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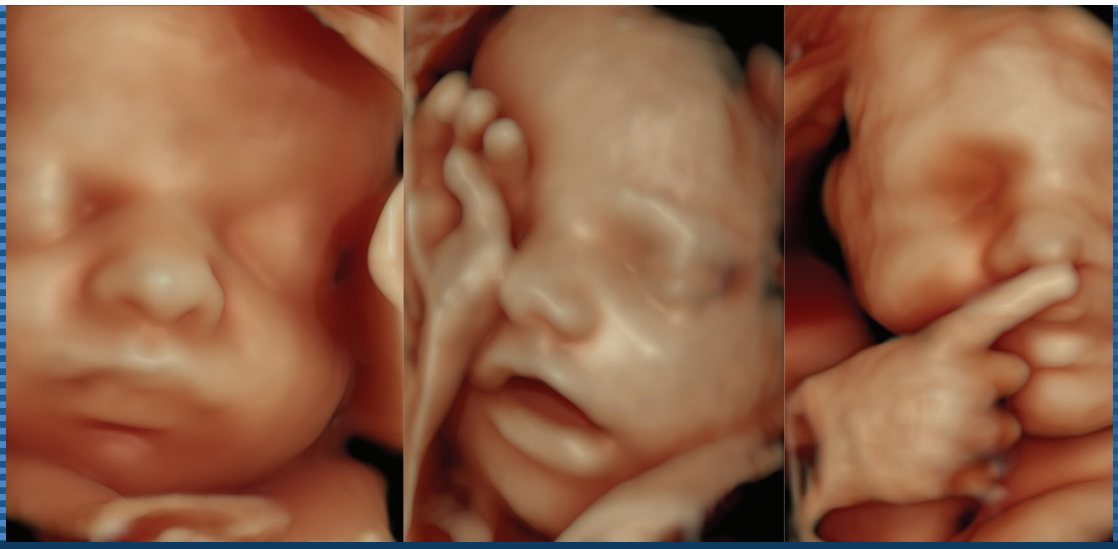

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Aims and Scope

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

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

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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.

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

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

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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. The abstract should be limited to 250 words for original articles and review articles.

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

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

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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.

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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.

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

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

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Contents

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Editorial

Dear Colleagues,

I am delighted to introduce the first issue of the *J Turk Ger Gynecol Assoc* in the publishing year of 2017.

The *J Turk Ger Gynecol Assoc* is the leading general clinical journal covering the whole world. It publishes peer reviewed original research articles, as well as a wide range of news, book reviews, biographical, historical and educational articles and a lively correspondence section. Fields covered include obstetrics, prenatal diagnosis, maternal-fetal medicine, perinatology, general gynecology, gynecologic oncology, uro-gynecology, reproductive medicine, infertility, reproductive endocrinology, sexual medicine and reproductive ethics. The *J Turk Ger Gynecol Assoc* provides a forum for scientific and clinical professional communication in obstetrics and gynecology throughout Turkey and the world.

J Turk Ger Gynecol Assoc has been in PubMed Central. Besides it is indexed in PubMed Central, EMBASE, Scopus, CINAHL, Gale/Cengage Learning, EBSCO, DOAJ, ProQuest and Index Copernicus, I am very glad and proud to say that it has been accepted for Emerging Sources Citation Index (ESCI).

The ESCI was launched in late 2015 as a new database within Clarivate Analytics' (formally Thomson Reuters') Web of Science (WoS). Around 3,000 journals were selected for coverage at launch, spanning the full range of subject areas. As of February 2017, the database contains 5,578 journals. Indexing in the ESCI will improve the visibility of our journal, provides a mark of quality and is good for authors. We have already seen examples of institutions and funders suggesting publication in an ESCI listed journal, similar to what already takes places with other WoS databases.

Dear Young Researchers,

Successful production of a written product for submission to a peer-reviewed scientific journal requires substantial effort. Clear communication of the findings of research is essential to the growth and development of science and professional practice. To begin it might be interesting to learn why reviewers accept manuscripts! Reviewers consider the following five criteria to be the most important in decisions about whether to accept manuscripts for publication:

1) The importance, timeliness, relevance, and prevalence of the problem addressed;
2) The quality of the writing style (i.e., that it is well-written, clear, straightforward, easy to follow, and logical);
3) The study design applied (i.e., that the design was appropriate, rigorous, and comprehensive);
4) The degree to which the literature review was thoughtful, focused, and up-to-date; and
5) The use of a sufficiently large sample.

For these statements to be true there are also reasons that reviewers reject manuscripts. The following are the top five reasons for rejecting papers:

1) Inappropriate, incomplete, or insufficiently described statistics;
2) Over-interpretation of results;
3) Use of inappropriate, suboptimal, or insufficiently described populations or instruments;

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4) Small or biased samples; and

5) Text that is poorly written or difficult to follow.

With these reasons for acceptance or rejection in mind, it is time to review basics and general writing tips to be used when performing manuscript preparation.

We have an interesting manuscript from India that highlighted urinary fistula. You will be able to read a manuscript from USA that searchs “Effect of sacrocolpopexy and retropubic sling on overactive bladder symptoms”. CIN I regression is possible? You will see the answer in the journal. “Maternal mortality due to hypertensive disorders in pregnancy, childbirth, and the puerperium between 2012 and 2015 in Turkey: A nation-based study” will be very important paper for reading and understanding the most dangerous situation in these high risk pregnant patients. We will see a manuscript that investigates whether serum levels of estradiol affect reproductive outcomes of normoresponder women undergoing fresh embryo transfer versus frozen-thawed embryo transfer. Controlled ovarian hyperstimulation is a key step for successful outcomes of assisted reproductive technique cycle outcomes. Many medications are available, which are commonly used solely or in combination to achieve multiple follicular development. We will be able to evaluate the current literature to determine the most reliable and relevant information about the most used ovulation induction drugs with an interesting review. I hope you will see and read all other manuscripts either from local or international like USA, India, Spain and Greece. Please also enjoy solving a challenging quiz.

We are very proud to announce our 12th Turkish - German Gynecology Congress which will be held in Cyprus between April 27 and May 1 of 2018. We are confident that this global meeting in Cyprus will attract many participants. As usual the high standard of the scientific program will be attractive for the international gynecology and obstetrics community world and we look forward to welcoming you to Cyprus.

I would like to wish you a happy new scientific year in 2017 and we are looking forward to receiving your valuable submissions.

Sincerely,

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

Do clinical data and human papilloma virus genotype influence spontaneous regression in grade I cervical intraepithelial neoplasia?

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Abstract

Objective: To determine whether medical history, clinical examination and human papilloma virus (HPV) genotype influence spontaneous regression in cervical intraepithelial neoplasia grade I (CIN-I).

Material and Methods: We retrospectively evaluated 232 women who were histologically diagnosed as have CIN-I by means of Kaplan-Meier curves, the pattern of spontaneous regression according to the medical history, clinical examination, and HPV genotype.

Results: Spontaneous regression occurred in most patients and was influenced by the presence of multiple HPV genotypes but not by the HPV genotype itself. In addition, regression frequency was diminished when more than 50% of the cervix surface was affected or when an abnormal cytology was present at the beginning of follow-up.

Conclusion: The frequency of regression in CIN-I is high, making long-term follow-up and conservative management advisable. Data from clinical examination and HPV genotyping might help to anticipate which lesions will regress. (J Turk Ger Gynecol Assoc 2017; 18: 1-8)

Keywords: Human papilloma virus, cervical intraepithelial neoplasia grade I, human papilloma virus genotyping, regression, low-grade squamous intraepithelial lesion

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Introduction

Cervical cancer and its precancerous lesions represent a major health issue, which needs early intervention in order to prevent high morbidity and mortality rates. Extensive research has revealed the existence of a close relationship between the onset of malignant lesions in the female tract and the presence of human papilloma virus (HPV) (1), especially regarding high oncogenic risk genotypes (2, 3). The natural history of HPV infection indicates that following an initial HPV infection, a number of patients develop low-grade squamous intraepithelial lesions (LSIL), also known as cervical intraepithelial neoplasia grade I (CIN-I) (4), which occasionally progresses to high-grade squamous intraepithelial lesion (HSIL) (5, 6) requiring exhaustive management and follow-up. However, the majority of CIN-I lesions regress without medical intervention, making treatment at this stage superfluous and cost-ineffective (7). Consequently, over-treatment at early stages should be avoided (8), especially in young women, and follow-up periods should be encouraged to make HPV clearance and histologic regression amenable (9).

The ability to identify patients whose lesions regress in advance would help to diminish costs, increase follow-up intervals, and decrease morbidity resulting from invasive diagnosis and unnecessary treatments (10-13). However, the means to anticipate this information are still unavailable. The aim of this study was to determine whether clinical information (medical history and examination) and HPV genotyping were useful to predict the frequency of regression and the need of treatment in CIN-I (14-16).

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Material and Methods

We retrospectively studied 232 women who were histologically diagnosed as having CIN-I at cervical pathology unit between 1995 and 2007. Most patients were referred for consultation because of an altered cytology. All women's medical histories and clinical examinations including an initial cytology and colposcopy were evaluated. We took a biopsy in the first consultation to make the CIN-I diagnosis. The rest of the follow-ups were done using cytology. The patients were subsequently followed up every 6 months during the first two years, checking for the presence of progression, persistence or regression. When cytology results were normal, annual follow-ups were done for 5 additional years in some cases. CIN-I regression was defined as the disappearance of the lesion without treatment after two consecutive negative follow-ups, considering a negative follow up as both normal cytology and colposcopy, or in cases of altered colposcopy, negative follow up was considered as negative cytology and biopsy. If the cytology follow up resulted in HSIL, the biopsy was repeated. Progression was diagnosed if any change to HSIL was histologically detected. Finally, persistence was considered if no progression or regression was observed during the two-year follow-up. In case of persistence, appropriate treatment was applied.

Unfortunately, being a retrospective analysis, many exceptions applied. These circumstances determined the method of analysis, using Kaplan-Meier curves and excluding contingency tables to avoid comparison biases among the different clinical situations and HPV genotypes. A number of persistent lesions were followed up without treatment for more than 2 years and were seen to regress months afterwards. According to the Kaplan-Meier analysis, these cases were considered "still alive" at the end of the study and were consequently censored data (value=0). Some lesions were precipitately treated in the first or second year of follow-up and we therefore disregarded whether they regressed afterwards. According to the Kaplan-Meier analysis, these cases were considered as cases that "dropped off the study" and were consequently again treated as censored data (value=0). A third situation was when lesions progressed within the two-year follow-up interval and were treated. We considered them in the same group as those that dropped off the study, and were also classified as censored data (value=0). The time value in months assigned to these censored data was either the month of treatment or the limit of the study in case of follow-up for more than 2 years. Kaplan-Meier curves evaluated the pattern of regression using the log-rank (Mantel-Cox) test and also provided the median survival and hazard ratio with their 95% confidence intervals.

Regarding HPV, we evaluated the frequency of the different HPV genotypes (6, 11, 16, 18, 31, 33, 51, and 53) and also the different combinations of HPV genotypes (low- and high-risk HPV, single and multiple HPV). Regression was also evaluated in women with different clinical characteristics including age (<25, 25-34, 35-44, 45-54, and ≥55 years), menopause, age of first intercourse, parity, cigarette smoking, oral contraceptive use, condom use, of intrauterine device (IUD) use, results of the cytologic and colposcopic examination, and cervical extension of colposcopic findings.

Exclusion criteria were: pregnancy, immunodeficiency, existence of concurrent vaginal lesions, follow-up less than two years, lack of data in the patient files, inability to obtain DNA, and treatment immediately after the diagnosis of CIN-I.

HPV genotype was obtained by means of polymerase chain reaction (PCR) according to earlier descriptions using a commercially available kit, SPF10 primers and line probe assay detection system (INNO-LiPA) HPV Genotyping Extra Amp. (Innogenetics, Ghent, Belgium). This system allowed the identification of 9 low-risk HPV genotypes (6, 11, 34, 40, 43, 44, 54, 70, 71), 16 high-risk HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 69, 73, 82) and 3 probable high-risk HPV genotypes (26, 53, 66). The kit also determined additional HPV genotypes (69/71/74) and unclassified HPV genotypes (types X).

The study was undertaken with a protocol authorized by the hospital. Statistics and graphs were constructed using Graph Pad Prism 5.0 (Graph Pad Software, La Jolla, CA, USA). Statistical significance was established at $p<0.05$.

Results

A description of the studied population at the onset of follow-up is shown in Table 1. A total of 232 women were included with a mean age of 34.6 years. Of them, 15 (6.5%) were menopausal, 55 (23.7%) had their first sexual intercourse prior to age 18 years, and 140 (60.3%) had delivered at least once. The most frequent family planning method was the condom, which was used by 88 women (37.9%), followed by hormonal contraception used by 47 women (20.2%), surgical methods 30 (12.9), and IUDs 19 (8.2). The majority of patients (n=218, 94%) were referred due to an abnormal cytology in the absence of any other symptoms, but only 162 (69.8%) presented with this finding at the onset of follow up. Other patients presented with vaginitis (4.3%), postmenopausal bleeding (0.4%) or intercourse bleeding (1.3%). Nearly all patients had abnormal colposcopy (94.4%), 68.1% of which was due to low risk changes. Finally, half of the patients (59.5%) smoked.

HPV infection was found in 224 patients (96.5%). Of these, 212 (91.4%) presented one or several high-risk HPV, 68 (29.3%) had one or several low-risk HPVs, and 4 (1.7%) additional or X HPV. Multiple HPV genotypes were found in 125 women (53.9%) with the following distribution: two different HPV genotypes in 81 women (34.9%), three in 25 (10.8%), four in 11 (4.7%), five in

Table 1. Clinical description of the population with histologic diagnosis of cervical intraepithelial neoplasia grade I

Clinical data	n	%
Age (mean 34.6)		
<25	29	12.5
25-34	91	39.2
35-44	81	34.9
45-54	29	12.5
≥55	2	0.9
Menopause		
At pre-menopause	217	93.5
Already at menopause	15	6.5
Onset of sexual intercourse		
First intercourse before age 18 years	55	23.7
First intercourse at or after age 18 years	177	76.3
Parity		
No children	92	39.7
At least 1 child	140	60.3
Smoking		
No smoking	94	40.5
Less than 10 cigarettes per day	67	28.9
Between 10-20 cigarettes per day	55	23.7
More than 20 cigarettes per day	16	6.9
Family planning method		
Condom	88	37.9
Oral contraceptives	47	20.2
No family planning methods	36	15.5
Surgical method	30	12.9
IUD	19	8.2
No intercourse	8	3.4
Presence of clinical symptoms		
Absence of symptoms	218	94
Vaginitis	10	4.3
Intercourse bleeding	3	1.3
Postmenopausal bleeding	1	0.4
Cytology results		
Normal cytology	70	30.2
ASCUS	23	9.9
AGUS	1	0.4
Low-risk SIL	116	50.0
High-risk SIL	22	9.5
Colposcopy results		
Normal colposcopy	13	5.6
Metaplasia	50	21.6
ATZ + Low-risk colposcopic changes	158	68.1
ATZ + High-risk colposcopic changes	11	4.7
Colposcopy image: percentage of cervix affected		
Less than 50% of the cervix surface affected	171	73.7
More than 50% of the cervix surface affected	61	26.3

IUD: intrauterine device; ASCUS: atypical squamous cells of undetermined significance; AGUS: atypical glandular cells of undetermined significance; SIL: squamous intraepithelial lesion; ATZ: atypical transformation zone.
Cytology and colposcopy were performed in unison in all patients.
Only patients with histologic diagnosis of cervical intraepithelial neoplasia grade I were included.
In patients with normal colposcopy, an endocervical curettage was subsequently performed.
In 171 (73%) patients atypical transformation zone extended to less than 50% of cervical surface. These included cases where lesion extended into the cervical channel.

6 (2.6%), seven in 1 (0.4%), and eight in 1 (0.4%). Table 2 shows the percentage of infection caused by each virus, isolated or combined with other HPV genotypes. The three most frequent high-risk HPV genotypes were HPV 16, 51, and 53. In 99 (42.7%) women, the etiology of infection was HPV 16. Of those, 38 (16.4% of the total number) presented with HPV16 as the only viral genotype. For HPV 51, this occurred in 46 (19.8%) and 18 (7.7%) cases, and for HPV 53 in 44 (19%), and 8 (3.4%) cases. In 12 women (5.2%), both HPV 16 and 18 were found. HPV 45 was found in only 2 (0.9%) women and always in association with other viral genotypes. In 99 (42.6%) women, HPV infection was caused by other high-risk viruses different to HPV 16 and 18. Finally, the most frequent low-risk virus was HPV 11 and less frequently, HPV 6.

Of the 232 CIN-I lesions, 116 regressed in the 2-year follow-up and 9 reached the end of the interval without regression. In addition, 93 were precipitately treated within the two years of follow-up and 14 progressed to HSIL and were treated during the follow-up interval. These last three groups were considered as censored data in Kaplan-Meier analyses.

The analysis of the clinical parameters (Figure 1) showed that the regression frequency was significantly lower only in women who had an abnormal cytology at the onset of follow-up or who showed colposcopical abnormalities on more than 50% of the cervical surface. However, there was large influence on regression of characteristics related with sexuality such as condom use and age at first sexual intercourse, but it did not reach significance ($p=0.0565$ and $p=0.0741$). Influence of other characteristics such as age, colposcopical images (high-versus low-risk changes and normal versus abnormal findings), contraceptives use, IUD use, parity, menopause and smoking did not reach statistical significance. Comparison statistics are described in Table 3.

With regards to the HPV genotypes, the analysis showed that regression frequency was significantly influenced only in the presence of multiple combinations of high-risk HPV

Table 2. Distribution of human papilloma virus infection. n may reflect, isolated or combined human papilloma virus genotypes

HPV infection according to genotype	n	%
HPV infection (isolated or combined with similar risk HPV)		
All HPV genotypes	232	100.0
High-risk HPV	212	91.4
Low-risk HPV	8	3.4
No HPV infection	8	3.4
Additional HPV, HPV X	4	1.7
Most frequent isolated HPV infection		
HPV 16	38	16.4
HPV 51	18	7.7
HPV 31	13	5.6
HPV 53	8	3.4
HPV 33	6	2.6
HPV 18	5	2.1
HPV 11	5	2.1
HPV 6	2	0.9
High-risk HPV infection (isolated or combined with other High-risk-HPV)		
HPV 16	99	42.7
HPV 51	46	19.8
HPV 53	44	19.0
HPV 31	32	13.8
HPV 18	26	11.2
HPV 33	18	7.8
HPV 58	17	7.3
HPV 52	15	6.5
HPV 39	14	6
HPV 66	12	5.2
HPV 56	11	4.7
HPV 68	11	4.7
HPV 35	5	2.2
HPV 82	4	1.7
HPV 45	2	0.9
HPV 26	1	0.4
Low-risk HPV infection (isolated or combined with other Low-risk-HPV)		
HPV 11	22	9.5
HPV 6	5	2.2
HPV 44	4	1.7
HPV 54	4	1.7
HPV 70	3	1.3
X and additional HPV infection (isolated or combined with similar HPV)		
HPV X (Unknown HPV)	11	4.7
HPV 69/71	6	2.6
HPV 74	2	0.9
Specific combination of HPV genotypes		
Low risk HPV + additional HPV + HPV X	12	5.2
HPV 16 + any other HPV	87	37.5
HPV 16 + any other High-risk HPV	49	21.1
HPV 18 + any other HPV	14	6.0
HPV 18 + any other High-risk HPV	9	3.9
HPV 16 + HPV 18	12	5.2
High-risk HPV different to HPV 16 and HPV 18	99	42.7

HPV: human papilloma virus

(p=0.0353). Neither the presence of high-risk HPV versus low-risk HPV (p=0.1717) or the different combinations of high-risk HPV (p=0.4307) or the sum of any low-risk plus any high-risk HPV (p=0.4667) were seen to influence regression (Figure 2). Comparison statistics are also described in Table 3. The probabilities of CIN-I regression in relation to each HPV genotype, HPV16/18 or HPV 16/18/31/33/35/51/53/45/52/58 are shown in Table 4.

Discussion

Our results show that most of the CIN-I lesions disappeared in the two-year follow-up period, which is in line with earlier works quoting similar percentages (17-20). Although HPV is the etiology of cervical cancer and intraepithelial neoplasia, the importance of the different viral genotypes is unclear (21). According to our data, 96.6% of CIN-I had HPV infection, HPV 16 being the most frequent genotype. This agreed with earlier investigations although with slightly lower percentages (22, 23). Also, although HPV 18 was the world's second most frequent HPV genotype (24), it represented only 11.2% of our cases, a lower frequency than that of HPV 51 (19.8%), HPV 53 (19%), and HPV 31 (13.8%), in line with other works (25, 26).

Our results showed that HPV genotype (including HPV 16 and 18) was not a determinant of regression frequency as the survival curves did not show significant differences either in the pattern of regression between HPV 16/18 and the remaining high risk HPV or in the pattern of regression between high and low risk HPV. Concerning earlier references, very few studies examined the influence of the viral type in relation with regression. In addition, previous results were unclear. Some works showed a higher progression to CIN-III for HPV 16 (27), a higher progression to CIN-III and cervical cancer for HPV 16 (28)

Table 3. Kaplan-Meier statistics for the different parameters analyzed in Figures 1, 2

(Figure number) Parameters analyzed	n	n regression	Mean RT	Ratio	95 CI-R	HzR	95 CI HzR	p-value
(1) Colposcopy: cervical surface affected >50%	61	25	21	1.7	1.13-2.37	0.53	0.34-0.81	0.0037
(1) Colposcopy: cervical surface affected <50%	171	100	12					
(1) Cytology at the onset of follow-up: abnormal	162	71	13	1.3	0.61-1.99	0.47	0.31-0.71	0.0004
(1) Cytology at the onset of follow-up: normal	70	54	10					
(1) Colposcopy: normal or metaplasia	63	37	12	1	0.33-1.67	1.31	0.84-2.03	0.2356
(1) Colposcopy: low and high-risk changes	169	88	12					
(1) Colposcopy: LR changes	158	85	12	0.6	0.35-0.84	2.14	0.87-5.28	0.0970
(1) Colposcopy: HR changes	11	3	20					
(2) Oral contraception: no	185	99	12	1	0.36-1.64	1.01	0.63-1.61	0.9682
(2) Oral contraception: yes	47	26	12					
(2) Intercourse onset below 18 years old	55	23	14	1.17	0.55-1.78	0.66	0.42-1.04	0.0741
(2) Intercourse onset at or after 18 years old	177	102	12					
(2) Condom use: no	146	71	13	1.3	0.61-1.99	0.67	0.45-1.01	0.0565
(2) Condom use: yes	86	54	10					
(2) IUD use: no	213	117	12	1	0.51-1.49	1.37	0.71-2.66	0.8810
(2) IUD use: yes	19	8	12					
(3) Pregnancies: no children	92	58	11	0.85	0.15-1.54	1.31	0.88-1.94	0.1818
(3) Pregnancies: at least 1 children	140	67	13					
(3) Menopause: yes	15	7	14	1.17	0.70-1.63	1.03	0.45-2.34	0.9365
(3) Menopause: no	217	18	12					
(3) Smoking: yes	138	73	12	1	0.31-1.69	0.98	0.66-1.45	0.9316
(3) Smoking: no	94	52	12					
(3) Age: 34 or less	123	66	11	0.85	0.15-1.54	1.10	0.75-1.63	0.2590
(3) Age: >34	109	59	13					
(4) High risk HPV	212	112	12	1.09	0.53-1.65	0.61	0.30-1.24	0.1717
(4) Low risk, X HPV, additional HPV, no HPV	20	13	11					
(4) High risk HPV: 16 and/or 18	113	58	12	1.08	0.40-1.76	0.85	0.56-1.28	0.6209
(4) High risk HPV: other HR HPV	99	54	13					
(4) Multiple HR HPV infection	125	63	14	1.00	0.46-1.54	1.26	0.67-2.36	0.5298
(4) Multiple HR, LR, X, and additional HPV	212	119	14					
(4) Single HR HPV	87	49	10	1.40	0.75-2.05	0.62	0.39-0.97	0.0353
(4) Multiple HR HPV	125	63	14					

HR: high risk; LR: low risk; mean RT: mean regression time in months; 95 CI-R: 95% confidence interval of the ratio; HzR: hazard ratio; 95 CI-HzR: 95% confidence interval of the Hazard ratio; p-value: p-value according to the log-rank (Mantel-Cox) test.

or a higher progression to CIN-III for HPV 16, 18, 31, 33, 35, 45, 52, and 58 (29). Conversely, others showed no increase to CIN-III for HPV 16/18 when compared with other high-risk HPV (30). Regarding the combination of different HPV, our analysis showed that the number of high-risk viruses present in the epithelium (single or multiple high-risk HPV infections) influenced the frequency of regression. This was not in line with some previous works (31), but agreed with others that found differences, especially when one of the viruses was HPV 16 (32-34).

