic carpal and metacarpal bones, and radial deviation of the hand on the right-upper extremity; a severely hypoplastic humerus, aplastic radius and ulna, fusion of carpal bones, triphalangeal thumb, and two aplastic fingers on the left side (Figure 2a, b). The ventricular septal defect was confirmed, and no other gross pathology was observed in the postmortem examination. The fetus weighed 150 g. Venous blood sampling was performed in I-2 (the father), I-3 (the mother), and II-1 (the living child) (Figure 1), and dermal tissue sampling in mutation analysis revealed heterozygous c.241A>T alteration in the exon-3 of the *TBX5* gene, causing p.Arg81Trp alteration in the protein. Because we were not able to conduct functional studies to document the effect of this amino acid change in protein, we checked how conserved it is among different species and observed that it was highly conserved (Figure 3). In support of this observation, SIFT (Fred Hutchinson Cancer Research Center, Seattle, WA, USA) (6), and PolyPhen (Department of Genetics, Harvard University, Cambridge, MA, USA) (7) analyses (involving predicting whether an amino acid substitution affects protein function) also indicated that Arg>Trp change at residue 81 would be damaging. A written informed consent was obtained from the patient to present this clinical report. The patient was discharged 2 days after the termination of pregnancy without any problems. We informed the family about the recurrence risk of the disease (50%) and preimplantation genetic diagnosis (PGD) for the determined specific *TBX5* missense mutation prior to future conceptions.

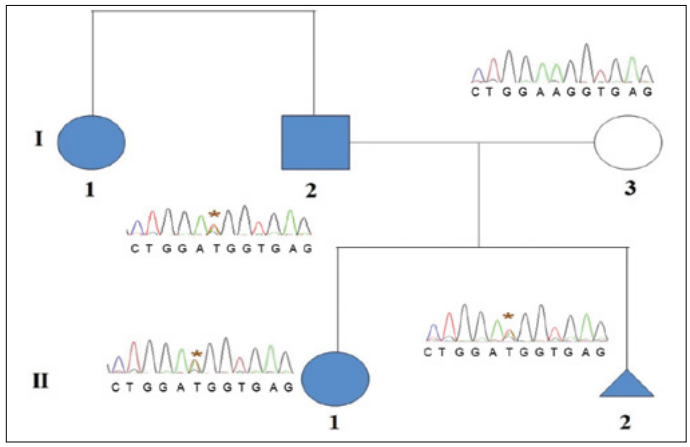

Figure 1. Pedigree is consistent with an autosomal-dominant inheritance pattern. Mutation analysis in affected individuals revealed c.241A>T alteration in exon 3 of *TBX5*

Figure 2. a, b. External view of the fetus (a), X-ray radiogram of the fetus (b)

Discussion

We reported three individuals in the same family with a missense mutation (c.241A>T), causing amino acid change at residue 81 in the T-Box domain. All individuals in the family of the present study had cardiac and limb abnormalities, being less severe in the father and his sister. This observation was in accordance with a previous report in which 19 individuals from the same family had an amino acid change at residue 80, and all of them were found to have cardiac and limb abnormalities (5). These observations provided convincing evidence that alterations around these residues may have more deleterious phenotypic outcomes compared with other residues in *TBX5*.

Individuals with HOS should be properly counseled regarding the disease. As it is clear from the family in the present study, HOS is characterized with full penetrance. We realized that skeletal phenotypes were more severe in fetus and living child than in father, possibly as a result of anticipation. Cardiac and skeletal phenotypes were different between father and two siblings, which is indicative of variable expression in HOS. Molecular genetic analysis is very important to know the type of mutation and which part of the protein is being affected to estimate genotype–phenotype correlation. All of these information will help in discussing the condition with the family in detail during genetic counseling.

This rare genetic syndrome dramatically affects the destiny of families. Sequential cardiac and/or orthopedic operations may be warranted. The family in our case had to travel frequently due to health problems of their first child.

The same kind of mutated sequence may show phenotypic heterogeneity among individuals in a family.