The method by which the sample was obtained (cytology or biopsy) and the PCR method applied are also important issues, because they could influence the number of HPV genotypes detected (35, 36). We applied SPF10 primers, genotyping with PCR-SPF10/LiPA. This was an excellent method to detect single and multiple HPV infection in paraffin fixed tissues (37-39), thanks to which we were able to identify HPV in a very high percentage of cases.

In consideration to clinical parameters, it has been suggested that smoking, use of oral contraceptives, sexual behavior,

parity, and age at first sexual intercourse had an influence on the natural history of HPV intraepithelial lesions. However, most studies dealt with the influence on progression and very few on the influence on regression. Our data, in line with earlier works (27), showed a high influence of the onset of sexual intercourse or the use condom during sexual relationships, which may have yielded significant results with a slightly higher number of patients.

Although colposcopy is an excellent method to investigate abnormal cytologies (40), it cannot substitute histologic evaluation. Our data did not support an influence of colposcopy findings (suggesting lower or higher grade lesions) on the frequency of regression. However, similar to earlier works (41), the presence of a more extended lesion (>50% of the cervix surface) indicated that this was less likely to regress. Finally, we showed that regression was less frequent when anomalies were present in the initial cytology. Similar results were obtained in earlier studies, which showed a higher progression to CIN-III in these cases (42).

Finally, several strengths and limitations in the study should be underlined. Concerning the strengths: 1- We used only histologic diagnosis (a biopsy indicating CIN-I) avoiding the high false positive rate of cytology. 2- Our progression reference was CIN-III, avoiding the low correlation among pathologists in CIN-II lesions (43, 44). 3- The PCR method applied allowed a wide identification of HPV genotypes and the detection of multiple HPV infections with high sensibility, specificity, and celerity. The number of cases was also low. In addition, many lesions were precipitately treated prior to end of follow up, and

Table 4. Probabilities of cervical intraepithelial neoplasia grade I regression in relation to human papilloma virus genotypes

Human papilloma virus genotype	n patient	Regression %
6	2	50%
11	5	60%
16	35	51%
18	6	11%
31	13	69%
33	6	17%
51	18	61%
53	8	62%
16/18	113	51%
16/18/31/33/35/51/53/45/52/58	212	52.8%

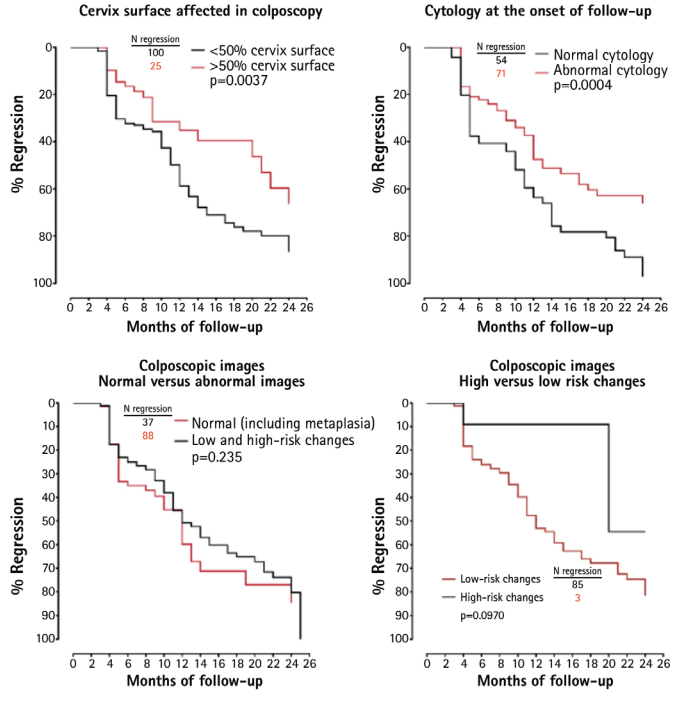

Figure 1. Kaplan-Meier curves evaluating the regression frequency of the cervical intraepithelial neoplasia grade I infections according to the percentage of cervical surface affected, the existence of an abnormal cytology at the onset of follow up, and the presence of different images (normal versus abnormal and high- versus low-risk changes) in the colposcopic examination

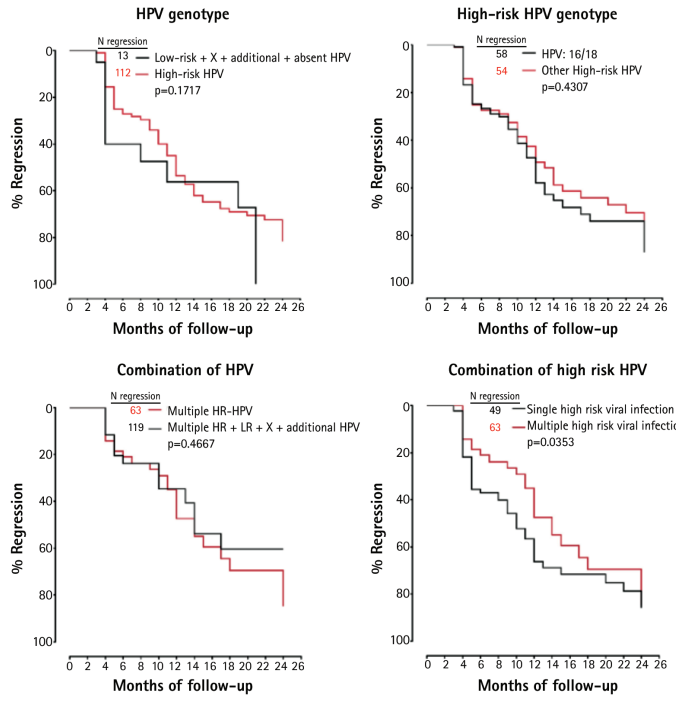

Figure 2. Kaplan-Meier curves evaluating the regression frequency of cervical intraepithelial neoplasia grade I infections according to the human papilloma virus genotype: high-risk versus low-risk, high-risk versus other high-risk, multiple high-risk versus any other multiple and finally single versus multiple high-risk human papilloma virus infection

therefore, the information of what would have happened if the follow-up had continued was unavailable.

In summary, most CIN-I lesions tend to a spontaneously resolve in the first two years of follow-up. However, the existence of abnormal cytology, an infection in more than 50% of the cervical surface or the presence of multiple viral high-risk HPV genotypes might influence the frequency of regression making this less likely.

Ethics Committee Approval: *Ethics committee approval was received for this study from the ethics committee of Complex Hospital of Albacete University (Number: 06/09, Date: 25.06.2009).*

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

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References

1. Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 1999; 189: 12-9.
2. Bosch FX, Lorincz A, Muñoz N, Meijer CJ Shah KV. The causal relation between human papillomavirus and cervical cancer. J Clin Pathol 2002; 55: 244-65.
3. Schiffman M, Castle PE, Jeronimo J, Rodriguez AC, Wacholder S. Human papillomavirus and cervical cancer. Lancet 2007; 370: 890-907.
4. IARC monographs on the evaluation of. Human Papillomaviruses/ IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Lyon, France; 2007.
5. Ostör AG. Natural history of cervical intraepithelial neoplasia: a critical review. Int J Gynecol Pathol 1993; 12: 186-92.
6. Khan MJ, Castle PE, Lorincz AT, Wacholder S, Sherman M, Scott DR, et al. The elevated 10-year risk of cervical precancer and cancer in women with human papillomavirus (HPV) type 16 or 18 and the possible utility of type-specific HPV testing in clinical practice. J Natl Cancer Inst 2005; 97: 1072-9.
7. Ho GY, Bierman R, Beardsley L, Chang CJ, Burk RD. Natural history of cervicovaginal papillomavirus infection in young women. N Engl J Med 1998; 338: 423-8.
8. Insinga RP, Glass AG, Myers ER, Rush BB. Abnormal outcomes following cervical cancer screening: event duration and health utility loss. Med Decis Mak 2007; 27: 414-22.
9. Moscicki AB, Hills N, Shiboski S, Powell K, Jay N, Hanson E, et al. Risks for incident human papillomavirus infection and low-grade squamous intraepithelial lesion development in young females. JAMA 2001; 285: 2995-3002.
10. Koutsky L. Role of epidemiology in defining events that influence transmission and natural history of anogenital papillomavirus infections. J Natl Cancer Inst 1991; 83: 978-9.
11. Nobbenhuis MA, Walboomers JM, Helmerhorst TJ, Rozendaal L, Remmink AJ, Risse EK, et al. Relation of human papillomavirus status to cervical lesions and consequences for cervical-cancer screening: a prospective study. Lancet 1999; 354: 20-5.
12. Koshiol J, Lindsay L, Pimenta JM, Poole C, Jenkins D, Smith JS. Persistent human papillomavirus infection and cervical neoplasia: a systematic review and meta-analysis. Am J Epidemiol 2008; 168: 123-37.
13. Chen HC, Schiffman M, Lin CY, Pan MH, You SL, Chuang LC, et al. Persistence of type-specific human papillomavirus infection and increased long-term risk of cervical cancer. J Natl Cancer Inst 2011; 103: 1387-96.
14. Quint WG, Scholte G, van Doorn LJ, Kleter B, Smits PH, Lindeman J. Comparative analysis of human papillomavirus infections in cervical scrapes and biopsy specimens by general SPF(10) PCR and HPV genotyping. J Pathol 2001; 194: 51-8.
15. Melchers WJ, Bakkers JM, Wang J, de Wilde PC, Boonstra H, Quint WG, et al. Short fragment polymerase chain reaction reverse hybridization line probe assay to detect and genotype a broad spectrum of human papillomavirus types. Clinical evaluation and follow-up. Am J Pathol 1999; 155: 1473-8.
16. Holowaty P, Miller AB, Rohan T, To T. Natural history of dysplasia of the uterine cervix. J Natl Cancer Inst 1999; 91: 252-8.
17. Melnikow J, Nuovo J, Willan AR, Chan BK, Howell LP. Natural history of cervical squamous intraepithelial lesions: a meta-analysis. Obstet Gynecol 1998; 92: 727-35.
18. Song SH, Lee JK, Oh MJ, Hur JY, Park YK, Saw HS. Risk factors for the progression or persistence of untreated mild dysplasia of the uterine cervix. Int J Gynecol Cancer 2006; 16: 1608-13.
19. Bansal N, Wright JD, Cohen CJ, Herzog TJ. Natural history of established low grade cervical intraepithelial (CIN 1) lesions. Anticancer Res 2008; 28: 1763-6.
20. Ho GYF, Einstein MH, Romney SL, Kadish AS, Abadi M, Mikhail M, et al. Risk factors for persistent cervical intraepithelial neoplasia grades 1 and 2: managed by waiting. J Low Genit Tract Dis 2011; 15: 268-75.
21. Guan P, Howell-Jones R, Li N, Bruni L, de Sanjosé S, Franceschi S, et al. Human papillomavirus types in 115,789 HPV-positive women: a meta-analysis from cervical infection to cancer. Int J Cancer 2012; 131: 2349-59.
22. Sandri MT, Riggio D, Salvatici M, Passerini R, Zorzino L, Boveri S, et al. Typing of human papillomavirus in women with cervical lesions: prevalence and distribution of different genotypes. J Med Virol 2009; 81: 271-7.
23. Mazarico E, Gonzalez-Bosquet E. Prevalence of infection by different genotypes of human papillomavirus in women with cervical pathology. Gynecol Oncol 2012; 125: 181-5.
24. Muñoz N, Bosch FX, de Sanjosé S, Herrero R, Castellsagué X, Shah KV, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 2003; 348: 518-27.
25. Herrero R, Castle PE, Schiffman M, Bratti MC, Hildesheim A, Morales J, et al. Epidemiologic profile of type-specific human papillomavirus infection and cervical neoplasia in Guanacaste, Costa Rica. J Infect Dis 2005; 191: 1796-807.
26. Ho GY, Kadish AS, Burk RD, Basu J, Palan PR, Mikhail M, et al. HPV 16 and cigarette smoking as risk factors for high-grade cervical intra-epithelial neoplasia. Int J Cancer 1998; 78: 281-5.

27. Brisson J, Morin C, Fortier M, Roy M, Bouchard C, Leclerc J, et al. Risk factors for cervical intraepithelial neoplasia: differences between low-and high-grade lesions. Am J Epidemiol 1994; 140: 700-10.
28. Rodríguez AC, Schiffman M, Herrero R, Wacholder S, Hildesheim A, Castle PE, et al. Rapid clearance of human papillomavirus and implications for clinical focus on persistent infections. J Natl Cancer Inst 2008; 100: 513-7.
29. Matsumoto K, Oki A, Furuta R, Maeda H, Yasugi T, Takatsuka N, et al. Predicting the progression of cervical precursor lesions by human papillomavirus genotyping: a prospective cohort study. Int J Cancer 2011; 128: 2898-910.
30. Bello BD, Spinillo A, Alberizzi P, Cesari S, Gardella B, D'Ambrosio G, et al. Cervical infections by multiple human papillomavirus (HPV) genotypes: Prevalence and impact on the risk of precancerous epithelial lesions. J Med Virol 2009; 81: 703-12.
31. Wheeler CM, Hunt WC, Schiffman M, Castle PE; Atypical Squamous Cells of Undetermined Significance/Low-Grade Squamous Intraepithelial Lesions Triage Study Group. Human papillomavirus genotypes and the cumulative 2-year risk of cervical precancer. J Infect Dis 2006; 194: 1291-9.
32. Clifford GM, Rana RK, Franceschi S, Smith JS, Gough G, Pimenta JM. Human papillomavirus genotype distribution in low-grade cervical lesions: comparison by geographic region and with cervical cancer. Cancer Epidemiol Biomarkers Prev. 2005; 14: 1157-64.
33. Trottier H, Mahmud S, Costa MC, Sobrinho JP, Duarte-Franco E, Rohan TE, et al. Human papillomavirus infections with multiple types and risk of cervical neoplasia. Cancer Epidemiol Biomarkers Prev 2006; 15: 1274-80.
34. Campos NG, Rodriguez AC, Castle PE, Herrero R, Hildesheim A, Katki H, et al. Persistence of concurrent infections with multiple human papillomavirus types: a population-based cohort study. J Infect Dis 2011; 203: 823-7.
35. Quint WG, Scholte G, van Doorn LJ, Kleter B, Smits PH, Lindeman J. Comparative analysis of human papillomavirus infections in cervical scrapes and biopsy specimens by general SPF(10) PCR and HPV genotyping. J Pathol 2001; 194: 51-8.
36. Castle PE, Porras C, Quint WG, Rodriguez AC, Schiffman M, Gravitt PE, et al. Comparison of two PCR-based human papillomavirus genotyping methods. J Clin Microbiol 2008; 46: 3437-45.
37. Odida M, de Sanjose S, Sandin S, Quiros B, Alemany L, Lloveras B, et al. Comparison of human papillomavirus detection between freshly frozen tissue and paraffin embedded tissue of invasive cervical cancer. Infect Agent Cancer 2010; 5: 15.
38. Perrons C, Kleter B, Jelley R, Jalal H, Quint W, Tedder R. Detection and genotyping of human papillomavirus DNA by SPF10 and MY09/11 primers in cervical cells taken from women attending a colposcopy clinic. J Med Virol 2002; 67: 246-52.
39. Van Hamont D, van Ham MA, Bakkers JM, Massuger LF, Melchers WJ. Evaluation of the SPF10-INNO LiPA human papillomavirus (HPV) genotyping test and the roche linear array HPV genotyping test. J Clin Microbiol 2006; 44: 3122-9.
40. Kyrgiou M, Tsoumpou I, Vrekoussis T, Martin-Hirsch P, Arbyn M, Prendiville W, et al. The up-to-date evidence on colposcopy practice and treatment of cervical intraepithelial neoplasia: the Cochrane colposcopy & cervical cytopathology collaborative group (C5 group) approach. Cancer Treat Rev 2006; 32: 516-23.
41. Burghardt E, Ostör AG. Site and origin of squamous cervical cancer: a histomorphologic study. Obstet Gynecol 1983; 62: 117-27.
42. Castle PE, Gage JC, Wheeler CM, Schiffman M. The clinical meaning of a cervical intraepithelial neoplasia grade 1 biopsy. Obstet Gynecol 2011; 118: 1222-9.
43. Carreon JD, Sherman ME, Guillén D, Solomon D, Herrero R, Jerónimo J, et al. CIN2 is a much less reproducible and less valid diagnosis than CIN3: results from a histological review of population-based cervical samples. Int J Gynecol Pathol 2007; 26: 441-6.
44. Castle PE, Stoler MH, Solomon D, Schiffman M. The relationship of community biopsy-diagnosed cervical intraepithelial neoplasia grade 2 to the quality control pathology-reviewed diagnoses: an ALTS report. Am J Clin Pathol 2007; 127: 805-15.

Effect of sacrocolpopexy and retropubic sling on overactive bladder symptoms

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Abstract

Objective: In this study, we aimed to evaluate the effect of sacrocolpopexy and retropubic midurethral sling, or transvaginal tape (TVT) procedure, on overactive bladder (OAB) symptoms. Our null hypothesis was that concomitant sacrocolpopexy and TVT exacerbate OAB symptoms.

Material and Methods: This is a prospective cohort study. All subjects had apical/anterior prolapse and underwent robotic-assisted sacrocolpopexy and TVT, with or without concomitant hysterectomy. All subjects completed a standardized one-year follow-up between 2009 and 2014. To assess for OAB symptoms, we used the Urogenital Distress Inventory subscale questions #15 and/or question #16. Reponses to these questions are based on a five-point 0 to 4 Likert scale (0 represents a negative response or no symptoms, and 4 represents the most problems). Any patient who answered 1 or higher on the Likert scale, either on the frequency or urge incontinence question, was defined as having OAB symptoms.

Results: Sixty-six subjects completed 12 months of visits. Preoperatively, 54 patients (83%) had OAB symptoms, and postoperatively 29 patients (45%) had OAB symptoms ($p<0.001$). Patients with postoperative OAB had a lower patient global impression of improvement (PGI-I) scores, PGI-I 5.8 with OAB, and PGI-I 6.6 without OAB ($p<0.003$).

Conclusion: We found that sacrocolpopexy and concomitant retropubic midurethral sling does not contribute to additive OAB symptoms, and symptoms actually resolved in 38% of women in our cohort. The presence of postoperative OAB contributes to lower global impression of improvement. (J Turk Ger Gynecol Assoc 2017; 18: 9-14)

Keywords: Overactive bladder, sacrocolpopexy, midurethral sling, transvaginal tape, prolapse repair

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Introduction

Pelvic organ prolapse (POP) and urinary incontinence are common healthcare problems, with one in four adult women in the United States reporting at least one pelvic floor disorder (1). The lifetime risk of pelvic floor surgery is estimated as 11%-19% (2, 3).

Symptoms of POP and overactive bladder (OAB) are often present together. The International Continence Society defines OAB as urgency with or without urge incontinence, usually with frequency and nocturia (4). Hospital- and community-based studies have shown that the prevalence of OAB is higher in women with POP (5, 6). Presence of OAB symptoms have been reported in up to 88% of women with POP (7). The estimated national cost of OAB in the United States was projected to be $76.2 billion in 2015 and $82.6 billion in 2020, which highlights the magnitude of the economic burden (8, 9).

Several studies have shown improvement in symptoms of OAB after POP surgery (10-14). The majority of these studies included women who underwent repair in the anterior or apical vaginal compartment. Unfortunately, women who underwent a concomitant stress incontinence procedure were excluded out of concern that an incontinence procedure might aggravate OAB symptoms.

OAB subjectively improves in more than 60% of patients undergoing transvaginal tape (TVT) retropubic midurethral sling

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(15). However, there is little guidance in the literature regarding women who undergo surgery of the apical compartment of the vagina, along with a concomitant incontinence procedure. We aimed to evaluate the effect of apical POP surgery (sacrocolpopexy) and TVT on OAB symptoms.

Material and Methods

This is a secondary analysis of subjects who participated in a prospective cohort study whose primary aim was to measure the effect that prolapse surgery had on the ability to cure stress urinary incontinence in patients also receiving a TVT (16). The subjects were recruited from two sites with a Female Pelvic Medicine and Reconstructive Surgery fellowship (an academic institution and a community-based institution in the Pacific Northwest of the United States) and followed for one year between January 2009 and January 2014.

All participants had apical/anterior prolapse and underwent robotic-assisted sacrocolpopexy and TVT, with or without concomitant hysterectomy, and completed a standardized one-year follow-up.

The specific surgical steps were similar at both sites. We used 5 abdominal ports (1 camera arm, 3 robotic arms, and 1 assist port) with the patient in the deep Trendelenburg position. The sacrocolpopexy was started by opening the presacral space to expose the anterior longitudinal ligament at the level of S1. The peritoneal opening was then extended along the right pelvic sidewall into the pouch of Douglas. We performed a deep dissection on both the anterior and posterior vaginal walls to facilitate placement of an adequately sized piece of mesh to address prolapse in all three compartments. We used polyproplylene Gynemesh PS Mesh (Ethicon, USA) for the sacrocolpopexy. We used 4-6 delayed absorbable Maxon sutures (Covidien-Medtronic) anteriorly and 6-8 Maxon sutures posteriorly on the vagina to affix the mesh. The sacral end of the mesh was attached to the anterior longitudinal ligament at the level of S1, with two non-absorbable sutures of 0-Ti-Cron (braided polyester). TVT was performed after the laparoscopic portion of the case had been completed. The TVT procedure was performed as described by Ulmsten et al. (17). Tensioning of the sling was performed by placing Metzenbaum scissors between the sling and the mid urethra until the plastic sheaths were withdrawn.

Inclusion criteria were women with symptomatic prolapse with the leading edge of the prolapse extending beyond the hymen, and symptoms of stress incontinence and documented stress incontinence on urodynamics. All subjects had a complete preoperative evaluation and physical exam, including POP quantification (POP Q) examination, and each completed the validated short form of the Pelvic Floor Distress Inventory (PFDI-20) questionnaire. Informed consent was obtained from all subjects. The subjects subsequently completed 12 months follow-up with a POP Q examination and the same questionnaires, and also completed the Patient Global Impression of Improvement (PGI-I).

Ethics committee approval was obtained from the Institutional Review Board (IRB) at both sites, and we used an IRB approved data repository Filemaker Pro (FileMaker, Inc. CA). Data from both sites were entered into a standardized template in Filemaker. At one year, the subjects returned for a research evaluation. Urogynecology fellows who did not participate in the original surgery conducted the follow-up exams.

To assess for OAB symptoms, we used Urogenital Distress Inventory subscale (UDI) questions #15 ("Do you usually experience frequent urination?") and/or question #16 ("Do you usually experience urine leakage associated with a feeling of urgency, a strong sensation of needing to go to the bathroom?"). Reponses to these questions are based on a five-point 0 to 4 Likert scale, where 0 represents a negative response or no symptoms, and 4 represents the most problems. Any patient who answered 1 or higher on the Likert scale, either on the frequency or urge incontinence question, was defined as having OAB symptoms.

In order to assess the role of POP Q stages in OAB symptoms following sacrocolpopexy, we also dichotomized the POP Q staged by combining stages 1-2 and 3-4. This was done to perform analysis once prolapse was divided into less severe (stages 1-2) and more severe (stages 3-4) anatomic groups based on the POP Q.

Statistical analysis was performed using SPSS (Version 22.0. Armonk, NY: IBM). Based on prior reports focusing only on POP surgery and its effect on OAB symptoms, we assumed a prevalence of OAB symptoms of about 70% in women with POP, and 50% improvement in symptoms after surgery that included both prolapse repair and surgery for stress incontinence (10, 11, 13). Assuming an alpha of 0.05 and 80% power, we anticipated that 62 subjects would be required. Univariate analysis was performed using Student's t-test for continuous variables and Chi-square for categorical variables. We used Fisher's exact test when assumptions for Chi-square distribution were violated. Wilcoxon's signed-rank test was performed to compare the urinary frequency and urge incontinence scores between the preoperative and postoperative groups, and McNemar's test was used to compare the OAB symptoms between these groups. We used the Mann-Whitney U test to compare PGI-I scores between those with and without OAB postoperatively.

Results

We enrolled 77 subjects from both sites. Eleven subjects did not attend the 12 monthly visits and were excluded. Sixty-six subjects completed 12 monthly visits; one had missing

data. Sixty-five subjects were included in the final analysis. Our population was predominantly white (96%). Patient characteristics are shown in Table 1.

Prolapse measurements, as examined using the POP Q system, showed significant improvement at one year postoperatively (Table 2).

Preoperative urinary frequency was present in 43 (67%) subjects; 18 (28%) had urinary frequency postoperatively. Preoperatively, 41 (64%) had urge incontinence. In the postoperative group, 25 (39%) subjects reported urge incontinence.

The median preoperative score for urinary frequency was 2.5 and this reduced postoperatively to 0, and was significantly different with a p value of <0.001. The median preoperative score for urge incontinence was 2 and this reduced to 0 postoperatively; this was significantly different with a p value of 0.004.

Preoperatively, 54 (83%) patients had OAB symptoms, and 31 (48%) patients had OAB symptoms postoperatively. This difference was statistically significant with a p value of <0.001 (Table 3). Patients with postoperative OAB had a lower global impression of improvement, PGI-I 5.8 with OAB, PGI-I 6.6 without OAB (p<0.003).

We also looked at *de novo* OAB symptoms. In our cohort, 54 out of 66 subjects had OAB preoperatively, it resolved in 26 of these subjects postoperatively, showing a 48% reduction in OAB (p<0.001). Of the 12 patients who did not have OAB preoperatively, only five developed it postoperatively, a *de novo* OAB rate of five out of 66 (7.5%).

In a multivariate logistic regression: age, body mass index (BMI), parity, concomitant hysterectomy, and pre- and postoperative stage of prolapse revealed no significant relationship between these variables and the presence of postoperative OAB (Table 4).

We assessed the role of urodynamics, and 5/66 patients had preoperative detrusor overactivity (DO) on urodynamics. OAB resolved in 3 out 5 (60%) of these patients.

In order to assess the impact of prolapse severity, we dichotomized the POP Q stages by combining stages 1-2 and 3-4. These baseline characteristics are shown in Table 5. Women with stages 1-2 POP were younger than women with stages 3-4 (mean age: 58.8 vs. 69.3 years, p<0.001). There was no difference in BMI and parity.

Thirteen (57%) women in stages 1-2 reported urinary frequency compared with 30 (71%) women in the stage 3-4 group on PFDI-20, which was not statistically different (p=0.25). Similarly, 14 (61%) women in the stage 1-2 group and 27 (64%) women in the stage 3-4 group reported baseline urge incontinence (p=0.86). There was no difference postoperatively between the

Table 1. Baseline demographics

Variables	n=65
Age (SD) (years)	65.5 (10.5)
Race	96% white
BMI (SD)	28.1 (4.8)
Smoking	5%
Any hormone replacement	25%
Prior hysterectomy	51%
Parity (SD)	2.7 (4.8)
SD: standard deviation; BMI: body mass index	

Table 2. Prolapse measurements

POP-Q measurements	Preoperative mean (SD)	Postoperative mean (SD)	p value
POP-Q GH	4.9 (1.1)	3.4 (1.1)	<0.001
POP-Q Aa	0.3 (1.4)	-2.8 (1.1)	<0.001
POP-Q Ba	1.9 (2.3)	-2.2 (1.2)	<0.001
POP-Q C	-1.9 (4.2)	-8.8 (1.3)	<0.001
POP-Q Ap	-1.2 (1.5)	-2.3 (1.1)	<0.001
POP-Q Bp	-0.8 (2.4)	-2.1 (1.2)	<0.001
POP-Q PB	3.4 (0.9)	2.9 (0.9)	0.004
POP-Q leading edge	2.3 (2.0)	-1.5 (1.4)	<0.001
POP-Q: pelvic organ prolapse quantification; SD: standard deviation			

Table 3. Preoperative and postoperative overactive bladder symptoms

	Preoperative number (%)	Postoperative number (%)
OAB symptoms present	54 (83)	31 (48)
OAB symptoms absent	11 (17)	34 (52)
OAB: overactive bladder. p<0.001		

two groups. Only 4 (17%) women reported urinary frequency from the stage 1-2 group and 14 (32%) in the stage 3-4 group (p=0.18). Postoperatively, urge incontinence was present in 7 (30%) women in the stage 1-2 group and 18 (42%) women in the stage 3-4 group (p=0.35).

Discussion

The primary aim of our study was to evaluate the effect of sacrocolpopexy and TVT on OAB symptoms. We found that almost half of the patients with OAB symptoms preoperatively had resolution of symptoms at 12 months after surgery. OAB significantly affects the quality of life of women. The National Overactive Bladder Evaluation study found the overall prevalence of OAB in women as 16.9% (18). However, the prevalence of OAB is even higher in women with POP (5, 6), and has been reported to be up to 88% (7). OAB causes a dramatic reduction in quality of life of women (19). Stress, anxiety, and generalized irritability are higher in women with OAB (20). In our cohort, a significant proportion (83%) of women with apical POP and stress incontinence had OAB symptoms. However, postoperatively, only 45% percent of women reported OAB symptoms at one year. We found that sacrocolpopexy and concomitant TVT does not contribute to additive OAB symptoms, and these symptoms resolved in 38% of women.

Prior reports have shown a reduction in OAB symptoms after prolapse surgery (10-14), though out of concern for aggravation of OAB symptoms, incontinence procedures were excluded from these studies. Although they may have been counseled otherwise, women undergoing surgery for stress incontinence expect their symptoms of OAB to improve as well (21). Recognizing this as a potential source of disappointment, our study found that participants with postoperative OAB had lower global impression of improvement.

Midurethral slings have been shown to improve OAB symptoms in women with mixed incontinence without prolapse (15, 22). Similarly, prolapse repairs alone have shown improvement in OAB symptoms (10-14). However, whether that same effect can be seen in women receiving slings and undergoing complex prolapse surgery is not as well defined. One of the reasons for this concern is that by adding the incontinence sling procedure at the time of prolapse surgery, we are adding another variable that might exacerbate OAB symptoms. However, we found

Table 4. Multivariate logistic regression

Variables	B	SE	Wald	df	Sig	Exp (B)	95% Confidence interval	
							Lower	Upper
BMI	-.002	.058	.002	1	.967	.998	.891	1.117
Age	.004	.031	.017	1	.895	1.004	.944	1.068
Parity	.353	.252	1.966	1	.161	1.424	.869	2.332
Stage_Postop	.120	.311	.149	1	.700	1.127	.613	2.073
Stage_Preop	.372	.531	.490	1	.484	1.450	.512	4.105
Hysterectomy	.558	.571	.954	1	.329	1.747	.570	5.354
Constant	-2.518	2.537	.985	1	.321	.081		

B: coefficient for the constant; SE: standard error; Wald: wald, Chi-square test; df: degrees of freedom; Sig: p value; Exp (B): odds ratio; BMI: body mass index

Table 5. Baseline characteristics after dichotomizing stages

Variable	POP-Q Stage 1-2 n=(23)	POP-Q Stage 3-4	p value
Age mean (SD)	58.8 (10.4)	69.3 (8.8)	<0.001
BMI mean (SD)	27.5 (4.9)	28.4 (4.6)	0.48
Parity mean (SD)	2.4 (1)	2.7 (1.2)	0.26
Concomitant hysterectomy n (%)	12 (52.1)	23 (54.7)	0.84
POP-Q measurements mean (SD)			
Ba	0.08 (0.9)	2.9 (2.2)	<0.001
C	-4.8 (2.3)	-0.3 (4.1)	<0.001
Bp	-1.5 (1.3)	-0.5 (2.7)	0.10
GH	4.5 (1)	5.1 (1)	0.03

SD: standard deviation; BMI: body mass index; POP-Q: pelvic organ prolapse quantification

that OAB symptoms improved, despite the concomitant TVT procedure. We also studied urinary frequency and urgency/urge incontinence separately and found improvement in both facets. Almost half of the patients with OAB symptoms preoperatively had resolution of symptoms at 12 months after surgery. The incidence of *de novo* OAB was only 7.5% in our cohort, with only 5 patients developing *de novo* OAB at 12 months.

In our cohort, 5/66 patients had preoperative DO on urodynamics, and symptoms resolved in 3 out 5 (60%) of these patients. Although our numbers are small, we found that presence of DO on urodynamics might not confer persistent OAB symptoms postoperatively.

We also dichotomized the preoperative prolapse stage as stages 1-2 and 3-4. We found no difference in baseline urinary frequency and urge incontinence in the two groups. Furthermore, we found no difference in OAB symptoms postoperatively based on preoperative stage.

Most prior studies included women undergoing mixed prolapse surgeries or anterior vaginal wall repairs. Our cohort included women who underwent sacrocolpopexy and TVT (with or without concomitant procedures). It is possible that the symptoms improved because POP causes obstruction of the urethra, which results in OAB symptoms in women, similar to benign prostatic hypertrophy causing OAB symptoms in men (23). Petros and Ulmsten (24) proposed the "integral theory," in which they suggested that the anterior vaginal wall relaxation was associated with OAB symptoms. It is plausible that women with prolapse, stress incontinence, and OAB do not have two separate conditions, and the symptoms of mixed urinary incontinence are a spectrum of stress urinary incontinence. This could explain the improvement of OAB symptoms in women undergoing concomitant incontinence sling and prolapse repair.

To our knowledge, this is the first study to assess the effect of concomitant sacrocolpopexy and TVT on OAB symptoms. The strengths of our study are that it is a prospective study, and we used validated questionnaires to assess for OAB symptoms. Everyone in our cohort underwent sacrocolpopexy, which is widely considered as the gold standard procedure for apical prolapse. A limitation of our study is the lack of urodynamic data for the postoperative assessment of resolution of DO. However, we used validated questionnaires and urodynamics have a low predictive value to reproduce clinical findings of OAB (25). Another limitation is that we did not account for concurrent medication use in these subjects. In conclusion, in this prospectively recruited cohort of women, sacrocolpopexy and concomitant TVT did not contribute to additive OAB symptoms, which actually resolved in almost half of the women. The presence of postoperative OAB contributes to lower global impression of improvement. These findings will be helpful to providers, and will assist in counseling patients.

Ethics Committee Approval: *Ethics committee approval was obtained from the Institutional Review Board.*

Informed Consent: *Informed consent was obtained from all subjects.*

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References

1. Wu JM, Vaughan CP, Goode PS, Redden DT, Burgio KL, Richter HE, et al. Prevalence and trends of symptomatic pelvic floor disorders in U.S. women. Obstet Gynecol 2014; 123: 141-8.
2. Smith FJ, Holman CD, Moorin RE, Tsokos N. Lifetime risk of undergoing surgery for pelvic organ prolapse. Obstet Gynecol 2010; 116: 1096-100.
3. Olsen AL, Smith VJ, Bergstrom JO, Colling JC, Clark AL. Epidemiology of surgically managed pelvic organ prolapse and urinary incontinence. Obstet Gynecol 1997; 89: 501-6.
4. Abrams P, Cardozo L, Fall M, Griffiths D, Rosier P, Ulmsten U, et al. The standardisation of terminology of lower urinary tract function: report from the Standardisation Sub-committee of the International Continence Society. Am J Obstet Gynecol 2002; 187: 116-26.
5. de Boer TA, Salvatore S, Cardozo L, Chapple C, Kelleher C, van Kerrebroeck P, et al. Pelvic organ prolapse and overactive bladder. Neurourol Urodyn 2010; 29: 30-9.
6. Lawrence JM, Lukacz ES, Nager CW, Hsu JW, Luber KM. Prevalence and co-occurrence of pelvic floor disorders in community-dwelling women. Obstet Gynecol 2008; 111: 678-85.
7. Digesu GA, Chaliha C, Salvatore S, Hutchings A, Khullar V. The relationship of vaginal prolapse severity to symptoms and quality of life. BJOG 2005; 112: 971-6.
8. Coyne KS, Wein A, Nicholson S, Kvasz M, Chen CI, Milsom I. Economic burden of urgency urinary incontinence in the United States: a systematic review. J Manag Care Pharm 2014; 20: 130-40.
9. Ganz ML, Smalarz AM, Krupski TL, Anger JT, Hu JC, Wittrup-Jensen KU, et al. Economic costs of overactive bladder in the United States. Urology 2010; 75: 526-32.
10. Basu M, Wise B, Duckett J. Urgency resolution following prolapse surgery: is voiding important? Int Urogynecol J 2013; 24: 1309-13.
11. Miranne JM, Lopes V, Carberry CL, Sung VW. The effect of pelvic organ prolapse severity on improvement in overactive bladder symptoms after pelvic reconstructive surgery. Int Urogynecol J 2013; 24: 1303-8.

12. Lensen EJ, Withagen MI, Kluivers KB, Milani AL, Vierhout ME. Urinary incontinence after surgery for pelvic organ prolapse. Neurourol Urodyn 2013; 32: 455-9.
13. de Boer TA, Kluivers KB, Withagen MI, Milani AL, Vierhout ME. Predictive factors for overactive bladder symptoms after pelvic organ prolapse surgery. Int Urogynecol J 2010; 21: 1143-9.
14. Digesu GA, Salvatore S, Chaliha C, Athanasiou S, Milani R, Khullar V. Do overactive bladder symptoms improve after repair of anterior vaginal wall prolapse? Int Urogynecol J Pelvic Floor Dysfunct 2007; 18: 1439-43.
15. Duckett JR, Tamilselvi A. Effect of tension-free vaginal tape in women with a urodynamic diagnosis of idiopathic detrusor overactivity and stress incontinence. BJOG 2006; 113: 30-3.
16. Osmundsen B, Gregory WT, Denman MA, Adams K, Edwards R, Clark A. Tension-Free Vaginal Tape Failure After Robotic Sacrocolpopexy and Tension-Free Vaginal Tape for Concomitant Prolapse and Stress Incontinence. Female Pelvic Med Reconstr Surg 2015; 21: 244-8.
17. Ulmsten U, Henriksson L, Johnson P, Varhos G. An ambulatory surgical procedure under local anesthesia for treatment of female urinary incontinence. Int Urogynecol J Pelvic Floor Dysfunct 1996; 7: 81-5.
18. Stewart WF, Van Rooyen JB, Cundiff GW, Abrams P, Herzog AR, Corey R, et al. Prevalence and burden of overactive bladder in the United States. World J Urol 2003; 20: 327-36.
19. Dubeau CE, Simon SE, Morris JN. The effect of urinary incontinence on quality of life in older nursing home residents. J Am Geriatr Soc 2006; 54: 1325-33.
20. Chiara G, Piccioni V, Perino M, Ohlmeier U, Fassino S, Leombruni P. Psychological investigation in female patients suffering from urinary incontinence. Int Urogynecol J Pelvic Floor Dysfunct 1998; 9: 73-7.
21. Wagg A, Das Gupta R, Assassa P, Shaw C, Mayne C, Martin M. Secondary-care treatment patterns in the UK for women with urinary incontinence. BJU Int 2005; 96: 839-42.
22. Han JY, Choo MS, Lee YS, Seo JT, Kim JH, Kim YH, et al. Effectiveness of retropubic tension-free vaginal tape and transobturator inside-out tape procedures in women with overactive bladder and stress urinary incontinence. Int Neurourol J 2013; 17: 145-51.
23. Kwon JK, Han JH, Choi HC, Kang DH, Lee JY, Kim JH, et al. Clinical significance of peripheral zone thickness in men with lower urinary tract symptoms/benign prostatic hyperplasia. BJU Int 2016; 117: 316-22.
24. Petros PE, Ulmsten UI. An integral theory of female urinary incontinence. Experimental and clinical considerations. Acta Obstet Gynecol Scand Suppl 1990; 153: 7-31.
25. Caruso DJ, Kanagarajah P, Cohen BL, Ayyathurai R, Gomez C, Gousse AE. What is the predictive value of urodynamics to reproduce clinical findings of urinary frequency, urge urinary incontinence, and/or stress urinary incontinence? Int Urogynecol J 2010; 21: 1205-9.

Urinary fistula-A continuing problem with changing trends

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Abstract

Objective: Urinary fistula is a distressing complication after difficult vaginal deliveries, obstetric, and gynecologic surgeries. The present study describes a single center's experience in the management of urinary fistula at a tertiary care hospital. It was performed to analyze the etiology of genitourinary fistula, to assess the outcome after surgical repair, and to determine the changing trends in the etiology and management of urinary fistula.

Material and Methods: This retrospective study was conducted over 5 years in the department of obstetrics and gynecology, All India Institute of Medical Sciences, New Delhi. Twenty patients who underwent surgical repair of urinary fistula were included in the study and analyzed for their etiology, presentation, site, size, previous failed repair, approach of surgical repair, and outcome. The findings of the present study were compared with a previous study at our center to determine the changing trends of urinary fistula.

Results: The mean age of the study population was 37.05±8.08 years. The majority (65%) of the fistulae occurred following gynecologic surgeries, whereas 25% were due to obstructed labor, and 10% after cesarean section for other indications. The vaginal approach was used in all except one case of uterovesical fistula, which was repaired abdominally. The outcome was successful in 85% of cases. The success rate was similar in primary versus previous failed repair (p=0.270).

Conclusion: The most common cause of urinary fistula in the present study was gynecologic surgery. The vaginal approach can be used even in cases with previous failed repairs with a high success rate. (J Turk Ger Gynecol Assoc 2017; 18: 15-9)

Keywords: Urinary fistula, vesicovaginal, obstructed labor, iatrogenic

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Introduction

Urinary fistula is one of the most distressing complications after difficult vaginal deliveries, obstetric, and gynecologic surgeries. It has great impact on social, psychological, and sexual life of affected patients. It is a very old entity, the earliest case was reported in 1923 in a mummified body that was dated 2050 BC (1). The reported incidence of vesicovaginal fistula (VVF) in developed countries is 0.3-2% (2). However, the exact incidence in the developing world is not known, probably due to the underreporting of cases (3). A study estimated the prevalence of VVF in the reproductive age group as 1.60 per 1000 women in South Asia (4). The burden can be estimated by the fact that according to an estimation in Ethiopia alone, 9000 women develop fistula annually, and only 1200 of those were being treated (5).

In the past, the most common cause of VVF was prolonged and obstructed labor. Up to 90% of fistulae in developing countries were obstetric in origin (6), whereas almost 70% were due to gynecologic causes in developed countries such as the United States and United Kingdom (7). All this reflected poor access to skilled obstetric care in the majority of the population (8). In recent years, there has been a shift in etiology of urinary fistula. The trend has changed from obstetric to gynecologic causes, mainly due to the advancement in obstetric practices, increased institutional delivery, improved intrapartum care, and availability of emergency cesarean sections (CS) (9). In a recent metaanalysis, the gynecologic contribution was 81-91% (10). In a study, the incidence of VVF after abdominal hysterectomy for benign conditions and vaginal hysterectomies for prolapse was reported as 0.18% and 0.025%, respectively (11). Another study

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reported the incidence after total laparoscopic hysterectomy as 0.16% (12).

Repair of VVF can be achieved transvaginally or transabdominally. The choice of route depends on the characteristics of the fistula and the experience of the surgeon. The present study describes a single center's experience in the management of urinary fistula at a tertiary care hospital. The objective of study was to analyze the etiology of genitourinary fistula, to assess the outcome after surgical repair, and also to determine changing trends in the etiology and management of urinary fistulae.

Material and Methods

The study was conducted in the department of obstetrics and gynecology, All India Institute of Medical Sciences, New Delhi, over a period of 5 years (2011-2015). Twenty patients who underwent surgical repair of urinary fistula were included in the study after approval was obtained from the Ethics Committee of the institution. Informed written consent was given by all patients. The hospital records of these patients were reviewed. Patients with previous failed repair of fistula were also included. Detailed history was taken including age, parity, etiology of fistula, presentation, previous surgical repair and their outcome. All the patients were examined for site of fistula, number, size, fibrosis, and vaginal stenosis. A three-swab test was performed to clearly locate the fistula, with the patient in the lithotomy position. Three swabs were placed in the vagina, one above the other, followed by retrograde filling of the bladder with diluted and sterilized methylene blue by means of a per urethral catheter. Patients were then asked to walk around or perform the Valsalva manoeuvre. The swabs were then examined after 15 minutes to determine the site of the fistula. If the topmost swab was discolored, it indicated a VVF. Only wetting of the topmost swab without discoloration indicated a ureterovaginal fistula. Discoloration of only the lowest swab was suggestive of a low urethral fistula or back flow from the introitus. Cystoscopy was performed in all patients to see the number and location of fistula in relation to the trigone of the bladder. A urine culture and sensitivity was performed using a sterile speculum in all patients prior to surgery.

The fistulae were repaired via the transvaginal route using the flap-splitting technique in all cases but one case of uterovesical fistula, which was repaired transabdominally. In the flap-splitting technique, the fistula was identified and a pediatric Foley catheter was inserted through the fistulous opening. Traction on the catheter made the fistula more accessible and a circumferential incision was then made around the fistulous opening. A vaginal mucosal flap was lifted away from bladder to an extent that allowed tension-free closure of the defect. The bladder was closed in two layers using a delayed absorbable suture (Polyglactin 3-0). In the first layer, the bladder mucosa was closed with interrupted sutures. The second layer included bladder muscularis and perivesical fascia in an interrupted fashion and hemostasis ensured. After this, bladder integrity was checked using diluted methylene blue dye. The vaginal mucosa was then closed over it in a running interlocking manner with Polyglactin 2-0. Continuous bladder drainage was ensured in all patients for 14 to 21 days using a per urethral catheter. Broad-spectrum antibiotics were given and patients were followed up 1 month, 2 months, and 6 months after surgery. The success of the repair was assessed at the end of 6 months by examination and a repeat three-swab test. The findings of the present study were compared with a previous study at our center to determine changing trends of urinary fistula in a developing country such as India (13).

Statistical analysis was performed using Statistical Package for the Social Sciences software, International Business Management version 20.0. (Armonk, New York, USA). Descriptive statistics such as mean, standard deviation, range and median values were calculated for continuous variables such as age of patients and duration of symptoms. Frequencies of outcomes across the categories are represented as frequency and percentage values. To compare the frequency of occurrences of outcomes across categories, Chi-square/ Fisher's exact tests were used as appropriate. For all tests, a two-tailed probability of $p<0.05$ was considered statistically significant.