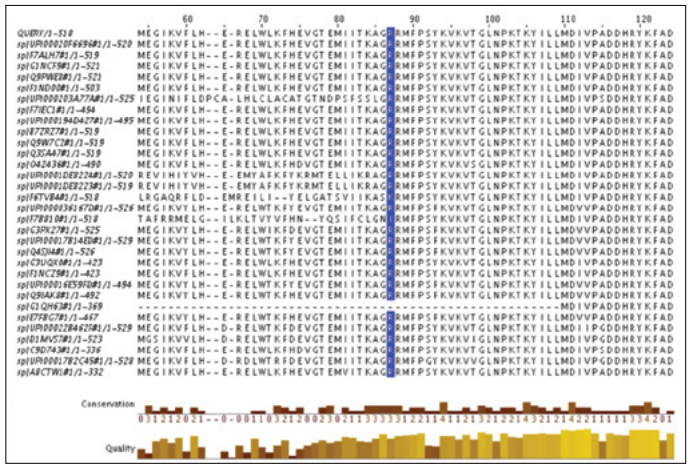

Figure 3. Multiple sequence alignment of TBX5 with its homologs. The altered amino acid is shown to be highly conserved evolutionarily across various species (http://genetics.bwh.harvard.edu)

In this study, the father and his sister had relatively mild cardiac and skeletal defects rather than the affected children. Conduction defects are less frequently seen cardiac problems in HOS (3).

Skeletal abnormalities are frequently more severe in upper-left limbs than in upper-right limbs, and an abnormal carpal bone is present in almost all of the affected cases. Furthermore, we observed these specifications in our case, as defined in the literature (2). Skeletal deformities as well as cardiac malformations were heterogeneous among individuals. The literature review provided no explanation for the predominant involvement of the left side of upper limbs, which therefore remains unclear.

The clinical implications of *TBX5* missense mutations, which are less frequently seen, are not clearly known, but it was denoted that these mutations seem to result in lesser severe cases than other types of mutations, predominantly cardiac or skeletal deformities (5, 8).

In a recent review by Al-Qattan and Abou Al-Shaar, the clinical heterogeneity of HOS was well established. They highlighted that there were three different mutation groups in *TBX5* gene: the first one was a single base change, e.g., missense mutations, causing a specific amino acid deficiency with regard to change in the associated nucleotide; second mutation caused extended protein changes; and third was the intragenic duplication of a region (8). A missense mutation altering an amino acid near the amino-terminal end of the T-box causes more considerable cardiac malformations, while a different missense mutation near the carboxyl end causes predominantly more severe upper limb deformities (frequently left-sided deformity) (5). Extended protein mutations are more inclined to cause severe bilateral skeletal malformations and more severe cardiac anomalies. Intragenic duplications have been reported to entail more severe cardiac anomalies rather than severe skeletal anomalies (8). In a recent case report by Kimura et al. (9), duplication screening was offered for families that had unidentified genetic structure. In the case of the family who presented at our clinic, the novel missense mutation (c.241A>T) seemed to cause both cardiac and skeletal malformations and showed a variable expressivity.

PGD is an advanced technique of molecular biology and genetics. It has been applied with high accuracy in HOS and used on affected families. Its combination with embryo cryopreservation and in vitro fertilization has been reported to be effective for the affected families to have several healthy children (10).

In conclusion, molecular genetic analysis is helpful in the management of inherited conditions such as the situation of the family in our study. Genetic counseling is additionally crucial for affected families. In practice, it merits great importance to inquire genetic conditions in family history, particularly when one of the parents raises suspicion of any genetic syndrome (short upper limb and arrhythmia in the father of the present family). This would help couples plan their pregnancies in advance and reduce emotional and economical damage overall. The severity of cardiac and concurrently skeletal defects should be discussed with the family when a decision remains unclear regarding the termination of pregnancy. PGD is an advanced technique and provides an opportunity to have a healthy baby with a normal genetic sequence. c.241A>T is a novel mutation found in a patient diagnosed with HOS according to our comprehensive literature search.

Ethics Committee Approval: *N/A.*

Informed Consent: *Written informed consent was obtained from patient who participated in this case.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Concept - A.Ö.E., V.T., İ.K.; Design - A.Ö.E., V.T., E.E.; Supervision - A.Ö.E., S.Ö., N.D.; Resource - V.T., İ.K., E.E.; Materials - V.T., İ.K., E.E.; Data Collection and/or Processing - A.Ö.E., S.Ö., N.D.; Analysis and/or Interpretation - V.T., İ.K., S.Ö.; Literature Search - A.Ö.E., E.E., N.D.; Writing - A.Ö.E., V.T., İ.K.; Critical Reviews - E.E., S.Ö., N.D.*

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What is your diagnosis?