Results

The mean age of the study population was 37.05±8.08 years (range, 20-50 years). Patients presented with symptoms for a median duration of 8 months. Table 1 shows the antecedent events that led to fistula in our cases. The majority (65%) were due to gynecologic causes, most of which occurred after abdominal hysterectomy (45%). All except one fistulae in our study were vesicovaginal, the remainder was a uterovesical fistula that occurred following CS. There were two cases of fistulae following CSs performed for indications other than obstructed labor, one was VVF following CS performed for a morbidly adherent placenta, and the other was a uterovesical fistula where there were dense adhesions of the bladder involving the uterus due to previous cesarean section. Based on the cystoscopy findings, the majority (75%) of the fistulae was supratrigonal. This was followed by fistula on the bladder neck in 15%, and trigonal in 10%.

We also classified fistula as simple and complicated based on characteristics of fistula, such as number, site, size, urethral involvement, scarring of tissue, and number of previous repairs (14). Simple fistulae were present in 11 (55%) women, and

9 (45%) had complicated fistulae. Thirteen (65%) patients underwent primary repair, and 7 (35%) had at least one previous failed repair elsewhere. All the repairs in our study except one were performed vaginally, the remainder was a uterovesical fistula, which was repaired abdominally. At the 6th month follow-up, 17 patients had a successful result (85%) and 3 (15%) had failed repairs. The outcome of surgery was compared in simple versus complicated group, and also regarding the number of previous repairs (Table 2). There was no statistically significant difference between the success rates of simple and complicated fistulae, or between primary and previous repair.

Discussion

The mean age of the patients in the present study was 37.05 ±8.08 years. In recent studies by Wadie and Kamal (15) in 2011 and Karateke et al. in 2010 (16), the median age at presentation was >35 years, which is similar to the present study. Wall et al. (17) in 2004 reported a higher incidence of VVF in a younger population; the mean age was 27 years (range, 13-20 years). The difference in age of presentation over the years is due to different antecedent events leading to fistula. Obstetric fistula used to be a major cause of genitourinary fistula. Wall et al. (17) reported almost 96.5% cases of urinary fistula due to obstetric causes in developing parts of the world like sub-Saharan African

Table 1. Etiology of fistula

Etiology of fistula		**n=20 (%)**
Obstetric	Obstructed labor with vaginal delivery	4 (20)
	Obstructed labor with caesarean section	1 (5)
	Cesarean section for other indications	2 (10)
	Total	7 (35)
Gynecologic	Total abdominal hysterectomy	9 (45)
	Laparoscopic hysterectomy	2 (10)
	Vaginal hysterectomy	1 (5)
	Vaginoplasty	1 (5)
	Total	13 (65)

Table 2. Outcome of fistula repair based on type of fistula and number of previous repair

Parameter		**Outcome, n (%)**		**p value**
		Successful	**Failed**	
Complexity of fistula (WHO)	Simple	10 (90.9)	1 (9.1)	0.566
	Complicated	7 (77.8)	2 (22.2)	
No. of previous repair	No previous repair	12 (92.3)	1 (7.7)	0.270
	Previous ≥1 repair	5 (71.4)	2 (28.6)	
WHO: World Health Organization				

Table 3. Comparison between present study and a previous study from same center

	Present study	**Previous study (13)**
Number of patients	20	23
Time period of study	5 years (2011-2015)	5 years (1999-2004)
Age (years), Mean ± SD	37.05±8.08	25.4±3.7
Median duration, months	8	4.7
Obstetric fistula, %	35	73.9
Gynecological fistula, %	65	21.7
Others (traumatic), %	-	4.34
Success rate, %	85	83.3
SD: standard deviation		

nations, due to poor obstetric care. In the previous study from our center, 73.9% of fistulae were obstetric related (12). This is in contrast to the present study, where the majority of fistulae were gynaecologic in origin (Table 3). Similar results were reported by another study where hysterectomy accounted for 91% of fistulae (83% abdominal, 9% vaginal surgeries) (2). In our study too, abdominal hysterectomy accounted for most fistulae. This is partly due to improved obstetric care services, and the increasing number of gynecologic surgeries. Obstructed labor as a cause of fistula is showing a declining trend due to an increase in delivery rates by skilled birth attendants and the availability of round-the-clock emergency cesarean services. During abdominal hysterectomy, VVF can result due to direct injuries resulting from sharp dissection of the bladder in the wrong plane, especially in cases of previous pelvic surgeries, endometriosis or malignancies. It could also be due to iatrogenic injury by inadvertent sutures in the bladder wall or thermal cautery burn leading to necrosis of the wall.

There is a long gap between the appearance of symptoms and presentation to health care facility in these women. The median duration of symptoms in a study was 11.5 months (range, 3-228 months) (15). In the present study, this duration was 8 months (range, 1-228 months). Even today, there is a delay in seeking health care in these women. This condition is socially debilitating and these women are usually neglected in their families, which could be the reason for this delay. A difference in opinion exists regarding the route of repair. The choice of route of repair depends on the nature of the fistula. However, the most important factor is the choice of surgeons and their experience. Eilber et al. (2) used the vaginal approach in most cases because the abdominal approach was associated with greater morbidity such as greater blood loss, longer hospital stay, more pain, and cost. Nevertheless, they considered that the surgeon's choice was the most important. In another study, the same authors used the abdominal approach in 68% cases, vaginal in 25%, and a combined approach in 7% (15). We used the vaginal approach in all patients except one who had a uterovesical fistula. Even patients with previous failed repairs underwent vaginal access procedures. The abdominal approach has greater blood loss, prolonged hospitalization, and increased postoperative pain, but the success rates are similar (18, 19).

We achieved an overall success rate of 85% in the present study. Complicated fistulae were also managed using the vaginal approach. The outcome of surgery in relation to fistula type and previous failed repair is shown in Table 3. Another study by Wadie and Kamal (15) also reported a cure rate of 91% with abdominal access and 70% with the vaginal approach, the reason for choosing a particular route was surgeon's preference. Kapoor et al. (20) achieved very a high success rate of 94.2% in their study, but they used the abdominal approach for complex fistulas. The findings of the present study were compared with a previous study from the same center that was conducted between 1999 and 2004 (12). The important points are summarized in Table 3. This table highlights the change in etiology of fistula from obstetric to gynaecologic causes. In both the studies, the preferred approach was vaginal and the success of repair was almost the same. The limitations of our study are its small sample size and retrospective nature.

Conclusion

The causes of urinary fistula have changed significantly over the last few decades, even in developing countries such as India. The trend is shifting from obstetric to gynecologic causes. The vaginal approach has a high success rate and less morbidity, even in patients with previous failed repairs. Emphasis should be laid on preventing this dehumanizing condition.

Ethics Committee Approval: *The study was approved from the ethics committee of the institution.*

Informed Consent: *Informed written consent was given by all patients.*

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References

1. Riley VJ. Vesicovaginal fistula. E Med Web MD 2006, June 25.
2. Eilber KS, Kavaler E, Rodriguiz LV, Rosenblum N, Raz S. Ten-year experience with transvaginal vesicovaginal fistula repair using tissue interposition. J Urol 2003; 169: 1033-6.
3. Cowgill KD, Bishop J, Norgaard AK, Ruben CE and Gravett MG. Obstetric fistula in low-resource countries: an under-valued and under-studied problem - systematic review of its incidence, prevalence, and association with stillbirth. BMC Pregnancy and Childbirth 2015; 15: 193.
4. Adler AJ, Ronsmans C, Calvert C, Filippi V. Estimating the prevalence of obstetric fistula: a systematic review and meta analysis. BMC Pregnancy Childbirth 2013; 13: 246.

5. Report on the meeting for the prevention and treatment of obstetric fistula. 18-19 July, London, New York: Technical Support Division; 2001.
6. Hilton P. Obstetric fistulae. In: Cardozo L, Staskin D, editors. Textbook of female urology and urogynaecology. London: Isis Medical Media Ltd; 2001. p. 711-9.
7. Hilton P. Surgical fistulae. In: Cardozo L, Staskin D, editors. Textbook of female urology and urogynaecology. London: Isis Medical Media Ltd; 2001. p. 691-709.
8. Gessessew A, Mesfin M. Genitourinary and rectovaginal fistulae in Adigrat Zonal Hospital, Tigray, north Ethiopia. Ethiop Med J 2003; 41: 123-30.
9. Hilton P. Trends in the aetiology of urogenital fistula: a case of 'retrogressive evolution'? Int Urogynecol J 2016; 27: 831-7.
10. Ghoniem GM, Warda HA. The management of genitourinary fistula in the third millennium. Arab J Urol 2014; 12: 97-105.
11. Hilton P, Cromwell DA. The risk of vesicovaginal and urethrovaginal fistula after hysterectomy performed in the English National Health Service--a retrospective cohort study examining patterns of care between 2000 and 2008. 2012; 119:1447-54.
12. Brummer TH, Seppälä TT, Härkki PS. National learning curve for laparoscopic hysterectomy and trends in hysterectomy in Finland 2000-2005. Hum Reprod 2008; 23: 840-5.
13. Roy KK, Malhotra N, Kumar S, Seth A, Nayar B. Genitourinary fistula -An experience from a tertiary care centre. J & K Sciences 2006; 8: 144-7.
14. Obstetric Fistula: Guiding principles for clinical management and programme development, World Health Organization, 2006. p. 45.
15. Wadie BS, Kamal MM. Repair of vesicovaginal fistula: Single centre experience and analysis of outcome predictors. Arab J Urol 2011; 9: 135-8.
16. Karateke A, Asoglu MR, Selçuk S, Cam C, Tuğ N, Ozdemir A. Experience of our surgery in iatrogenic vesicovaginal fistulas. J Turkish-German Gynecol Assoc 2010; 11: 137-40.
17. Wall LL, Karshima JA, Kirschner C, Arrowsmith SD. The obstetric vesicovaginal fistula. Characteristics of 899 patients from Jos. Nigeria. Am J Obstet Gynecol 2004; 190: 1011-6.
18. Kumar S, Kekre NS, Gopalkrishnan G. Vesicovaginal fistula: An update. Indian J Urol 2007; 23: 187-91.
19. Blaivas JG, Heritz DM, Romanzi LJ. Early versus late repair of vesicovaginal fistulas: vaginal and abdominal approaches. J Urol 1995; 153: 1110-2.
20. Kapoor R, Ansari MS, Singh P, Gupta P, Khurana N, Mandhani A, et al. Management of vesicovaginal fistula: An experience of 52 cases with a rationalized algorithm for choosing the transvaginal or transabdominal approach. Indian J Urol 2007; 23: 372-6.

Maternal mortality due to hypertensive disorders in pregnancy, childbirth, and the puerperium between 2012 and 2015 in Turkey: A nation-based study

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Abstract

Objective: To analyze maternal deaths in Turkey due to hypertensive disorders.

Material and Methods: In this retrospective study 812 maternal deaths were analyzed. Maternal demographic features, presence of antenatal care, medical and obstetric history, mode of delivery, and use emergency antihypertensive therapy were recorded. The delay model for each case was investigated.

Results: Hypertensive disorders accounted for 15.5% (n=126) out of all maternal mortality. They were the third most frequent cause among all causes and the 2nd among direct causes of maternal deaths. Sixty-one (48.4%) cases were in severe preeclampsia or pre-existing hypertensive disorder with increased/superimposed proteinuria, 30.1% were in eclampsia, 9.5% cases were diagnosed as hemolysis, elevated liver enzymes, low platelet count syndrome, and 11.1% in pre-existing hypertension complicating pregnancy, childbirth, and puerperium without increased or superimposed proteinuria. The median age was 32 years, 37.3% women were ≥35 years. All deaths except for 2 cases occurred during the postpartum period. Twenty-three percent of deaths occurred in the first 48 hours postpartum, and 51.6% between 8-42 days. Intracranial hemorrhage was the major final cause of death with a rate of 41.3%. With the exception of fifteen patients with intracranial hemorrhage, emergency antihypertensive agents were not implemented in optimal dose and/or duration. A first and/or third delay was identified in 36.5% of cases.

Conclusion: Approximately one third of maternal death due to hypertensive disorders could be prevented. The importance of acute antihypertensive treatment should be emphasized because of most frequent cause of death was intracranial hemorrhage. (J Turk Ger Gynecol Assoc 2017; 18: 20-5)

Keywords: Hypertensive disorders, maternal mortality, Turkey

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Introduction

One of the three health-related goals of the Millennium Developmental Goals was the reduction of maternal mortality by three-quarters by the year 2015 (1). Maternal deaths are not uniformly distributed throughout the world and most maternal deaths occur in undeveloped regions such as sub-Saharan Africa (1). High maternal mortality ratios (MMR) are related with development and cultural factors that are not easy to change. The MMR of Turkey between 2007 and 2009 was 19.7 per 100,000 live births (2). Most maternal deaths are preventable and a review of the underlying clinical and social risk factors is important to decrease the number of these deaths (3). It has been estimated that 74% of maternal mortality can be averted if all women receive appropriate emergency obstetric care (4).

Hypertensive disorders in pregnancy, childbirth, and the puerperium are one of the most frequent causes of maternal and perinatal mortality (5). There is no proven effective method for the prevention of preeclampsia.

Our aim was to analyze maternal deaths in Turkey due to hypertensive disorders in pregnancy, childbirth, and the puerperium.

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Material and Methods

In this retrospective study, medical records of all pregnancy-associated deaths recorded in Turkey between 2012 and 2015 were reviewed. The Turkish Statistical Institute (TURKSTAT) has been collecting data on the number of deaths and causes of death using the vital registration (VR) system since 2009 in details of ICD-10 codes, and underlying cause is the main concern as the World Health Organization (WHO) suggests. The VR system of TURKSTAT collects data through forms that include a check box to mark whether the death was a maternal death. Maternal deaths are also debated by the Ministry of Health (MoH) because the MoH assigns a committee of doctors and specialists to discuss suspicious maternal deaths in detail to determine whether the death fulfilled the WHO maternal death criteria. The MoH made an act that stated that death notifications would no longer be received from the field through paper forms but through the death notification system so that deaths were registered to the VR. The VR system works well in terms of completeness and accuracy of data.

The underlying cause as "disease or injury that initiated a chain of events, which lead to death" was noted. Deaths related to hypertensive disorders of pregnancy were evaluated. All maternal deaths in Turkey are reported to the Preliminary Investigation Committee for Maternal Deaths at the MoH of Turkey. A team of clinicians including 2 nurses/midwives, 1 perinatologist, 2 obstetricians, 1 internal medicine specialist, and 1 anesthetist evaluate the medical records in a group setting in order to arrive at consensus on clinical determinations. Identifying the cause and preventability of maternal mortality includes medical hospital records, death certificates, autopsy reports, local and national registries, and verbal autopsy. Each case is analyzed separately in the first month after death occurs. The WHO application of "ICD-10 to deaths during pregnancy, childbirth, and the puerperium: ICD-MM" manual was used for the definitions (6). Under the ICD-MM, maternal death is defined as the death of a woman while pregnant or within 42 days of termination of the pregnancy, irrespective of the duration and site of the pregnancy, and of any cause related to or aggravated by the pregnancy or its management with the exception of accidental or incidental causes. Late maternal deaths (more than 42 days but less than 1 year after the termination of pregnancy) were excluded in the present study. MMR was calculated as the number of maternal deaths during the given time period per 100,000 live births during the same time period.

A death was classified as preventable by consensus of the expert committee. An event was considered preventable if one of the three delays was reported (7, 8). Phase 1 delay: delay in deciding to seek appropriate medical help for an obstetric emergency, phase 2 delay: delay in reaching an appropriate health facility; phase 3 delay: delay in receiving adequate emergency obstetric care when a facility was reached.

In the present study hypertensive disorders in pregnancy, childbirth, and the puerperium that caused maternal death were distributed into four groups; 1- Pre-existing hypertensive disorder complicating pregnancy, childbirth, and the puerperium with increased/superimposed proteinuria and severe preeclampsia; 2- Hemolysis, elevated liver enzymes, low platelet count (HELLP) syndrome; 3- Eclampsia; and 4- Pre-existing hypertension complicating pregnancy, childbirth, and the puerperium without increased/superimposed proteinuria.

Data on maternal demographic features, presence of antenatal care, medical and obstetric history, mode of delivery, and medication (including emergency antihypertensive therapy and eclampsia prophylaxis with magnesium sulphate) were recorded. This investigation was reviewed and approved by the Public Health Agency of the MoH of Turkey.

Data were entered into a database and analyzed. The Statistical Package for the Social Sciences statistical software package version 16.0 (SPSS Inc., Chicago, IL, USA) was used for the analyses. The results were presented as frequencies, percentages, and descriptive summary statistics. The Chi-square test was used for comparison of the categorical data between the groups. $P<0.05$ was considered statistically significant.

Results

In Turkey, a total of 812 maternal deaths were recorded between 2012 and 2015. In this period, hypertensive disorders in pregnancy, childbirth, and the puerperium accounted for 15.5% (n=126) of all maternal mortality. They were the third most frequent cause among all causes after diseases of the circulatory system complicating pregnancy, childbirth, and the puerperium (n=180, 22.2%) and obstetric hemorrhage groups (n=146, 18.0%), and they were second among direct causes of maternal death (Table 1).

The distribution of hypertensive disorders in pregnancy, childbirth, and the puerperium was 61 cases in severe preeclampsia or pre-existing hypertensive disorder with increased/superimposed proteinuria (48.4%), 39 in eclampsia (31.0%), 12 in HELLP syndrome (9.5%), and 14 cases in pre-existing hypertension complicating pregnancy, childbirth, and the puerperium without increased or superimposed proteinuria. The rate of severe preeclampsia or pre-existing hypertensive disorder with increased/superimposed proteinuria decreased gradually and was statistically significant from 2012 to 2015 ($\chi^2=23.135$, $p=0.006$) (Table 2).

Sociodemographic and clinical characteristics of the women with hypertensive disorders of pregnancy are depicted in Table 3. The ages of the 126 women with hypertensive disorders of

pregnancy ranged from 19 to 48 years with a median of 32 years (mean; 31.6±6.3 years). Out of 126 women, 47 (37.3%) women were aged ≥35 years. The majority of women (n=102, 81%) were found to receive adequate antenatal care from any health care practitioners according to the WHO criteria. All women who did not receive antenatal care were illiterate (Table 3).

With the exception of 2 cases, all deaths occurred during the postpartum period. Twenty-three percent (n=29) of deaths occurred in the first 48 hours postpartum, 23.8% (n=30) occurred between 2-7 days, and 51.6% (n=65) between 8-42 days. The time interval from admission to any medical facility to death was 7.29±0.83 days. A total of 89.7% (n=113) women underwent cesarean section. The major cesarean delivery indications in the study were preeclampsia complications, i.e. hypertensive emergencies or HELLP syndrome (n=82), fetal distress (n=13), and abruption placenta (n=15). Cesarean section performed because of previous cesarean and breech presentation without any evidence of hypertension at delivery was performed in only 3 women. We report no cases of prolonged or obstructed labor.

Out of 39 maternal deaths due to eclampsia, 27 (69.2%) were diagnosed during the antenatal period, whereas 10 (25.6%) had

Table 1. Maternal deaths based on diagnosis

		Years				
	Diagnosis	**2012**	**2013**	**2014**	**2015**	**Total**
		n (%)	**n (%)**	**n (%)**	**n (%)**	**n (%)**
Direct causes (n=415, 51.1%)	Obstetric hemorrhage	35 (18.2)	42 (18.8)	41 (19.2)	28 (15.3)	146 (18.0)
	Hypertensive disorders	33 (17.2)	38 (17.0)	29 (13.6)	26 (14.2)	126 (15.5)
	Obstetric embolism	37 (19.3)	19 (8.5)	26 (12.2)	11 (6.0)	93 (11.5)
	Pregnancy-related infection	5 (2.6)	9 (4.0)	14 (6.6)	4 (2.2)	32 (3.9)
	Other direct causes	3 (1.6)	4 (1.8)	3 (1.4)	8 (4.4)	18 (2.2)
Indirect causes (n=372, 45.8%) (Disease complicating pregnancy, childbirth and the puerperium)	Diseases of the circulatory system	42 (21.9)	54 (24.1)	44 (20.7)	40 (21.9)	180 (22.2)
	Mental disorders and diseases of the nervous system	7 (3.6)	6 (2.7)	8 (3.8)	8 (4.4)	29 (3.6)
	Indirect maternal infectious disease, including respiratory system infections	8 (4.2)	28 (12.5)	21 (9.9)	25 (13.7)	82 (10.1)
	Other specified diseases and conditions	20 (10.4)	19 (8.5)	21 (9.9)	21 (11.5)	81 (10.0)
	Undiagnosed	2 (1.0)	5 (2.2)	6 (2.8)	12 (6.6)	25 (3.1)
	Total	192 (100)	224 (100)	213 (100)	183 (100)	812 (100)

Table 2. Distribution of the cases and delay models according to years

	2012	**2013**	**2014**	**2015**	**Total**	**p**
	n (%)	**n (%)**	**n (%)**	**n (%)**	**n (%)**	
Hypertensive disorders in pregnancy, childbirth, and the puerperium						
Severe preeclampsia or pre-existing hypertension with increased/superimposed proteinuria	23 (69.7)[1]	19 (50.0)	13 (44.9)	6 (23.1)[1]	61 (48.4)	0.006
HELLP syndrome	1 (3.0)	3 (7.9)	4 (13.8)	4 (15.4)	12 (9.5)	
Eclampsia	9 (27.3)	12 (31.6)	7 (24.1)	11 (42.3)	39 (31.0)	
Pre-existing hypertension without increased/ superimposed proteinuria	-	4 (10.5)	5 (17.2)	5 (19.2)	14 (11.1)	
Delay model						
No delay	24 (72.7)	28 (73.7)	16 (55.2)	12 (46.2)	80 (63.5)	0.052
Phase 1 delay	8 (24.2)[2]	6 (15.8)	8 (27.6)	7 (26.9)	29 (23.0)	
Phase 3 delay	3 (9.0)[2]	4 (10.5)	5 (17.2)	7 (26.9)	19 (15.1)	

[1]$p<0.05$, [2]Two cases both of phase 1 and 3 delays were identified and the numbers included those
HELLP syndrome: hemolysis, elevated liver enzymes, low platelet count syndrome

a postnatal onset. Only 2 patients had a seizure during labor. Out of the 27 cases diagnosed during the antenatal period, 5 were diagnosed before 32 weeks of gestation, and 22 at or after 32 weeks of gestation. All women with postnatal eclampsia delivered after 32 weeks of gestation. The rate of specific use of magnesium sulfate in eclampsia was (37/39) 94.9%.

Intracranial hemorrhage was the major final cause of death with a rate of 41.3% (n=52) and was noted in 24 patients with eclampsia, five patients with HELLP syndrome, and 23 patients with severe preeclampsia or pre-existing hypertensive disorder with/without increased/superimposed proteinuria. Except for fifteen patients with intracranial hemorrhage, emergency antihypertensive agents (nifedipine, hydralasine or labetalol) were not implemented in optimal dose and/or duration before the event had occurred. Pulmonary edema was the final cause of death in 11 patients with severe preeclampsia or pre-existing hypertensive disorder with/without increased/superimposed proteinuria, and in two patients with HELLP syndrome.

Table 3. Demographic characteristics of the maternal deaths based on years

Variables	**2012**	**2013**	**2014**	**2015**		
	n (%)	**n (%)**	**n (%)**	**n (%)**	**χ^2**	**p**
Total	33 (100.0)	38 (100.0)	29 (100.0)	26 (100.0)		
Educational status					11.716	0.469
Illiterate	9 (27.3)	8 (21.1)	13 (44.9)	5 (19.2)		
Education ≤4 years	12 (36.4)	11 (28.9)	6 (20.7)	9 (34.6)		
Education 5-8 years	1 (3.0)	6 (15.8)	2 (6.9)	2 (7.7)		
Education ≥9 years	10 (30.3)	9 (23.7)	7 (24.1)	8 (30.8)		
Not known	1 (3.0)	4 (10.5)	1 (3.4)	2 (7.7)		
Obesity					0.459	0.928
Normal	24 (72.7)	29 (76.3)	23 (79.3)	19 (73.1)		
Obese	9 (27.3)	9 (23.7)	6 (20.7)	7 (26.9)		
Age (years)					0.389	0.943
<35	21 (63.6)	25 (65.8)	17 (58.6)	16 (61.5)		
≥35	12 (36.4)	13 (34.2)	12 (41.4)	10 (38.5)		
Parity					4.888	0.180
<5	30 (90.9)	34 (89.5)	26 (89.7)	26 (100.0)		
≥5	3 (9.1)	4 (10.5)	3 (10.3)	-		
Additional risk factors					8.239	0.221
No risk	10 (30.3)	14 (36.8)	11 (37.9)	10 (38.5)		
1 risk	6 (18.2)	8 (21.1)	12 (41.4)	7 (26.9)		
More than 1 risk	17 (51.5)	16 (42.1)	6 (20.7)	9 (34.6)		
Number of antenatal visits					5.719	0.126
None	-	2 (5.3)		1 (3.8)		
<4	10 (30.3)	5 (13.2)	3 (10.3)	3 (11.5)		
≥4	23 (69.7)	31 (81.6)	26 (89.7)	22 (84.6)		
Mode of delivery					-	-
No delivery	-	-	2 (6.9)	-		
Vaginal birth	1 (3.0)	5 (13.2)	2 (6.9)	3 (11.5)		
Cesarean section	32 (97.0)	33 (86.8)	25 (86.2)	23 (88.5)		
Inter-pregnancy interval					10.819	0.288
First pregnancy	10 (30.3)	14 (36.8)	8 (27.6)	10 (38.5)		
<2 years	9 (27.3)	6 (15.8)	3 (10.3)	3 (11.5)		
≥2 years	13 (39.4)	13 (34.2)	12 (41.4)	12 (46.2)		
Not known	1 (3.0)	5 (13.2)	6 (20.7)	1 (3.8)		

When the delay models were analyzed, no delay was found during the mortality process in 80 (63.5%) cases. A phase 1 delay was determined delay in 29 (23.0%) cases, i.e. delay in seeking care by the patient, and a phase 3 delay in 19 (15.1%), i.e. preventable factors, before death occurred. In two cases, both phase 1 and 3 delays were present.

Twenty-three patients with delay in seeking care (23/29, 79.3%) were illiterate or had primary level (≤4 years) education. However, this rate was 62.5% (50/80) in cases in which no delay was identified. There was no correlation between educational status of women and phase 1 delay (p=0.099).

Discussion

Globally, an estimated 287,000 maternal deaths occurred in 2010. MMR is one of the most important public health indicators that reveals the development of a country's economy, culture, and healthcare system. The global MMR in 2010 was reported as 210 maternal deaths per 100,000 live births. Although one of the aims of MDG was to reduce the MMR by three quarters till the year 2010 with respect to the rates of 1990 the rate of reduction is still well short of the 5.5 per cent annual decline needed to meet the target (9). However Turkey has experienced an annual decline above this rate with 5.8% (9). Preeclampsia, which affects 2% of pregnancies, leads to considerable maternal and fetal morbidity and mortality (5). In a cross-sectional study designed by the WHO (10), which was conducted at health facilities in 29 countries from Africa, Asia, Latin America, and the Middle East, incidences of pre-eclampsia, eclampsia, and chronic hypertension were reported as 2.16%, 0.28%, and 0.29%, respectively. The present report provides a national estimate of the incidence of maternal mortality from hypertensive disorders in pregnancy, childbirth, and the puerperium in Turkey between 2012 and 2015. Maternal mortality due to hypertensive disorders in pregnancy, childbirth, and the puerperium was 16.4% between 2007 and 2009 in Turkey (2). Similar to the 2007- 2009 period, 15.5% of maternal deaths were due to the same reason between 2012 and 2015.

In the present study, the highest ratio of maternal deaths caused by hypertensive disorders (62.7%) was in mothers aged <35 years. All deaths except 2 occurred during the postpartum period. Eclampsia represented approximately 31.0% of hypertensive disorders. We found high rates of administration of magnesium sulfate in eclampsia. The cesarean delivery rate was high in the study but we found that it was widely used as a life-saving procedure. In the present study, 36.5% of hypertensive deaths were classified as preventable and the key preventable factor was delay in seeking care by the patient (23%). Moodley (11) reported 507 deaths associated with hypertensive disorders in pregnancy, childbirth, and the puerperium, and similar to our results, the author showed that the most common preventable factors were patient-oriented problems, i.e. women who either presented late for antenatal care or late to hospital when they became symptomatic.

Adu-Bonsaffoh et al. (12) reported that eclampsia, acute renal failure, intracranial hemorrhage and pulmonary edema were the major immediate causes of hypertension-related maternal death in their population. Bentata et al. (13) reported hypertensive disorders were the main reason for admission to the intensive care unit. In the present study, we found that the major final cause of death in hypertensive disorders of pregnancy was intracranial hemorrhage. Intracranial hemorrhage is an overwhelming cause of death in women with hypertensive disorders, which reflects a failure of effective treatment of systolic hypertension, although the explosive nature of fulminating preeclampsia may also cause intracranial hemorrhage. Maybe these patients would have a better prognosis if a standardized clinical protocol was adopted for the management of hypertensive disorders in pregnancy. We recommend that all maternity units have clear guidelines for the management of severe preeclampsia and treatment protocols should include emergency antihypertensive agents (nifedipine, hydralazine or labetalol), which were preferred as first-line therapy for emergency therapy of acute-onset severe hypertension by the American College of Obstetricians and Gynecologists in 2015 (14). Clark et al. (15) also stated that disease-specific protocols were beneficial in the reduction of maternal mortality because of hypertensive disorders of pregnancy. They reported that a policy of automatic and emergency antihypertensive therapy for hypertensive disorders of pregnancy eliminated deaths from in-hospital intracranial hemorrhage and decreased maternal deaths. Besides using antihypertensive agents, especially fluid restriction or at least giving under the controlled limits should be addressed as specific aspects of preeclampsia management because of the risk of acute renal failure development (16).

In conclusion, more efforts are needed to decrease maternal mortality in Turkey. At present, new strategies are being developed by the MoH for reducing maternal deaths. Eradicating preventable hypertensive maternal deaths will require an improvement in educational status of women, implementation of clear guidelines for the management of acute-onset severe hypertension, and the ability to easily and immediately obtain emergency antihypertensive agents, because the most frequent cause of deaths is intracranial hemorrhage.

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References

1. Hogan MC, Foreman KJ, Naghavi M, Ahn SY, Wang M, Makela SM, et al. Maternal mortality for 181 countries. 1980-2008: a systematic analysis of progress towards Millennium Development Goal 5. Lancet 2010; 375: 1609-23.
2. Engin-Üstün Y, Çelen Ş. Özcan A, Sanisoğlu S, Karaahmetoğlu S, Gül R, et al. Maternal mortality from cardiac disease in Turkey: a population-based study. J Matern Fetal Neonat Med 2012; 25: 2451-3.
3. Calvello EJ, Skog AP, Tenner AG, Wallis LA. Applying the lessons of maternal mortality reduction to global emergency health. Bull World Health Organ 2015; 93: 417-23.
4. Wagstaff A, Claeson M. The Millennium Development Goals for Health: rising to the challenges. Washington: World Bank; 2004.
5. World Health Organization. 2005. Make Every Mother and Child Count. World Health Report, 2005. Geneva. Switzerland.
6. The WHO application of ICD-10 to deaths during pregnancy, childbirth and puerperium: ICD MM. World Health Organization 2012, Geneva, Switzerland.
7. Thaddeus S, Maine D. Too far to walk: maternal mortality in context. Soc Sci Med 1994; 38: 1091-110.
8. Combs Thorsen V, Sundby J, Malata A. Piecing together the maternal death puzzle through narratives: the three delays model revisited. PLOS One 2012; 7: e52090.
9. WHO, UNICEF, UNFPA and The World Bank estimates. 1990-2010. Trends in maternal mortality 1990-2010.
10. Abalos E, Cuesta C, Carroli G, Qureshi Z, Widmer M, Vogel JP, et al. Pre-eclampsia, eclampsia and adverse maternal and perinatal outcomes: a secondary analysis of the World Health Organization Multicountry Survey on Maternal and Newborn Health. BJOG 2014; 121(Suppl 1): 14-24.
11. Moodley J. Maternal deaths associated with hypertensive disorders of pregnancy: a population-based study. Hypertens Pregnancy 2004; 23: 247-56.
12. Adu-Bonsaffoh K, Oppong SA, Binlinla G, Obed SA. Maternal deaths attributable to hypertensive disorders in a tertiary hospital in Ghana. Int J Gynaecol Obstet 2013; 123: 110-3.
13. Bentata Y, Madani H, Berkhli H, Saadi H, Mimouni A, Housni B. Complications and maternal mortality from severe pre-eclampsia during the first 48 hours in an intensive care unit in Morocco. Int J Gynaecol Obstet 2015; 129: 175-6.
14. Committee on Obstetric Practice. Committee Opinion No. 623: Emergent therapy for acute-onset, severe hypertension during pregnancy and the postpartum period. Obstet Gynecol 2015; 125: 521-5.
15. Clark SL, Christmas JT, Frye DR, Meyers JA, Perlin JB. Maternal mortality in the United States: predictability and the impact of protocols on fatal postcesarean pulmonary embolism and hypertension-related intracranial hemorrhage. Am J Obstet Gynecol 2014; 211: 32.
16. Mehrabadi A, Liu S, Bartholomew S, Hutcheon JA, Magee LA, Kramer MS, Liston RM, et al. Hypertensive disorders of pregnancy and the recent increase in obstetric acute renal failure in Canada: population based retrospective cohort study. BMJ 2014; 349: g4731.

Comparison of bilateral transversus abdominis plane block and wound infiltration with bupivacaine for postoperative analgesia after cesarean delivery

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Abstract

Objective: The study aimed to compare efficacy, safety, pain intensity and analgesic consumption in patients receiving either bilateral transversus abdominis plane (TAP) block or wound infiltration with bupivacaine after cesarean delivery (CD).

Material and Methods: A total of 216 parturient women undergoing CD under general anesthesia were randomly allocated into five groups: i) controls (group 1), ii) TAP placebo (group 2), iii) TAP (group 3), iv) wound infiltration placebo (group 4), and, v) wound infiltration (group 5). Pain intensity was assessed using a visual analogue scale (VAS). Analgesic consumptions were recorded by a blinded nurse at 6, 12, and 18 hours postoperatively.

Results: The baseline characteristics of the five groups were similar in terms of age, history of CD, and body mass indices ($p>0.05$). There were significant intergroup differences in VAS scores between all groups at the zero time-point ($p=0.03$), at the 6^{th} hour ($p=0.02$), 12^{th} hour ($p=0.02$), and at the 18^{th} hour ($p=0.02$). Group 3 patients had lower pain scores and consumed less diclofenac than group 2 patients only within 12 hours postoperatively whereas pain intensity and analgesic consumption were not different between group 5 and group 4 patients. Group 5 patients received significantly less pethidine than group 4 and group 1 patients ($p<0.001$).

Conclusion: TAP block provided better pain relief and less analgesic requirement than bupivacaine wound infiltration early after CD. Given the similar amounts of diclofenac but lower amounts of pethidine administered in the wound infiltration group, wound infiltration of bupivacaine seems promising in terms of reducing opioid use after CD under general anesthesia, especially when TAP block is not used. (J Turk Ger Gynecol Assoc 2017; 18: 26-32)

Keywords: Cesarean delivery, transversus abdominis plane block, wound block, bupivacaine

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Introduction

Cesarean delivery (CD) rates have been substantially rising worldwide and Turkey is among the countries where the increasing trend is most prominent (1). The number of women showing a tendency to have CD is rising possibly because this type of delivery sounds less frightening and less painful than normal vaginal birth. CD has become an appealing option requested by women in the general population and by health care providers, owing to the concerns commonly raised about complications and pain experience during labor (2).

A painless CD is achievable using various anesthesia techniques while each technique has its own criteria to be met by the given patient. However, post-operative pain, which appears after elimination of the anesthesia, continues for days after surgery and still constitutes a major problem in patients receiving CD (3). It was reported that about 10% of women still experience substantial pain after CD even though a programmed analgesic regimen was implemented (4). Women with depressive symptoms during the postnatal period report pain more commonly than those without depression (5). Furthermore, high pain levels after CD was reported to be associated with loss of ability for breast feeding and taking care of the newborn (6).

Transversus abdominis plane (TAP) block has gained popularity among physicians owing to the ease of the procedure and its

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effectiveness in reducing pain after lower abdominal surgery. In recent years, there have been a number of comparative studies demonstrating its effectiveness in postoperative pain control after CD under spinal anesthesia (7, 8). Wound infiltration of non-steroid anti-inflammatory drugs or local anesthetics during wound closure is an alternative for providing pain relief after CD. A systematic review of studies on wound infiltration of local anesthetics during CD under spinal anesthesia established that the technique provided a significant decrease in morphine consumption (9). Data is limited regarding its effectiveness in patients receiving general anesthesia. Even a single injection of local anesthetics within wound layers was shown to decrease morphine requirements within 12 hours after CD (10). Therefore, we conducted a placebo-controlled study to compare the efficacy and safety of these two aforementioned techniques in patients undergoing primary elective CD under general anesthesia.

Material and Methods

The Ethics Committee of Ankara Numune Hospital approved the study, which was conducted in accordance with the Declaration of Helsinki 2013 Brasil version (March 27. 2014, 20796219-E-14-159). All patients gave written informed consent to take part in the research. This prospective randomized double blinded five-arm study was conducted at the Hitit University Hospital and comprised patients undergoing elective CD between April and August 2014. Eligible patients were those at minimum 37 weeks of gestational age, with American Society of Anesthesiologists physical status I-II, aged between 18-45 years, non-laboring at the time of allocation, and those requesting general anesthesia. Patients with a body mass index (BMI) of more than 40 kg/m^2, with a history of chronic pain, drug abuse, cardiac and pulmonary disease, and those undergoing emergency CD were excluded. Based on the above criteria, a total of 216 patients were randomly allocated into two treatment and two placebo groups and one control group, according to randomly sequenced numbers generated using a computer-based random number generator in blocks of two methods, to ensure a near-equal distribution of patients into treatment arms. Group allocation was concealed with sealed envelopes including the code of the group that an individual patient would be included in. The patient and the investigator who collected study data were blinded to the group allocation.

The patients were divided into five groups so as to receive the planned procedure for them: i) group 1 (G1)- controls, ii) group 2 (G2)- TAP placebo, iii) group 3 (G3)- TAP, iv) group 4 (G4)- wound infiltration placebo, and, v) group 5 (G5)- wound infiltration. G1 served as controls and received no additional procedures. G3 patients received ultrasound (US)-guided TAP block with 20 mL of 0.25% bupivacaine after closure of the wound. Likewise, G5 patients received wound infiltration of 20 mL of 0.25% bupivacaine before closure of the wound. G2 patients received a 20 mL physiologic saline injection identical to G3, and G4 patients received a 20 mL infiltration of physiologic saline identical to G5.

In the operating room, standardized monitoring including electrocardiogram, non-invasive blood pressure, and pulse oximetry was provided before induction of anesthesia. Anesthesia was induced with 5 mg/kg of thiopental sodium and orotracheal intubation was performed. In all patients, skin preparation and sterile draping included whole upper and lower abdomen to allow for bilateral anterolateral wall approach of the abdomen. Anesthesia was maintained using sevoflurane with an end-tidal concentration of 2.5%.

CD procedures were performed by the same surgeon who used the same CD technique. After delivery, the surgeon operating on the patient continued in sterile conditions in all groups and performed all procedures himself after ensuring the investigator who would be involved in data collection was outside the operating room. In the TAP block group (G3), after closing the wound, the anesthesiologist performed the TAP block as described previously by Costello et al. (11). A linear transducer US probe was placed midway between the costal margin and the iliac crest at the anterolateral wall of the abdomen and fasciae of the external oblique, internal oblique and transversus abdominis muscles and the TAP was identified. A 22-gauge 80-mm aspiration needle was introduced into the TAP under real-time US guidance. After confirmation of the needle position by injecting 1 mL of test dose and negative aspiration, 20 mL of 0.25% bupivacaine was injected with negative aspirations performing at every 5 mL. The procedure was repeated on the contralateral side. In the TAP placebo group (G2), the procedure was identical to that in G3 except for injecting 20 mL of normal physiologic saline after entering the TAP. The skin was covered at needle insertion sites with dressing to ensure blinding.

In the wound infiltration group (G5), infiltration was performed before closure of the wound. The surgeon administered 20 mL of 0.25% bupivacaine within the fascia and also to the subcutaneous fat tissue above the fascia, as described previously by Niklasson et al. (10). In the wound infiltration placebo group (G4), the procedure was identical to that in G5 except for infiltrating 20 mL of normal physiologic saline to the fascia. In the control group (G5), no additional procedures were performed after delivery and the surgeon proceeded to skin closure without any delay. In G1, G4, and G5 where TAP procedure was not performed, sham skin dressings were applied at sites corresponding to needle insertion sites for TAP to ensure blinding of the data collectors.

After completion of the treatment, 0.1 mg/kg of morphine and 15 μg of sufentanil was given. All patients received 2.5 mg of neostigmine to eliminate any residual neuromuscular blockade. The patients were extubated soon after they opened their eyes on command and spontaneously breathing. In the postoperative care unit, patients received no type of patient-controlled anesthesia (PCA). Patients received a multimodal analgesic regimen including intramuscular administration of diclofenac sodium (Dikloron 75 mg, Deva Drugs, Turkey) and pethidine (Aldolan Gerot 100 mg, Liba Drugs, Turkey). Nausea and vomiting was treated using intramuscular metoclopramide 10 mg, as needed. Patients were asked by a blinded investigator to rate their pain using a 100-mm visual analogue scale (VAS) (0 hours) before receiving the first administration dose of 75 mg intramuscular diclofenac on arrival in the postoperative care unit. Patients were strongly advised that should ask for additional analgesics if needed at any time after surgery. For pain not relieved with diclofenac, pethidine 50 mg muscular injection was administered. Repeated doses of diclofenac and pethidine were administered if pain was not relieved, avoiding excessive doses of the same analgesics. Analgesic use was recorded by a blinded nurse at 6, 12, and 18 hours postoperatively, and the pain assessment was repeated to record the worst VAS score by requesting the patient to cough or change their position from supine to sitting.

Statistical analysis

A priori sample size calculation was performed for a five-group fixed effects one-way ANOVA test. The effect size (Cohen's f) in this study was found as 1.75 (12). According to Cohen (13) an effect size of more than 0.40 was defined as large. Therefore, the effect size in that study was considered as large and was not used. In G power, assuming a medium effect size (f=0.25), with a significance level of α=0.05, and β=0.20, we found that 40 subjects were required in each group.

Statistical analyses were performed using the Statistical Package for the Social Sciences version 21 (SPSS IBM Inc. Chicago, USA). The distribution of variables was tested using visual histograms and the Kolmogorov-Smirnov test to determine normality. Descriptive statistics for continuous variables were reported as mean ± standard deviation and categorical variables were represented as frequency and percentage. Categorical variables were compared using Chi-square test or Fisher's exact test where appropriate. One-way ANOVA or Welch ANOVA were used to compare normally distributed continuous variables among the five groups, based on the homogeneity of variances, which was tested using the Levene test. Post-hoc tests were performed using Hochberg's or Tamhane test according to the presence of homogeneity. Dunn-Sidak's method was used to calculate the level of significance for multiple five-group comparisons [p<1-(1-0.05)1/5=0.0102 was considered as statistically significant].

Results

The baseline characteristics of the patients are given in Table 1. The five groups were similar in terms of age, history of CD and BMIs. The procedure for TAP block took a mean of 4.6±0.8 min and 4.2±0.9 min in patients who received local analgesic and placebo, respectively (p=0.74). The procedure for wound infiltration of bupivacaine took 2.8±0.4 min and 2.9±0.6 min in patients receiving local analgesic and placebo, respectively (p=0.86). No patients had complications during application of TAP injection under US guidance. TAP injections caused no hematoma, bleeding or pain sensitivity at the site of application.

Table 1. Baseline characteristics of the patients

	G1 control (n=42, 19.4%)	G2 TAP placebo (n=39, 18.1%)	G3 TAP block (n=42, 19.4%)	G4 wound infiltration placebo (n=47, 21.8%)	G5 wound infiltration (n=46, 21.3%)	p
Age (years)	30.0±6.5	29.9±5.2	28.3±4.7	29.1±5.4	29.2±5.2	0.60
History of CD						
0	9 (21.4%)	5 (12.8%)	6 (14.3%)	12 (25.5%)	11 (23.9%)	0.47
1	27 (64.3%)	25 (64.1%)	24 (57.1%)	24 (51.1%)	23 (50.0%)	0.50
2	6 (14.3%)	9 (23.1%)	9 (21.4%)	8 (17.0%)	12 (26.1%)	0.66
>2	0 (0.0%)	0 (0.0%)	3 (7.1%)	3 (6.4%)	0 (0.0%)	0.06
BMI (kg/m^2) (pre-pregnancy)	24.8±4.1	26.38±6.3	25.1±4.4	25.53±5.1	25.7±5.0	0.67
BMI (kg/m^2) (at pregnancy)	29.2±4.3	30.5±6.3	30.0±4.2	29.7±4.8	30.5±5.1	0.69

TAP: transverse abdominis plane blockade; CD: cesarean delivery; BMI: body mass index; G: group.
*p-values indicate statistical significance (p<0.05).

There were significant intergroup differences in VAS scores between the treatment, placebo, and control groups at the zero time point (p=0.03), at 6 hours (p=0.02), 12 hours (p=0.02), and at 18 hours (p=0.02) as shown in Table 2. At the zero time point, patients in G3 reported significantly lower VAS scores than those in G5 (p=0.021), G2 (p=0.039), G4 (p<0.001), and the control group (G1) (p=0.009). The Post-hoc Tamhane test indicated that the difference between TAP block and wound infiltration placebo groups were the most pronounced. At the 6th hour, patients in TAP block group reported significantly lower VAS scores than those in the TAP placebo group (p=0.008), wound infiltration placebo group (p=0.004), and control group (p=0.02). When the Hochberg post-hoc test was applied, the most pronounced difference was found between the TAP block and wound infiltration placebo groups. At the 12th hour, patients in the TAP block group reported significantly lower VAS scores than those in TAP placebo group (p=0.017), and patients in the wound infiltration group reported significantly lower VAS scores than those in the TAP placebo group (p=0.017). As the Hochberg post-hoc test revealed, the difference between TAP block and TAP placebo groups were the most pronounced. At the 18th hour, patients in the TAP block group reported significantly lower VAS scores than those in the wound infiltration placebo group (p=0.02) and in the control group (p=0.002). Again, the Hochberg post-hoc test designated that the most pronounced difference was between TAP block and the control groups.

The analgesic requirements after surgery are summarized in Table 3. There were significant intergroup differences in diclofenac (p=0.004) and pethidine use (p<0.001) of the patients. According to the post-hoc pairwise comparisons for diclofenac use, patients in TAP block group used significantly less diclofenac than those in the wound infiltration group (p=0.007), TAP placebo group (p<0.001), and wound infiltration placebo group (p=0.002) where the difference between TAP block and TAP placebo groups were the most pronounced one. According to the post-hoc pairwise comparisons for pethidine use, patients in the TAP block group required significantly less pethidine than those in the TAP placebo group (p<0.001), wound infiltration placebo group (p<0.001), and control group (p<0.001). Also, patients in the wound infiltration group used significantly less pethidine than those in the TAP block placebo group (p=0.002), wound infiltration placebo group (p=0.009), and control group (p=0.004) in which the difference between TAP block and TAP placebo groups was again the most pronounced.