A woman aged 51 years was referred to our endocrinology outpatient clinic because of excessive hair growth for seven years. The Ferriman–Gallwey hirsutism score was 36. No abdominal palpable masses were found. Mild clitoromegaly was observed in the pelvic examination. Her hormonal evaluation revealed total testosterone (T) of 761.15 ng/dL (14.2–73.1 ng/dL), dehydroepiandrosterone sulfate (DHEA-SO4) of 160 μg/dL (35–430 μg/dL), 17-hydroxiprogesteron of 3.49 ng/mL, follicle-stimulating hormone of 1.1 IU/L (23.9–119.1 IU/L), luteinizing hormone of 0.2 IU/L (16.3–54.8 IU/L), and estradiol of 56 pg/mL (14.4–44.5 pg/mL). After a 1 mg dose of dexamethasone at night, plasma cortisol levels returned to a normal level of 0.7 mg/dL. A transvaginal ultrasound (US) examination showed uterine fibroid with normal ovaries. Magnetic resonance imaging (MRI) of the abdomen and pelvis revealed that all other organs including the adrenal gland and ovaries were normal (Figure 1, 2).

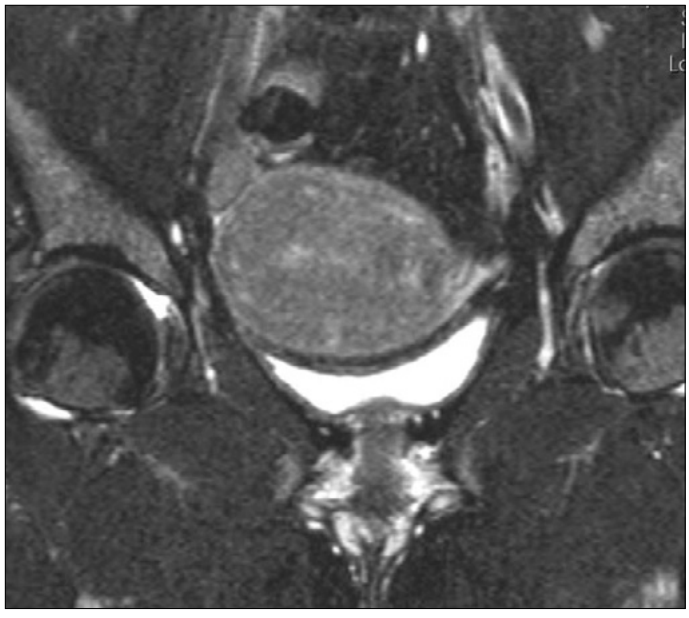

Figure 1. Fat-suppressed T2-weighted coronal pelvic magnetic resonance image shows normal uterus and adnexal structures

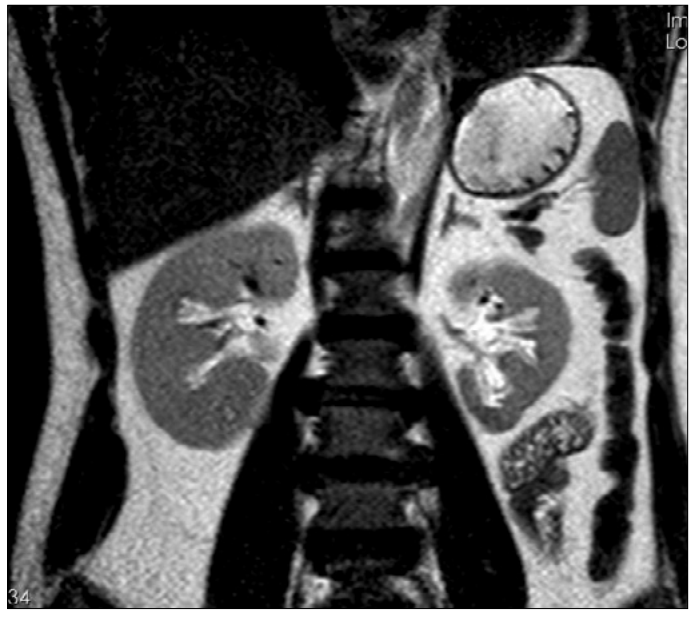

Figure 2. T2-weighted coronal pelvic magnetic resonance image shows normal adrenal gland

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Answer

Clinical findings of severe virilization in women with markedly increased serum androgens are indicative of androgen-secreting tumors, which may arise from the ovaries or adrenals (1). Leydig cell tumors are functioning testosterone-producing benign ovarian tumors that lead to hyperandrogenism and virilization (2). Diagnosis is often very challenging because Leydig cell tumors are small and are frequently missed with conventional imaging techniques owing to their size (3).