Table 2. Visual analogue scale, scores of the patients

VAS (after CD)	G1 controls	G2 TAP placebo	G3 TAP block	G4 wound infiltration placebo	G5 wound infiltration	p
0 hours	80.0±17.2	76.9±16.4	64.3±34.2	82.3±14.3	77.8±18.1	0.03*
6th hour	35.8±25.0	38.0±24.3	24.3±20.9	38.6±24.6	31.7±21.0	0.02*
12th hour	35.4±21.7	37.3±19.3	25.5±23.9	34.7±20.9	26.3±22.1	0.02*
18th hour	25.5±21.3	18.0±17.3	12.3±15.3	19.9±14.8	17.4±20.4	0.02*

TAP: transverse abdominis plane blockade; CD: cesarean delivery; VAS: visual analogue scale; G: group.
*p-values indicate statistical significance (p<0.05).

Table 3. Analgesic requirements after surgery

	G1 controls	G2 TAP placebo	G3 TAP block	G4 wound infiltration placebo	G5 wound infiltration	p
Diclofenac						
None	1 (2.4%)	0 (0.0%)	1 (2.4%)	1 (2.1%)	0 (0.0%)	0.733
Once	13 (31.0%)	7 (17.9%)	22 (52.4%)	10 (21.3%)	13 (28.3%)	0.006*
Twice	28 (66.7%)	32 (82.1%)	19 (45.2%)	36 (76.6%)	33 (71.7%)	0.004*
Mean ± SD	1.6±0.5	1.8±0.4	1.4±0.6	1.7±0.5	1.7±0.5	0.004*
Pethidine						
None	3 (7.1%)	4 (10.3%)	23 (54.8%)	4 (8.5%)	19 (41.3%)	<0.001*
Once	24 (57.1%)	18 (46.2%)	14 (33.3%)	28 (59.6%)	16 (34.8%)	0.031*
Twice	15 (35.7%)	17 (43.6%)	5 (11.9%)	15 (31.9%)	11 (23.9%)	0.020*
Mean ± SD	1.3±0.6	1.3±0.7	0.6±0.7	1.2±0.6	0.8±0.8	<0.001*

TAP: transverse abdominis plane blockade; SD: standard deviation.
*p-values indicate statistical significance (p<0.05).

Discussion

Our study showed that a single injection TAP block satisfactorily provided pain relief for 12 hours postoperatively in patients who underwent elective CD under general anesthesia whereas such benefit was limited in patients who received wound infiltration with local anesthetic at similar doses. TAP block provided the lowest VAS scores in all assessments with the largest difference being observed immediately after the patients arrived in the postoperative intensive care unit. The difference in VAS scores observed between TAP block and TAP placebo patients sustained until the 12th hour assessment although patients in the TAP placebo group received much more diclofenac and pethidine. The difference between the TAP block and TAP placebo groups was not sustained and receded at the 18th hour, indicating the rapid onset but short duration effect of bupivacaine when used in TAP block.

There have been several studies on TAP block in patients undergoing CD under spinal anesthesia (14). Although some of these showed no benefit and implied that TAP block may have a potential role in pain relief following general anesthesia, few studies have focused on this topic (11, 15). Continuous wound infiltration of local anesthetics has also gained popularity in recent years; however, it is yet to be established whether a single-dose wound infiltration of local anesthetics provides benefit in terms of postoperative pain relief.

In a study by Eslamian et al. (16) VAS scores were significantly lower over time (within 24 h) in patients who received TAP block after CD with general anesthesia. These authors also reported that patients in the TAP block group requested analgesics after a longer time than those in the control group. Similar to us, these investigators did not use PCA in their patients. In another study on patients who underwent CD under general anesthesia, Tan et al. (17) reported similar outcomes; they observed a significant decrease in morphine use in the TAP block group, whereas VAS scores were not different between patients in the TAP block and standard care groups. In their study, principal component analysis was implemented as a routine standard of practice, whereas such protocol is not in use in our institution. Based on the above facts, we postulate that TAP block is beneficial both in terms of limiting analgesic use and improving pain relief in patients undergoing CD under general anesthesia.

In our study, we found no differences between patients receiving wound infiltration of 20 mL of bupivacaine or placebo in regards to VAS scores. Although we observed a slight difference at the 6th and 12th hours postoperatively, the difference did not reach statistical significance. In a randomized controlled trial, Niklasson et al. (10) injected a single dose 40 mL (2.5 mg/mL) of bupivacaine and adrenaline into the fascial layers before closure of the wound after CD and compared these patients with placebo controls. In that study, wound infiltration with bupivacaine resulted in lower morphine requirement for the first 12 postoperative hours and also a lower pain intensity for 6 hours. Supporting this, in our study, diclofenac use did not differ between the bupivacaine and placebo groups, whereas patients in the bupivacaine group used significantly lesser amount of pethidine in the postoperative period.

In the present study, patients in the TAP block group had lower VAS scores than those in the wound infiltration group soon after the operation. However, the significance of the difference was not sustained at the 6th and 18th-hour assessments. Moreover, the difference in the mean amount of pethidine use was not significantly different between these two groups, whereas patients in the TAP block group used significantly less diclofenac than those in the wound infiltration group. Given the similar baseline characteristics of the patients in the two groups and similar amount and dose of bupivacaine used for both protocols, we postulate that TAP block provides better pain relief than wound infiltration soon after the operation only, but its benefit in terms of reducing opioid use should be questioned in further research. The reason why TAP block provided lower postoperative pain than wound infiltration may be explained by its better pain control effect. With TAP block, the anesthetic directly blocks the afferent nerves before these nerves enter the anterior abdominal wall. Visceral pain relief may be due to posteromedial diffusion of the anesthetic along the fascial plane. To our knowledge, no other studies have compared TAP block with wound infiltration of local anesthetics for postoperative pain relief after CD.

One may raise concern about the high number of elective cesarean deliveries under general anesthesia in this study. This is due to the low number of anesthesiologists in our hospital and reluctance of the pregnant women to regional anesthesia for CD. In one review on TAP block for transverse lower abdominal incisions, 8 out of 12 trials were on patients receiving CD (18). Among these, only two studies included patients who underwent surgery under general anesthesia. Similarly, in one review on patients undergoing wound infiltration during CD, only one out of 12 studies was on patients receiving general anesthesia (9). This inconsistency may be due to fact that women in our country are likely to have a tendency towards undergoing a totally painless and unconsciousness experience during birth, which makes postoperative pain control even more important.

A study from Japan by Tsuchiya et al. (19) -reported that combining TAP block with general anesthesia promoted intraoperative hemodynamic stability in patients with severe cardiovascular disease. Although this was not valid for our

study population, TAP block seems to provide an advantage for patients with severe hemodynamic instability.

In parallel with our study, Tharwat et al. (20) assessed the efficacy and safety of incisional infiltration of local lidocaine in patients undergoing CD. They demonstrated that lidocaine administration reduced the opioid analgesic dose postoperatively and enhanced patient recovery.

The main strength of the study was that, to our knowledge, there have been no comparative studies performed to investigate whether TAP block or wound infiltration of local anesthetics provides better postoperative analgesia after CD. There were certain limitations in our study. First, because patients in the TAP block and TAP placebo group had pain sensitivity at the needle insertion site and might have reported pain during the investigator assessment, a flawless blinding might not have been achieved in this study. Also, despite being strongly advised, patients might not always have asked for analgesics from nurses because of individual variations in pain conception and resistance against pain. Another limitation of the present study was that analgesic use was not quantified as doses per weight and per time, which may produce more accurate implications from study outcomes. The comparison of equal doses of bupivacaine given through two distinct routes of administration is also questionable because different routes of administration would have produced different amounts of drug distribution through the abdominal wall.

In conclusion, TAP block provided better pain relief and less analgesic requirement than bupivacaine wound infiltration early after CD under general anesthesia. Given the similar amounts of diclofenac but lower amounts of pethidine used in the wound infiltration group, wound infiltration of bupivacaine seems promising in terms of reducing opioid consumption after CD under general anesthesia, especially when TAP block is not used.

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Ethics Committee Approval: *The Ethics Committee of Ankara Numune Hospital approved the study.*

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References

1. Organization for Economic Cooperation and Development OECD. Health at a Glance 2011: OECD Indicators OECD 2011.
2. Koken G, Cosar E, Sahin FK, Tolga Arioz D, Duman Z, Aral I. Attitudes towards mode of delivery and cesarean on demand in Turkey. Int J Gynaecol Obstet 2007; 99: 233-5.
3. McDonnell NJ, Keating ML, Muchatuta NA, Pavy TJ, Paech MJ. Analgesia after caesarean delivery. Anaesth Intensive Care 2009; 37: 539-51.
4. Ismail S, Shahzad K, Shafiq F. Observational study to assess the effectiveness of postoperative pain management of patients undergoing elective cesarean section. J Anaesthesiol Clin Pharmacol 2012; 28: 36-40.
5. Howell EA, Mora PA, Chassin MR, Leventhal H. Lack of preparation, physical health after childbirth, and early postpartum depressive symptoms J Womens Health (Larchmt) 2010; 19: 703-8.
6. Karlström A, Engström-Olofsson R, Nystedt A, Sjöling M, Hildingsson I. Women's postoperative experiences before and after the introduction of spinal opioids in anaesthesia for caesarean section. J Clin Nurs 2010; 19: 1326-34.
7. Baaj JM, Alsatli RA, Majaj HA, Babay ZA, Thallaj AK. Efficacy of ultrasound-guided transversus abdominis plane (TAP) block for postcesarean section delivery analgesia--a double-blind, placebo-controlled, randomized study. Middle East J Anesthesiol 2010; 20: 821-6.
8. Belavy D, Cowlishaw PJ, Howes M, Phillips F. Ultrasound-guided transversus abdominis plane block for analgesia after Caesarean delivery. Br J Anaesth 2009; 103: 726-30.
9. Bamigboye AA, Hofmeyr GJ. Local anaesthetic wound infiltration and abdominal nerves block during caesarean section for postoperative pain relief. Cochrane Database Syst Rev 2009; 8: CD006954.
10. Niklasson B, Börjesson A, Carmnes UB, Segerdahl M, Ohman SG, Blanck A. Intraoperative injection of bupivacaine-adrenaline close to the fascia reduces morphine requirements after cesarean section: a randomized controlled trial. Acta Obstet Gynecol Scand 2012; 91:1433-9.
11. Costello JF, Moore AR, Wieczorek PM, Macarthur AJ, Balki M, Carvalho JC. The transversus abdominis plane block, when used as part of a multimodal regimen inclusive of intrathecal morphine, does not improve analgesia after cesarean delivery. Reg Anesth Pain Med 2009; 34: 586-9.
12. Faul F, Erdfelder E, Lang AG, Buchner A. G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods 2007; 39: 175-91.
13. Cohen J. A power primer. Psychological Bulletin 1990; 112: 155-9.
14. Abdallah FW, Halpern SH, Margarido CB. Transversus abdominis plane block for postoperative analgesia after Caesarean delivery performed under spinal anaesthesia? A systematic review and meta-analysis. Br J Anaesth 2012; 109: 679-87.
15. McKeen DM, George RB, Boyd JC, Allen VM, Pink A. Transversus abdominis plane block does not improve early or late pain outcomes after Cesarean delivery: a randomized controlled trial. Can J Anaesth 2014; 61: 631-40.
16. Eslamian L, Jalili Z, Jamal A, Marsoosi V, Movafegh A. Transversus abdominis plane block reduces postoperative pain intensity and analgesic consumption in elective cesarean delivery under general anesthesia. J Anesth 2012; 26: 334-8.

17. Tan TT, Teoh WH, Woo DC, Ocampo CE, Shah MK, Sia AT. A randomised trial of the analgesic efficacy of ultrasound-guided transversus abdominis plane block after caesarean delivery under general anaesthesia. Eur J Anaesthesiol 2012; 29: 88-94.
18. Abdallah FW, Laffey JG, Halpern SH, Brull R. Duration of analgesic effectiveness after the posterior and lateral transversus abdominis plane block techniques for transverse lower abdominal incisions: a meta-analysis. Br J Anaesth 2013; 111: 721-35.
19. Tsuchiya M, Takahashi R, Furukawa A, Suehiro K, Mizutani K, Nishikawa K. Transversus abdominis plane block in combination with general anesthesia provides better intraoperative hemodynamic control and quicker recovery than general anesthesia alone in high-risk abdominal surgery patients. Minerva Anestesiol 2012; 78: 1241-7.
20. Tharwat AA, Yehia AH, Wahba KA, Ali AE. 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. J Turk Ger Gynecol Assoc 2016; 17: 1-5.

Stage IIIC transitional cell carcinoma and serous carcinoma of the ovary have similar outcomes when treated with platinum-based chemotherapy

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Abstract

Objective: Previous studies reported better outcomes for transitional cell carcinoma (TCC) of the ovary when compared with more common histologic types such as serous epithelial ovarian cancers (EOCs). The aim of this study was to compare the survival outcomes of platinum- based chemotherapy in patients with stage IIIC TCCs and serous EOCs.

Material and Methods: Clinicopathologic features and survival data of patients with FIGO stage IIIC TCC and serous EOC who had undergone primary surgery followed by six cycles of intravenous platinum/taxane between 2007 and 2015 were retrieved from the database of Hacettepe University Hospital.

Results: We identified 14 (10.9%) TCCs and 114 (89.1%) serous EOCs. The median follow-up duration was 28 months (range, 3-101 months). Univariate analysis revealed that the TCCs and serous EOCs had similar progression-free survival (PFS) and overall survival (OS). Patients with residual disease less than 1 cm had longer OS than patients with residual disease greater than 1 cm (75.0 vs. 45.0 months, p=0.012). Cox regression analysis of all potential prognostic factors showed that the only independent prognostic factor significantly associated with OS was residual disease less than 1 cm [hazard ratio=0.38; 95% confidence interval: (0.19-0.77); p=0.007].

Conclusion: Surgically treated advanced stage TCCs did not have a significantly better prognosis after platinum/taxane-based chemotherapy when compared with serous EOCs. Residual tumor volume after primary surgery was the only independent predictor of OS in patients with EOC. Our results demonstrate the significance of achieving optimal cytoreduction in all histologic subtypes of EOC. (J Turk Ger Gynecol Assoc 2017; 18: 33-7)

Keywords: Epithelial ovarian cancer, serous papillary carcinoma, transitional cell carcinoma, platinum/taxane, chemotherapy, cytoreduction

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Introduction

Ovarian transitional cell tumors include pure transitional cell carcinomas (TCCs) and Brenner tumors. TCCs are high-grade carcinomas of surface epithelial origin, as distinct from benign, malignant or borderline Brenner tumors. Primary TCC of the ovary is a rare subtype of epithelial ovarian cancer (EOC), which was first described by Austin and Norris in 1987 as a neoplasm composed of epithelial elements resembling urothelium and lack of a benign or borderline Brenner tumor (1, 2).

Transitional cell tumors, including pure TCCs and Brenner tumors of the ovary, represent approximately 2% of all ovarian tumors, and the pure form accounts for 1% of surface epithelial tumors (3, 4). The clinical presentation is not different from other types of ovarian carcinoma, and most common presenting symptoms are abdominal distension and pain (3). The primary treatment of patients with TCC of the ovary is also similar in patients with other EOC, which consists of optimal surgical resection, followed by adjuvant platinum-based chemotherapy (5).

Although many previous studies reported that patients with TCCs had better prognoses compared with patients with all other types of ovarian carcinomas following standardized surgery and chemotherapy (3, 5, 6), results are conflicting regarding the prognosis of TCCs. Therefore, the aim of this study was to compare the survival outcomes of patients with similarly treated stage IIIC transitional cell and serous EOCs.

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Material and Methods

Clinicopathologic and outcome data of patients with International Federation of Gynecology and Obstetrics (FIGO) stage IIIC TCCs and serous EOC who had undergone primary surgery followed by six cycles of intravenous platinum/taxane between 2007 and 2015 were retrieved from the database of Hacettepe University Hospital. All operations were performed by gynecologic oncologists. All patients underwent total abdominal hysterectomy, bilateral salpingo-oophorectomy, and pelvic and para-aortic lymphadenectomy with omentectomy and other cytoreductive procedures to achieve optimal cytoreduction. The clinical and pathologic characteristics of patients with TCCs and serous EOC including age, histologic subtype, stage, grade, preoperative CA-125 level, and survival were determined and compared.

Overall survival (OS) was calculated from time of diagnosis until death or last follow-up. Progression-free survival (PFS) was calculated from time of diagnosis until disease recurrence. The Kaplan-Meier survival analysis was used to estimate OS and PFS, and survival differences were analyzed using the log-rank test. Cox regression analysis was performed to assess the potential influence of other prognostic factors. Mann-Whitney U test, Chi-square test or Fisher's exact test were used as appropriate. Statistical analyses were performed using Statistical Package for the Social Sciences statistical software (version 16.0, SPSS Inc, Chicago, IL, USA). Differences were considered statistically significant at $p<0.05$. Local Ethics Committee permission was not sought because this study represents a retrospective database review.

Results

We identified 14 patients with stage IIIC TCCs who were treated between 2004 and 2015 at Hacettepe University Hospital. These 14 patients' results were compared with 114 patients with stage IIIC serous EOC. The median ages of patients with TCC and serous EOC was 55 and 57 years, respectively. The median age, CA-125 level at diagnosis, and grade were similar for both groups. Optimal resection to <1 cm residual disease was achieved in 50% of patients with TCCs and 60.5% of patients with serous EOCs and there was no difference in the rates of optimal cytoreduction between the two groups. Histologically, all of the TCCs were grade 3. However, of the serous EOCs, 20 (17.5%) were grade 1-2 and the remainder (82.5%) was grade 3. All patients received 6 cycles of paclitaxel and carboplatin chemotherapy. Demographic, clinical, and pathologic characteristics of the study patients are presented in Table 1.

The median follow-up duration was 28 months (range, 3-101 months). The median OS of patients with TCCs was not obtained because <50% of patients died of disease at the time of analysis. Patients with serous EOCs had a median OS of 52 months. The median OS was not significantly different between the two groups ($p=0.135$). The median PFS for TCCs was 15 months, and patients with serous EOCs had a median PFS of 21 months ($p=0.242$). In substance, univariate analysis revealed that TCCs and serous EOCs had similar PFS and OS (Figure 1). Patients with residual disease less than 1 cm had longer OS than patients with residual disease greater than 1 cm (75.0 vs. 45.0 months, $p=0.012$). Figure 2 presents the median OS curves based on residual tumor volume in the whole study cohort. Cox regression analysis of potential prognostic factors showed that the only independent prognostic factor significantly associated with OS was residual disease less than 1 cm [hazard ratio=0.38; 95% confidence interval: (0.19-0.77); $p=0.007$] (Table 2).

Discussion

Limited data suggests that TCC is more chemosensitive and is associated with a better prognosis than serous carcinoma of

Table 1. Patient characteristics in the study cohort

Characteristic	TCC (n=14)	SOC (n=114)	p
Median age at diagnosis, years	55 (38-74)	57 (39-81)	NS*
Preoperative CA-125 (IU/mL)	251.5 (19-2854)	291 (13-4835)	NS*
Median follow-up, months (range)	38 (3-101)	26 (4-96)	0.019*
Tumor grade			NS†
1-2	0 (0.0%)	20 (17.5%)	
3	14 (100.0%)	94 (82.5%)	
Residual disease after primary surgery			NS†
<1.0 cm	7 (50.0%)	69 (60.5%)	
>1.0 cm	7 (50.0%)	45 (39.5%)	

Data is given as mean ± standard deviation, median (minimum-maximum value) or n (%).
TCC: transitional cell carcinoma; SOC: serous ovarian cancer; PFS: progression-free survival; OS: overall survival; NS: no significance.
*Mann-Whitney U test, †Chi-square test.

the ovary (7-9). Austin and Norris (2) who first described TCC of the ovary, also reported a better response to chemotherapy in patients with TCC. Kommoss et al. (6) found that TCCs had a significantly better prognosis when compared with all other types of ovarian carcinomas after standardized chemotherapy. However, they also documented that this better prognosis could be related with better surgical resectability and less tendency to the large extraovarian tumor spread of TCCs. In addition, they performed a subgroup analysis in patients with postoperative residual disease <1 cm; there was no statistical significant survival difference among patients with TCCs and other types of ovarian carcinomas (6). Guseh et al. (10) reported that TCCs of the ovary had a significantly lower risk of platinum resistance and also had improved overall survival when compared with patients with serous EOCs. In contrast to Kommoss et al. (6) study, they found a similar rate of surgical resectability between TCCs and controls (10). Robey et al. (9) performed a retrospective study and reported that although patients with non-TCC predominant tumors had a higher percentage of tumor recurrences after chemotherapy, most TCC predominant tumors responded completely to chemotherapy even if residual tumor was ≥2 cm. Similarly, Silva et al. (8) reported that TCCs of the ovary had an excellent response to different chemotherapy regimens.

It has been suggested by other authors that TCCs of the ovary have no significant difference in survival outcomes compared with serous EOCs after adjuvant chemotherapy. Hollingsworth et al. (11) evaluated 58 patients with EOC. Of these patients, 13 had TCC, 2 had mixed histology, 25 had serous ovarian

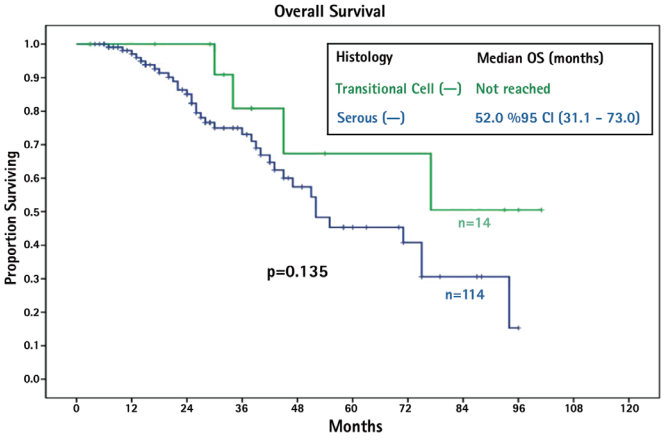

Figure 1. Median overall survival curves based on tumor histology

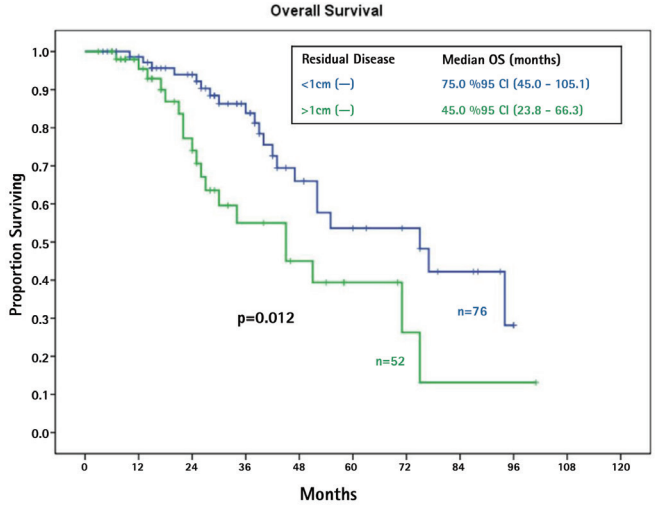

Figure 2. Median overall survival curves based on residual tumor volume after initial surgery in the whole study cohort

Table 2. Median progression-free survival and overall survival based on potential prognostic factors

		PFS		OS	
Characteristic	**No. of patients**	**Median (months)**	**p**	**Median (months)**	**p**
Age group, years					
<65	95	15.0	0.267	71.0	0.841
>65	33	20.0		55.0	
Tumor grade					
1-2	20	11.0	0.113	52.0	0.801
3	106	17.0		52.0	
Histology					
Transitional	14	15.0	0.242	Not reached*	0.135
Serous	114	21.0		52.0	
Residual disease after primary surgery					
<1.0 cm	76	16.0	0.528	75.0	0.012
>1.0 cm	52	16.0		45.0	

*<50% of patients were dead of disease at the time of analysis.
PFS: progression-free survival; OS: overall survival.

cancer, and the remaining 18 patients had other types of EOC. The authors found that TCC histologic subtype was not a significant predictor for OS or DFS and concluded that TCC did not provide a favorable prognosis (11). Mackay et al. (12) performed a meta-analysis to evaluate the prognosis of women with rare EOC histology. Thirty-six patients with TCC were included in their study. When controlled for prognostic factors as age, stage, and residual disease, patients with TCC did not significantly differ from those with serous carcinoma. In addition, they found that patients with transitional cell histology were more likely to be optimally debulked to no visible tumor compared with those with serous histology after surgery (12). Consistent with this study, our data suggests that TCCs have a similar OS or PFS after platinum/taxane-based chemotherapy when compared with serous EOCs. However, we found a similar rate of surgical resectability between TCCs and controls [50% vs. 60.5% ($p>0.05$), respectively]. Additionally, consistent with previous studies, residual tumor volume after primary surgery was the only independent predictor of OS in patients with EOC in the present study.