Our patient's DHEA-SO4 levels were within the normal range, whereas the testosterone and estrogen levels were significantly elevated, which was suggestive of a tumor of ovarian origin. No lesion was observed in the transvaginal US and pelvic MRI. Selective ovarian venous hormonal catheterization (SOVHS) was used to assist diagnosis and localization of androgen-secreting tumors. SOVHS is recommended when tumor identification remains unclear (4). When small androgen-producing tumors are suspected clinically and biochemically in postmenopausal patients, laparoscopic or open bilateral adnexectomy is recommended in the literature because conventional imaging is often unable to detect this variety of tumor (5, 6). Localization of the tumor was provided according to the hormonal findings obtained with SOVHS. An alternative method that can assist the localization of androgen-producing tumors is preoperative measurement of T levels in the ovarian vein (7).

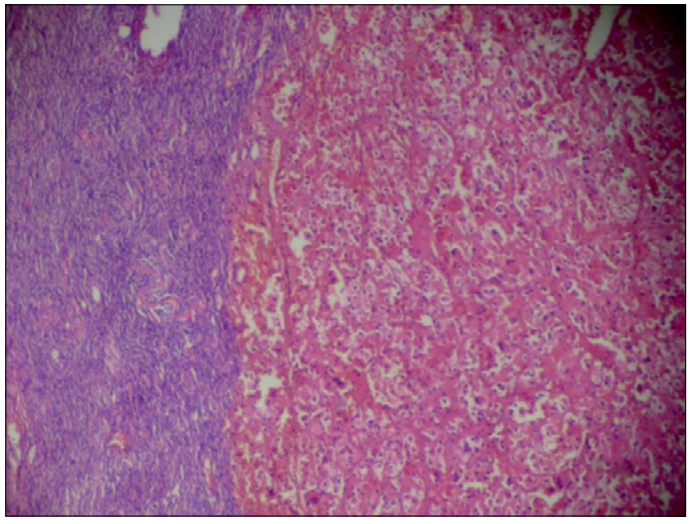

Figure 3. Normal ovarian tissue observed on the left side. Eosinophilic and vacuolated cytoplasm of tumor cells was seen on the right side (10X, H.E.)

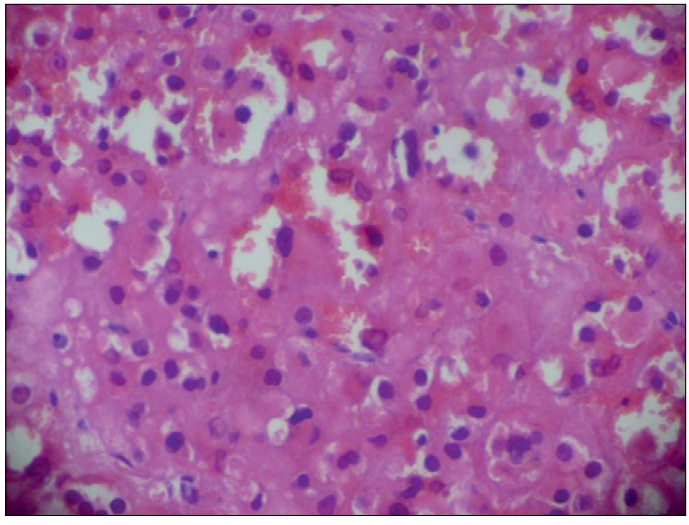

Figure 4. Eosinophilic cytoplasm of tumor cells and Reinke crystals in the cytoplasm were observed (20×, H.E.)

Hyperandrogenism is frequently observed in polycystic ovary syndrome (PCOS). Speed of virilization onset (<2 years) or late onset in life is a better predictor of neoplasm than androgen levels (8). Our patient was diagnosed with PCOS and received treatment accordingly. However, she developed hyperandrogenism associated with serious atherosclerotic vascular disease. Six months later she underwent surgery after being diagnosed with an ovarian tumor (Figure 3, 4). The differential diagnosis of PCOS and ovarian or adrenal tumors must be successfully evaluated and SOVHS should be remembered in challenging tumor localization.

This report demonstrates effective preoperative use of SOVHS in localization of an androgen-secreting ovarian tumor that could not be identified using conventional imaging. Early diagnosis of this tumor is very important to decrease the risk of cardiovascular and cerebrovascular events.

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