There are different results in the literature related with the prognosis and response to chemotherapy in patients with TCC of the ovary. These differences can be associated with lack of prospective studies due to the small number of patients. Other possible related factors include differences in pathologic diagnostic criteria and surgical practice patterns of individual oncology centers. Furthermore, some of the studies were published before the introduction of taxanes, which are routinely used in primary adjuvant therapy in patients with EOC. On the other hand, recent molecular and immunohistochemical data demonstrated that TCCs express the same immunophenotype and genetic mutations as high-grade serous carcinoma (13, 14). For instance, Cuatrecasas et al. (1) found that TCCs of the ovary showed p16 and p53 overexpression, and p53 mutations as in other high-grade ovarian carcinomas (1). These findings support that TCC could be a variant of serous EOC. Our data suggest that TCCs have a similar clinical and prognostic behavior as serous EOC. However, further research is needed to demonstrate the clinical and pathologic similarities between TCCs and serous EOCs. From a practical point of view, the results of our study strongly support the most important surgical oncology doctrine, which is to achieve no visible disease in patients with peritoneal carcinomatosis of epithelial ovarian origin. This principle should guide gynecologic oncologists until further robust data becomes available in the era of targeted therapy, which may nullify the heroic actions of dedicated surgeons.

Our study has inherent limitations due to its retrospective study design. The presence of other possible confounding variables such as selection and recall bias, which might have affected our results, cannot be ruled out because of the retrospective nature of the study. Another disadvantage of the present study is its limited sample size. Therefore, any conclusions maybe limited in their implications. Despite these limitations, our study adds to significant findings because of our specific group of stage IIIC TCCs.

In conclusion, TCC of the ovary is a rare type of EOC. Surgically treated advanced stage TCCs did not have a significantly better prognosis after platinum/taxane-based chemotherapy when compared with serous EOCs. Residual tumor volume after primary surgery was the only independent predictor of OS in patients with EOC. Our results demonstrate the significance of achieving optimal cytoreduction in all histologic subtypes of EOC. Furthermore, our study suggests that TCC does not relate with a favorable prognosis or better response rate to chemotherapy.

Ethics Committee Approval: *This study represents a retrospective database review.*

Informed Consent: *All patients signed an informed consent which allowed our institution to use their clinical data.*

Peer-review: *Externally peer-reviewed.*

Author Contributions*: Concept - G.B., M.C.S.; Design - G.B., M.C.S., D.B.; Supervision - M.C.S., N.Ö., K.Y.; Materials - G.B., D.B.; Data Collection and/or Processing - G.B., D.B.; Analysis and/or Interpretation - G.B., D.B., M.C.S.; Literature Review - G.B., N.Ö., K.Y.; Writer - G.B.; Critical Review - M.C.S., N.Ö., K.Y.*

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References

1. Cuatrecasas M, Catasus L, Palacios J, Prat J. Transitional cell tumors of the ovary: a comparative clinicopathologic, immunohistochemical, and molecular genetic analysis of Brenner tumors and transitional cell carcinomas. Am J Surg Pathol 2009; 33: 556-67.
2. Austin RM, Norris HJ. Malignant Brenner tumor and transitional cell carcinoma of the ovary: a comparison. Int J Gynecol Pathol 1987; 6: 29-39.
3. Ichigo S, Takagi H, Matsunami K, Murase T, Ikeda T, Imai A.Transitional cell carcinoma of the ovary (Review). Oncol Lett 2012; 3: 3-6.
4. Ingin RJ, Andola SK, Zubair AA. Transitional cell carcinoma of the ovary: case series and review of literature. J Clin Diagn Res 2014; 8: FD07-8.
5. Guseh SH, Rauh-Hain JA, Tambouret RH, Davis M, Clark RM, Boruta DM, et al. Transitional cell carcinoma of the ovary: a case-control study. Gynecol Oncol 2014; 132: 649-53.

6. Kommoss F, Kommoss S, Schmidt D, Trunk MJ, Pfisterer J, du Bois A, et al. Survival benefit for patients with advanced-stage transitional cell carcinomas vs. other subtypes of ovarian carcinoma after chemotherapy with platinum and paclitaxel. Gynecol Oncol 2005; 97: 195-9.
7. Gershenson DM, Silva EG, Mitchell MF, Atkinson EN, Wharton JT.Transitional cell carcinoma of the ovary: a matched control study of advanced-stage patients treated with cisplatin-based chemotherapy. Am J Obstet Gynecol 1993; 168: 1178-85.
8. Silva EG, Robey-Cafferty SS, Smith TL, Gershenson DM. Ovarian carcinomas with transitional cell carcinoma pattern. Am J Clin Pathol 1990; 93: 457-65.
9. Robey SS, Silva EG, Gershenson DM, McLemore D, el-Naggar A, Ordonez NG.Transitional cell carcinoma in high-grade high-stage ovarian carcinoma. An indicator of favorable response to chemotherapy. Cancer 1989; 63: 839-47.
10. Guseh SH, Rauh-Hain JA, Tambouret RH, Davis M, Clark RM, Boruta DM, et al. Transitional cell carcinoma of the ovary: a case-control study. Gynecologic Oncology 2014; 132: 649-53.
11. Hollingsworth HC, Steinberg SM, Silverberg SG, Merino MJ. Advanced stage transitional cell carcinoma of the ovary. Hum Pathol 1996; 27: 1267-72.
12. Mackay HJ, Brady MF, Oza AM, Reuss A, Pujade-Lauraine E, Swart AM, et al. Prognostic relevance of uncommon ovarian histology in women with stage III/IV epithelial ovarian cancer. Int J Gynecol Cancer 2010; 20: 945-52.
13. Ali RH, Seidman JD, Luk M, Kalloger S, Gilks CB. Transitional cell carcinoma of the ovary is related to high-grade serous carcinoma and is distinct from malignant brenner tumor. Int J Gynecol Pathol 2012; 31: 499-506.
14. Logani S, Oliva E, Amin MB, Folpe AL, Cohen C, Young RH. Immunoprofile of ovarian tumors with putative transitional cell (urothelial) differentiation using novel urothelial markers: histogenetic and diagnostic implications. Am J Surg Pathol 2003; 27: 1434-41.

Frozen embryo transfer prevents the detrimental effect of high estrogen on endometrium receptivity

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Abstract

Objective: To investigate whether serum levels of estradiol affect reproductive outcomes of normoresponder women undergoing fresh embryo transfer (ET) versus frozen-thawed ET (FET).

Material and Methods: Two hundred fifty-five normoresponder women underwent fresh ET in their first or second *in vitro* fertilization cycle. Ninety-two women with negative pregnacy test results underwent FET. Clinical and ongoing pregnancy rates, implantation, and live birth rates of women undergoing fresh ET versus FET were compared.

Results: One hundred forty-seven (57.65%) out of the 255 normoresponder women receiving FET had positive beta-human chorionic gonadotrophin (hCG) results. The remaining 108 women had negative beta-hCG results. The clinical pregnancy rates of the fresh ET group were found as 55.69% (n=142). Ninety-two of the 108 women with failed pregnancies underwent FET; 72.83% had positive beta-hCG results (n=67), and 70.65% had clinical pregnancy (n=65). Both biochemical and clinical pregnancy rates of women undergoing FET increased significantly ($p<0.012$ and $p<0.013$, respectively). Ongoing pregnancy (60.87% vs. 52.94%) and live birth rates (59.87% vs. 48.63%) were similar in both fresh and FET groups. Serum E2 levels of women who failed to conceive were significantly higher than those women did conceive. Serum progesterone levels of women who conceived versus those that did not were similar.

Conclusion: The detrimental effect of high serum estradiol levels on endometrial receptivity could be prevented by FET. (J Turk Ger Gynecol Assoc 2017; 18: 38-42)

Keywords: Frozen embryo transfer, fresh embryo transfer, clinical pregnancy rates, serum estradiol, serum progesterone

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Introduction

High estradiol levels on the day of human chorionic gonadotrophin administration were found to be detrimental upon endometrial receptivity in women undergoing *in vitro* fertilization (IVF)/intracytoplasmic sperm injection (ICSI) (1). By altering serum levels of estrogen and progesterone, controlled ovarian stimulation (COS) with recombinant or urinary follicle-stimulating hormone (FSH) might alter endometrial receptivity either positively or negatively (2, 3). Fluctuation in serum estradiol or progesterone levels might lead to asynchrony between embryo and endometrium. It is well known that synchrony between the implanted blastocyst and endometrium increases implantation rates. On the other hand, asynchrony between the transferred embryo and endometrium may lead to implantation failures, despite the transfer of sufficient numbers and good quality embryos (4-8).

Fresh embryo transfer (ET) and frozen-thawed (FET) are the most common ET methods. Implantation rates of frozen-thawed embryos reached the success rates of fresh embryos with the development of vitrification technologies (9, 10). In particular, infertile women with slowly developing embryos or premature progesterone peaks might benefit more from FET than with fresh ET. Endometrial priming in frozen ET with the use of exogenous hormones may lead to strict control of endometrial development. In FET cycles, the endometrium

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is artificially primed with E2 and progesterone and embryos are therefore transferred to an environment that has not been exposed to the effects of high estradiol and progesterone levels that occur during COS. Previous studies used different infertile participants to compare the impact of fresh and frozen ET on reproductive outcomes (11). Unlike others, we examined the clinical outcomes of a cohort of young patients underdoing fresh ET, and subsequently compared the outcomes of a subset of these patients who failed with fresh transfer and subsequently underwent frozen single ET. Thus, we will have the opportunity to more objectively analyze the influences of COS-related hormonal alterations on endometrium receptivity.

Material and Methods

Patient selection

This study included 255 patients who received fresh ET in their first or second treatment cycle. An increase in serum levels of human chorionic gonadotrophin (hCG) within 10-12 days after fresh or frozen ET was accepted as pregnancy. One hundred eight of 255 participants had negative beta-hCG results, 92 of whom underwent frozen ET. The clinical and ongoing pregnancy rates, implantation, and live birth rates of women undergoing fresh and frozen ET were compared. Ongoing pregnancy was accepted as the main outcome measure. Detection of fetal heart motion at 6-7 weeks' gestation was noted as clinical pregnancy. Fetuses with fetal heart motion at 12 weeks' gestation were accepted as ongoing pregnancy. The implantation rate was defined as the ratio of the number of transferred fresh or frozen embryos that resulted in fetal heart activity. Pregnancy losses within the early gestational period was pregnancy but it did not become ongoing pregnancy. Institutional review board approval was obtained before initiation of this retrospective study. The inclusion criteria were i) patients undergoing their first or second IVF cycle; ii) cycle day 3 FSH <10 IU/L; and iii) 10-15 antral follicles observed on baseline ultrasonography; iv) 10-15 oocyte collection at oocyte pick-up. Patients with a history of recurrent implantation failure, recurrent spontaneous abortions, poor responders, and high responders were excluded. The mean age of participants was less than 35 years.

Controlled ovarian stimulation protocol

The protocols for COS, embryo culture, cryopreservation, and luteal support were described previously. In brief, patients underwent COS with recombinant FSH (rFSH; Gonal-F; Merck Serono, Turkey) and gonadotrophin-releasing hormone (GnRH) antagonist (Cetrotide; Merck Serono, Turkey). When the leading follicle reached a diameter of 12-13 mm, the GnRH antagonist was administered 0.25 mg daily until the hCG injection. When two or more follicles had attained a minimum mean diameter of 18 mm, follicular maturation was achieved using 250 μg of r-hCG (Ovitrelle; Merck Serono, Switzerland). Oocyte retrieval was performed 36 h after the hCG injection. Luteal phase support was given with vaginal progesterone gel until the detection of the fetal heartbeat.

Endometrial priming for frozen embryo transfer

Participants in the frozen ET group underwent endometrial priming with oral 6.0 mg/daily estradiol (Estrofem; Novo Nordisk, Denmark). It began on day 3 of menses and continued for 10-14 days. E2 patch supplementation was used if needed. Priming was continued until the endometrial thickness reached at least 8 mm. Luteal phase support was given to both the fresh and frozen ET group with vaginal P gel (Crinone; Merck Serono, Turkey). P gel was used twice a day beginning from five days before the thawing and continued until week 12.

Fresh and frozen embryo selection

Embryo morphology was assessed according to the number, symmetry, percentage of fragmentation, presence of multinucleated blastomeres, and degree of compaction (12). Blastocysts were scored according to Gardner's classification. Blastocysts with best-morphology were selected for fresh ET. Grade 3AA and above blastocysts were vitrified in turn on a cryotop (Kitazato; Japan) using a commercially available kit (Vitrolife; Sweden). Blastocysts were also thawed using the same kit following the manufacturer's instructions.

Statistical analysis

Data analysis was performed using the Statistical Package for Social Sciences version 15.0 (SPSS Inc, Chicago, IL, USA). Normality of distributions were checked with the Kolmogorov-Smirnov test. Statistical differences in continuous variables were determined using Student's t-test and the Mann-Whitney U test, if appropriate. Chi-squared and Fisher's exact tests were used to analyze categorical data. $P<0.05$ was considered statistically significant.

Results

All participants had good ovarian response, which allowed the retrieval of 13 cumulus oocyte complexes and 10 metaphase II oocytes. The age and body mass index (BMI) of the participants were found to be 27.9±3.72 years and 24.7±3.24 (kg/m^2), respectively. All fresh cycles were transferred on the same day. One hundred forty-seven (57.65%) of the 255 normoresponder women who received FET had positive beta-hCG results. The remaining 108 women had negative beta-hCG results. The clinical pregnancy rate was found as 55.69% (n=142). A subgroup analysis of patients who failed fresh transfer and

subsequently underwent FET demonstrated that there was no demographic differences between the two groups. However, a cycle-based difference was detected regarding serum estradiol levels on hCG day between patients undergoing fresh transfers who conceived versus those that did not.

Serum E2 levels of women who failed to conceive were significantly higher than those who conceived succesfully ($p<0.01$; Table 1). Nevertheless, similar serum progesterone levels were noted in women who conceived versus those that did not ($p>0.65$). The time between fresh and frozen transfers were at least one year. The maturation and fertilization rates of fresh oocytes were found as 83.50% and 87.02%, respectively. The transferred blastocysts were of top quality for fresh cycles (55% vs. 27%) and of good quality for frozen-thawed cycles (72.8% vs. 27%). Ninety-two of 108 women who failed pregnancy underwent FET, 72.83% (n=67) of whom had positive beta-hCG results, and the clinical pregnancy rate was 70.65% (n=65). Both biochemical and clinical pregnancy rates of women undergoing FET increased significantly ($p<0.012$ and $p<0.013$, respectively). Ongoing pregnancy (60.87% vs. 52.94%) and live birth rates (59.87% vs. 48.63%) were similar in both fresh and frozen ET groups. Finally, the cumulative clinical pregnancy rate was 81.17%. Clinical miscarriage rates of women undergoing forzen ET cycles were significantly higher than those in fresh ET cycles ($p<0.045$). However, early pregnancy loss in the fresh ET group was significantly higher than in the frozen ET group ($p<0.03$).

Discussion

Great efforts have been made over the last two decades to improve clinical and embryologic strategies with the aim of improving outcomes of assisted reproductive technologies. The endometrium is accepted as a final destination allowing blastocysts to attach under sufficient amounts of biologically-relevant receptivity molecules. Understanding endometrial receptivity, or more accurately, detecting the window of implantation, has become crucial in ART practise in order to go one step further (13, 14). In line with this, improvements in blastocyst culture medium combined with robust development in vitrification protocols have undeniably improved the impact of COS cycles. Accorrdingly, two large retrospective studies reported that FET cycles had equivalent reproductive outcomes to fresh cycles (15, 16). Despite the positive impact of COS on the number of oocytes collected, COS may lead to defective endometrial receptivity. Supraphysiological estrogen production may be the main culprit responsible for the failed receptivity in women having high estradiol levels (1). Concordantly, it has been reported that high serum estradiol levels on the day of HCG stimulation in women who are high or normal responder are detrimental to endometrial receptivity (17). Therefore, we conducted a retrospective cohort study with the aim of comparing reproductive outcomes of fresh ET versus FET in the same cohort of 255 young patients who were normoresponders in order to assess possible impact of COS cycles on endometrial receptivity.

Table 1. Clinical characteristics and sub-group analysis of fersh versus frozen-thawed embryo transfer cycles

	Fresh ET	**Frozen ET**	**p value**
The number of COC	13.83±7.96	NA	-
The number of MII	10.88±6.82	NA	-
E2 on hCG day (pg/mL)	3077±1438	NA	-
P on hCG day (ng/mL)	1.13±0.55	NA	-
Biochemical pregnancy (%)	147 (57.65%)	67 (72.83%)	0.01
Clinical pregnancy (%)	142 (55.69%)	65 (70.65%)	0.01
Miscarriage (%)	7 (4.92%)	9 (13.84%)	0.04
Ongoing pregnancy (%)	135 (52.94%)	56 (60.87%)	0.22
Early pregnancy loss	10	0	0.03
Live birth rates (%)	124 (48.63%)	55 (59.87%)	0.06
	Subgroup analysis of patients who conceived versus those that did not and subsequently underwent FET		**p value**
	147 women with positive beta hCG	**108 women with negative beta hCG**	
E2 on hCG day (pg/mL)	2945±1056	3560±1233	<0.01
P on hCG day (ng/mL)	1.10±1.21	1.22±0.76	0.65

FET: frozen-thawed embryo transfer; ET: embryo transfer; hCG: human chorionic gonadotrophin; NA: not applicable; P: progesterone; COC: cumulus oocyte complexes

As clinical pregnancy rates were higher in the subset of patients undergoing frozen transfer as opposed to the entire first cohort undergoing fresh transfer, we conclude that fresh cycles are hindered by impaired endometrial receptivity. However, de Neubourg et al. (18) reported a 5% increase in cumulative live birth rate as an additional effect of frozen-thawed cycles. Conversely, in the present study, the cumulative increase in pregnancy rates was about 25%. The difference betwen the two studies could be accounted for by the fact that our study used blastocyst vitrification, whereas de Neubourg et al. (18) used a slow freezing protocol on day 3 embryos. There may be different reasons for the increase in success rates of frozen ET. First, endometrial priming with estradiol and progesterone provide a natural endometrial environment for the transferring embryo in frozen ET cycles. Secondly, detrimental impacts of COS-related hormonal fluctuations on endometrium receptivity can be prevented by using frozen ET cycles, in which ovarian stimulation with rFSH or urinary FSH are not used. Moreover, disturbed expression of the receptivity genes and molecules in the endometrium during the window of implantation might be a common factor among patients undergoing COS due to different etiologies. In good agreement, failed endometrial receptivity has been noted in some COS cycles with high serum estradiol levels (1). Treatment of rats with 100 ng estradiol per day on gestation days 1-5 lead to complete absence of implantation sites, which supports the detrimental effect of high estrogen levels on implantation site (19). Likewise, we found that serum estradiol levels of women who did not conceive were significantly higher than those of women who conceived successfully.

Sevaral mechanisms may be responsible for increased pregnancy rates after FET cycles. Similar ongoing and live birth rates in both transfer groups suggest that COS per se, or COS-related defects alone do not disturb the expression of endometrial receptivity molecules. However, we do not know whether the increase in clinical pregnancy rates after FET cycles is associated with removal of high E2 levels or a consequence of other factors associated with the underlying disease. Down-regulation of serum E2 levels could be a direct cause of increased pregnacy rates. FET-related improvement in COS-induced hormonal fluctuations including high E2 and proesterone levels may lead to an increase in clinical pregnancy rates. To avoid COS-related detrimental effects on endometrium receptivity in the present study, all FET cycles were made at least one year after the fresh ET.

In the present study, despite higher biochemical and clinical pregnancy rates in FET cycles possible explanation of equivalent live birth and higher miscarriage rates in both groups of subjects are unclear. We can propose that although frozen transfers may indeed benefit some patients who with impaired endometrial receptivity after COS, we have to select our results more carefully to discern whether there is a subset of patients at highest risk for fresh transfer failure (i.e., potential patients with higher E2 or progesterone levels). E2-stimulated endometrium may be the source of implantation failure in some fresh IVF cycles; however, this may not be the only reason (20, 21). If so, abortion rates and live birth rates were not the same in both groups. If we proclaim high serum E2 and progesterone levels as causative we have to show a maximum association between E2 and progesterone levels and reproductive outcomes. Nevertheless, in the current study, we did not find a strong association between serum progesterone and primary outcomes measures.

Improving receptivity in patients with high E2 levels is easy with suspension of ET to the next cycle. However, slightly increased miscarriage rates with FET further supports the possibility of a receptivity defect secondary to high E2 levels. Concordantly, infertile women with hyperandrogenism have low HOXA-10 and β3-integrin expression, which suggests high androgens may have a detrimental impact on the endometrium (22, 23). An increase in circulating androgens might antagonize the expression of estrogen-dependent receptivity genes. Therefore, we strongly propose that the decline in implantation rates in FET cycles is not exclusively due to defective follicle development but also the result of failed receptivity secondary to high circulating E2 levels.

As opposed to our results, some studies reported that high serum E2 levels were not detrimental to embryo implantation. For this reason, one may believe that an increase in E2 levels does not significantly impair the endometrial micro-environment. Conception despite high or low serum E2 levels suggests that receptivity of the endometrium was not strictly related to serum estradiol levels. It should be remembered that good quality embryos coming from fresh or FET cycles may come through an E2-associated implantation defect. Finally, before recommending the routine use of frozen ET for women with implantation failure who have high serum E2 or progesterone levels, we have to find answers to queries such as the timing of FET cycles and cut-off values of high serum E2 and progesterone on the day of hCG.

Ethics Committee Approval: *Ethics committee approval was received for this study from the Ethics Committee of Bahçeşehir University School of Medicine.*

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

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Concept, Design, Supervision, Resources, Materials, and Writing Manuscript- A.A.E., M.A; Data Collection*

and/or Processing, Analysis and/or Interpretation, Literature Search, and Critical Review- A.A.E., M.A., S.S.E., N.D.G.

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References

1. Valbuena D, Jasper M, Remohí J, Pellicer A, Simón C. Ovarian stimulation and endometrial receptivity. Hum Reprod 1999; 14(Suppl 2): 107-11.
2. Nikas G, Develioglu OH, Toner JP, Jones HW Jr. Endometrial pinopodes indicate a shift in the window of receptivity in IVF cycles. Hum Reprod 1999; 14: 787-92.
3. Bourgain C, Devroey P. The endometrium in stimulated cycles for IVF. Hum Reprod Update 2003; 9: 515-22.
4. Kolibianakis E, Bourgain C, Albano C, Osmanagaoglu K, Smitz J, Van Steirteghem A, et al. Effect of ovarian stimulation with recombinant follicle-stimulating hormone, gonadotropin releasing hormone antagonists, and human chorionic gonadotropin on endometrial maturation on the day of oocyte pick-up. Fertil Steril 2002; 78: 1025-9.
5. Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Thomas S. Large blastocyst diameter, early blastulation, and low preovulatory serum progesterone are dominant predictors of clinical pregnancy in fresh autologous cycles. Fertil Steril 2008; 90: 302-9.
6. Simón C, Garcia Velasco JJ, Valbuena D, Peinado JA, Moreno C, Remohí J, et al. Increasing uterine receptivity by decreasing estradiol levels during the preimplantation period in high responders with the use of a follicle-stimulating hormone step-down regimen. Fertil Steril 1998; 70: 234-9.
7. Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Hudson C, Thomas S. High ongoing pregnancy rates after deferred transfer through bipronuclear oocyte cryopreservation and post-thaw extended culture. Fertil Steril 2009; 92: 1594-9.
8. Richter KS, Shipley SK, Mcvearry I, Tucker MJ, Widra EA. Cryopreserved embryo transfers suggest that endometrial receptivity may contribute to reduced success rates of later developing embryos. Fertil Steril 2006; 86: 862-6.
9. Herrero L, Martínez M, Garcia-Velasco JA. Current status of human oocyte and embryo cryopreservation. Curr Opin Obstet Gynecol 2011; 23: 245-50.
10. Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Hudson C, Thomas S. Similar ongoing pregnancy rates after blastocyst transfer in fresh donor cycles and autologous cycles using cryopreserved bipronuclear oocytes suggest similar viability of transferred blastocysts. Fertil Steril 2010; 93: 319-21.
11. Shapiro BS, Daneshmand ST, Garner FC, Aguirre M, Hudson C, Thomas S. Evidence of impaired endometrial receptivity after ovarian stimulation for in vitro fertilization: a prospective randomized trial comparing fresh and frozen-thawed embryo transfer in normal responders. Fertil Steril 2011; 96: 344-8.
12. Veeck L, Zaninovic N. An Atlas Of Human Blastocysts. Taylor & Francis; 2003.
13. Salamonsen LA, Edgell T, Rombauts LJ, Stephens AN, Robertson DM, Rainczuk A, et al. Proteomics of the human endometrium and uterine fluid: a pathway to biomarker discovery. Fertil Steril 2013; 99:1086-92.
14. Garrido-Gómez T, Ruiz-Alonso M, Blesa D, Diaz-Gimeno P, Vilella F, Simón C. Profiling the gene signature of endometrial receptivity: clinical results. Fertil Steril 2013; 99: 1078-1085.
15. Takahashi K, Mukaida T, Goto T, Oka C. Perinatal outcome of blastocyst transfer with vitrification using cryoloop: a 4-year follow-up study. Fertil Steril 2005; 84: 88-92.
16. Roy TK, Bradley CK, Bowman MC, Mcarthur SJ. Single-embryo transfer of vitrified-warmed blastocysts yields equivalent live-birth rates and improved neonatal outcomes compared with fresh transfers. Fertil Steril 2014; 101: 1294-301.
17. Simón C, Cano F, Valbuena D, Remohí J, Pellicer A. Clinical evidence for a detrimental effect on uterine receptivity of high serum oestradiol concentrations in high and normal responder patients. Hum Reprod 1995; 10: 2432-7.
18. De Neubourg D, Daels C, Elseviers M, Mangelschots K, Vercruyssen M, Van Royen E. Cumulative live-birth delivery after IVF/ICSI since the progressive introduction of single-embryo transfer. Reprod Biomed Online 2010; 20: 836-42.
19. Thorpe JB, Burgess PS, Sadkowski M, Decatanzaro D. Estrogen-progesterone balance in the context of blastocyst implantation failure induced by predator stress. Psychoneuroendocrinology 2013; 38: 3048-56.
20. Bourgain C, Devroey P. The endometrium in stimulated cycles for IVF. Hum Reprod Update 2003; 9: 515-22.
21. Devroey P, Bourgain C, Macklon NS, Fauser BC. Reproductive biology and IVF: ovarian stimulation and endometrial receptivity. Trends Endocrinol Metab 2004; 15: 84-90.
22. Cermik D, Selam B, Taylor HS. Regulation of HOXA-10 expression by testosterone in vitro and in the endometrium of patients with polycystic ovary syndrome. J Clin Endocrinol Metab 2003; 88: 238-43.
23. Thornton JW. Evolution of vertebrate steroid receptors from an ancestral estrogen receptor by ligand exploitation and serial genome expansions. Proc Natl Acad Sci USA 2001; 98: 5671-6.

Fertility sparing approach as the standard of care in young patients with immature teratomas

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Abstract

Immature teratomas are quite rare tumors arising in young women. They are usually diagnosed in early stage and grade and have a good prognosis. In these young patients, fertility-sparing management is suggested as the standard of care. Bilateral immature teratoma is a rare condition with an incidence of 10%, with a five-year survival rate of 80%. The majority of patients received fertility-sparing treatment followed by adjuvant chemotherapy in 78%. Older age, advanced stage, and high grade are negative prognostic factors. The surgery-only, watch-and-wait approach was evaluated; however, after a median follow-up time of 42 months, 50% of patients experienced recurrence, but they were successfully salvaged with chemotherapy. In a retrospective study, 12 out of 27 patients tried to conceive, resulting in 10 pregnancies (8 after chemotherapy). We present a narrative review of the current literature regarding the essential multidisciplinary approach of such patients in order to achieve the best oncologic and fertility-sparing outcome. (J Turk Ger Gynecol Assoc 2017; 18: 43-7)

Keywords: Immature teratoma, ovarian germ cell tumor, fertility-sparing surgery, premenopausal, treatment

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Introduction

The term teratoma has its origin from the Greek word "teras" which means monster (1). The term began to be used about 300 years ago because people believed that these types of tumor were either aborted embryos or embryonic structures (2). Teratoma represents one of the most frequent germ cell neoplasms, which contain elements of all three germ cell layers (3). This type of neoplasm arises from totipotent cells in the ovary, which develop progressively into ectodermal, mesodermal, and endodermal tissue (4). Teratomas account for 10 to 20% of all ovarian malignancies in women younger than 20 years (5, 6).

There is a grading system based on the proportion of mature and immature neuroepithelial tissues, mitotic activity, and degree of differentiation. The following grades are proposed: grade 0= tumors with only mature tissue, and grades 1, 2, 3 all with mitotic activity, but with limited, moderate or large amounts of immature neuroepithelial tissue, respectively. Surgical management of teratomas is based on the following criteria: symptomatic relief, tumor size (often defined as greater than 5 cm in diameter given the propensity for torsion), and malignant potential (7). Immature teratomas are less common and definitely more aggressive tumors (8). The "immature" character of these tumors and the presence of not fully differentiated cells reveal a more aggressive behavior as the 30% of the ovarian cancer mortalities between the ages of 10 and 20 years is attributed to immature teratomas (4). As a consequence, the selection of proper treatment is fundamental because its priorities should include an oncologic approach to achieve the best possible prognosis by minimizing the risk of recurrence while considering the option of preserving the patients' future fertility.

The aim of this narrative review was to clarify the role of fertility-sparing surgery as a treatment option for young patients with immature teratomas based on the available current literature.

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Methods

An extensive electronic search was performed in PubMed (27/08/2015) and Scopus (27/08/2015). The adopted search strategy included the combination of the following keywords: treatment and fertility and (immature teratoma or immature ovarian teratoma). In order to retrieve additional studies, the references of the included studies were also searched. Studies written in languages other than English were not included. The literature search had a limitation at the search range, only studies written after 1990 were considered eligible for this review. Nine studies were eligible to be included in our review. Studies reporting data on fertility-sparing surgery in premenopausal patients with immature teratoma were regarded as eligible for this review. Abstracts, conference papers, book chapters, animal studies, commentaries, editorials as well as review articles were excluded from this review.

Discussion

Immature teratomas are usually unilateral tumors, which can be associated with the presence of ascites. They are usually curable at an early stage. The prognosis is correlated with the grade of immature elements. Stage Ia grade 1 tumors have an adequately good prognosis (94%) if treated surgically with complete staging procedures and are excised unruptured (9). Higher grade tumors have a survival of 85% (9, 10). The need for adjuvant chemotherapy in stage Ia G2-G3 and Ib-Ic is still controversial. Bilateral immature teratoma is a rare condition with an incidence of 10% and a five-year survival rate of 80% (11, 12). According to a recent study that included 27 patients with immature ovarian teratoma, the median age at diagnosis was 27.0 years (range, 18-36 years). Eighty-two percent presented with stage I disease, 11% had stage II, and 7% had stage III disease. Thirty-three percent of the patients were grade 1, 11% grade 2, and 56% grade 3. The majority of the patients received fertility-sparing treatment followed by adjuvant chemotherapy in 78% (13). Jorge et al. (14), in a recent multivariate analysis, showed that 90% of patients with immature teratoma were aged 18 to 39 years, three quarters of whom were early staged and could be treated conservatively combining fertility-sparing surgery and chemotherapy. Older age, advanced stage, and high grade are negative prognostic factors (14).

Fertility preservation in the cancer setting, known as oncofertility, became one of the hot topics in patients with cancer. An Oncofertility Consortium funded by the National Institute of Health has been established since 2006 and there are currently 19 countries engaged in the global oncofertility community. Medical awareness should be raised in young patients with ovarian tumors such as immature teratomas regarding fertility preservation options (15, 16). The main aims of such a multicenter international approach would be to standardize and improve the quality of treatment for every patient in each institute, to make a referral system that will further clarify outcomes and prognosis for the oncofertility approach of such rare tumors, and provide a policy to support each hospital (15).

Based on the good prognosis of patients with early-stage immature teratoma, fertility-sparing surgery, which could include removal of the affected ovary and preserving the contralateral ovary and uterus, followed by combination chemotherapy has become the standard of care of early-stages immature teratomas (10). Figure 1 presents an algorithm on the management of patients with immature teratoma, based on the National Comprehensive Cancer Network guidelines (17). The surgical treatment of ovarian teratomas should be based on ultrasound diagnosis regarding tumor size and possible extra ovarian findings, and a laparoscopic fertility-preserving approach is recommended (18). Specifically, based on the surgeon's experience and the size of the tumor >10 cm or not, a decision can be reached regarding the open or minimal invasive approach (19). After informed consent, open, laparoscopic or a robotic approach can be used in these patients with the aim of complete cytoreduction. More specifically, unilateral salpingo-oophorectomy with surgical staging is suggested including peritoneal exploration, cytology, and biopsies, omental biopsy or omentectomy and pelvic and/or para-aortic lymph node dissection. During surgery, routine biopsy of the normal-appearing contralateral ovary should be avoided because biopsy of the contralateral ovary could lead to future infertility related to peritoneal adhesions or ovarian failure (20). Park et al. (21) showed in their retrospective study that pelvic, para-aortic lymph node dissection, omentectomy and appendectomy was performed in only 36%, 26%, 59%, and 22%, respectively. Naturally, the role of systematic pelvic and para-aortic lymph node dissection could be questioned by several groups especially in patients with early-stage immature teratomas. The widespread use of a laparoscopic bag decreased the incidence of tumor spillage into the peritoneal cavity (22). However, minimal invasive techniques are characterized by increased operating times, increased cost, but also less postoperative pain, fewer adverse events of surgery, and a shorter length of stay in hospital (23, 24). Moreover, Chatchotikawong et al. (25) showed in an eight-year analysis that bilateral pelvic and paraaortic lymphadenectomy could offer information regarding disease extension and prognosis, elimination of some microscopic tumors, and clarify the further treatment options postoperatively. As Brown et al. (26) mentioned the lack of full surgical staging could lead to limited tissue evaluation and inappropriate over- or under-

treatment options. Additionally, the MITO-9 study showed that in cases of bilateral immature teratomas, although bilateral salpingo-oophorectomy could be performed, enucleation of contralateral tumor could be a treatment option based on their high chemosensitivity (27). Furthermore, Beiner et al. (28) showed that 5 out of 8 patients with bilateral immature teratoma who had conservative management received adjuvant chemotherapy, but all were disease free after 5 years' follow-up.

Regarding the role of chemotherapy, it is known that there is no need for adjuvant treatment in stage Ia grade 1 immature teratomas, whereas bleomycin, etoposide, and cisplatin (BEP) are used with excellent results in later stages and/or grades (6). More specifically, this surgery-only, watch-and-wait approach was evaluated; however, after a median follow-up time of 42 months, 50% of patients experienced recurrence, but they were successfully salvaged with chemotherapy (29). In addition, Park et al. (30) showed that fertility-sparing surgery alone with surveillance could be a safe treatment strategy in such patients and the majority of recurrences could be salvaged surgically and using BEP chemotherapy. Excessive chemotherapy may be harmful for the function of the preserved ovary. The reported histologic changes in the ovaries of patients receiving chemotherapy include cortical fibrosis, reduction in number of follicles, and impaired follicular maturation. These changes may lead to hypogonadism (31, 32). Post-operative radiotherapy has not proven to be of benefit (33).

Prognosis is excellent in early-stage and low-grade tumors. Hannan et al. (34) showed that all recurrences were found in patients advanced-stage tumors with recurrence rates reaching 13% in such patients. Fertility-sparing surgery should be attempted whenever possible under the care of subspecialists and after a thorough multidisciplinary decision and informed consent of the patients and/or her parents. Advice should be also taken from specialists in subfertility prior to surgery in cases of bilateral ovarian involvement in order to discuss the possibility of uterine conservation for possible future pregnancy with frozen or donor eggs. An international database that includes patients who have undergone fertility-sparing treatment could further clarify the prognosis, recurrence rates, reproductive and fertility outcomes, as well as the possible alternative treatment options in patients with recurrence. The first report of a successful pregnancy following conservative surgery and chemotherapy for advanced-stage immature teratoma appeared in 1989 (35). To date, several studies have shown that successful pregnancy is possible after treatment for immature teratoma, despite

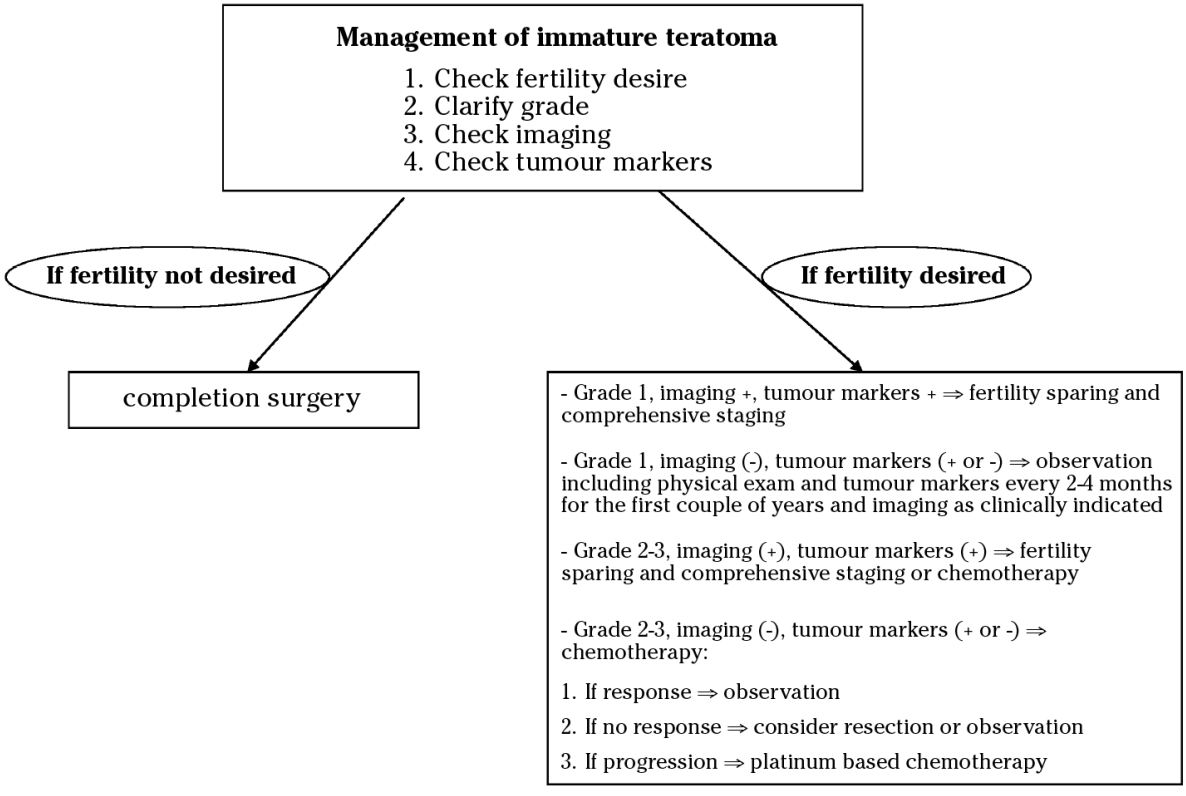

Figure 1. Algorithm on the management of patients with immature teratoma (adopted from National Comprehensive Cancer Network guidelines 2015)

the administration of chemotherapy and surgery (35, 36). Alwazzan et al. (13) showed in their retrospective study that 12 out of 27 patients tried to conceive resulting in 10 pregnancies (8 after chemotherapy). Long-term follow-up of patients with immature teratoma treated conservatively is very important to clarify recurrence rates and salvage options.

Conclusion

Fertility-sparing surgery could be offered to young patients with immature teratomas especially at early stage and grade. A multidisciplinary approach towards the patient including gynecologic oncologists, subfertility specialists, medical oncologists, and psychologists is suggested to optimize the care offered.

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References

1. Pantoja E, Noy MA, Axtmayer RW, Colon FE, Pelegrina I. Ovarian dermoids and their complications. Comprehensive historical review. Obstet Gynecol Surv 1975; 30: 1-20.
2. Sait K, Simpson C. Ovarian teratoma diagnosis and management: case presentations. J Obstet Gynaecol Can 2004; 26: 137-42.
3. Lu KH, Gershenson DM. Update on the management of ovarian germ cell tumors. J Reprod Med 2005; 50: 417-25.
4. Outwater EK, Siegelman ES, Hunt JL. Ovarian teratomas: tumor types and imaging characteristics. Radiographics 2001; 21: 475-90.
5. El-Lamie IK, Shehata NA, Abou-Loz SK, El-Lamie KI. Conservative surgical management of malignant ovarian germ cell tumors: the experience of the Gynecologic Oncology Unit at Ain Shams University. Eur J Gynaecol Oncol 2000; 21: 605-9.
6. Gershenson DM. Update on malignant ovarian germ cell tumors. Cancer 1993; 71: 1581-90.
7. Laufer M, Goldstein D, Benign and malignant ovarian masses. In: Emans S, Laufer M, Goldstein D, editors. Pediatric and Adolescent Gynecology. Philadelphia: Lippincott Williams and Wilkins; 2005. p. 706-10.
8. Iavazzo C, Kondi-Pafiti A, Germ Cell Tumours. In: Iavazzo C, Kondi-Pafiti A, editors. Non-epithelial ovarian tumours. Clinicopathological issues. New York, USA: Nova Science Publishers; 2013. p. 9-21.
9. Tewari K, Cappuccini F, Disaia PJ, Berman ML, Manetta A, Kohler MF. Malignant germ cell tumors of the ovary. Obstet Gynecol 2000; 95: 128-33.
10. Tzortzatos G, Sioutas A, Schedvins K. Successful pregnancy after treatment for ovarian malignant teratoma with growing teratoma syndrome. Fertil Steril 2009; 91: 936.
11. Mahdi H, Kumar S, Seward S, Semaan A, Batchu R, Lockhart D, et al. Prognostic impact of laterality in malignant ovarian germ cell tumors. Int J Gynecol Cancer 2011; 21: 257-62.
12. Saba L, Guerriero S, Sulcis R, Virgilio B, Melis G, Mallarini G. Mature and immature ovarian teratomas: CT, US and MR imaging characteristics. Eur J Radiol 2009; 72: 454-63.
13. Alwazzan AB, Popowich S, Dean E, Robinson C, Lotocki R, Altman AD. Pure Immature Teratoma of the Ovary in Adults: Thirty-Year Experience of a Single Tertiary Care Center. Int J Gynecol Cancer 2015; 25: 1616-22.
14. Jorge S, Jones NL, Chen L, Hou JY, Tergas AI, Burke WM, et al. Characteristics, treatment and outcomes of women with immature ovarian teratoma, 1998-2012. Gynecol Oncol 2016; 142: 261-6.
15. Ataman LM, Rodrigues JK, Marinho RM, Caetano JP, Chehin MB, Alves da Motta EL, et al. Creating a Global Community of Practice for Oncofertility. J Glob Oncol 2016; 2: 83-96.
16. Dursun P, Doğan NU, Ayhan A. Oncofertility for gynecologic and non-gynecologic cancers: fertility sparing in young women of reproductive age. Crit Rev Oncol Hematol 2014; 92: 258-67.
17. National Comprehensive Cancer Network guidelines 2015 (NCCN guidelines 2015, Less Common Ovarian Histologies. https://www.nccn.org/professionals/physician_gls/f_guidelines.asp#ovarian. Accessed on 28/07/2016.
18. Jalencas G, González Bosquet E, Guirado L, Fernandez Arias M, Ruiz de Gauna B, Fiores L, et al. Ovarian mature teratoma: a ten year experience in our institution. Clin Exp Obstet Gynecol 2015; 42: 518-22.
19. Ayhan A, Bukulmez O, Genc C, Karamursel BS, Ayhan A. Mature cystic teratomas of the ovary: case series from one institution over 34 years. Eur J Obstet Gynecol Reprod Biol 2000; 88: 153-7.
20. Buttram VC Jr, Vaquero C. Post-ovarian wedge resection adhesive disease. Fertil Steril 1975; 26: 874-6.
21. Park JY, Kim DY, Suh DS, Kim JH, Kim YM, Kim YT, et al. Outcomes of pediatric and adolescent girls with malignant ovarian germ cell tumors. Gynecol Oncol 2015; 137: 418-22.
22. Remorgida V, Magnasco A, Pizzorno V, Anserini P. Four year experience in laparoscopic dissection of intact ovarian dermoid cysts. J Am Coll Surg 1998; 187: 519-21.
23. Iavazzo C, Papadopoulou EK, Gkegkes ID. Cost assessment of robotics in gynecologic surgery: a systematic review. J Obstet Gynaecol Res 2014; 40: 2125-34.
24. Medeiros LR, Rosa DD, Bozzetti MC, Fachel JM, Furness S, Garry R, et al. Laparoscopy versus laparotomy for benign ovarian tumour. Cochrane Database Syst Rev 2009: CD004751.
25. Chatchotikawong U, Ruengkhachorn I, Leelaphatanadit C, Phithakwatchara N. 8-year analysis of the prevalence of lymph nodes metastasis, oncologic and pregnancy outcomes in apparent early-stage malignant ovarian germ cell tumors. Asian Pac J Cancer Prev 2015; 16: 1609-13.
26. Brown KL, Barnett JC, Leath CA 3rd. Laparoscopic staging of ovarian immature teratomas: a report on three cases. Mil Med 2015; 180: e365-8.
27. Sigismondi C, Scollo P, Ferrandina G, Candiani M, Angioli R, Viganò R, et al. Management of bilateral malignant ovarian germ cell tumors: a MITO-9 retrospective study. Int J Gynecol Cancer 2015; 25: 203-7.

28. Beiner ME, Gotlieb WH, Korach Y, Shrim A, Stockheim D, Segal Y, et al. Cystectomy for immature teratoma of the ovary. Gynecol Oncol 2004; 93: 381-4.
29. Billmire DF, Cullen JW, Rescorla FJ, Davis M, Schlatter MG, Olson TA, et al. Surveillance after initial surgery for pediatric and adolescent girls with stage I ovarian germ cell tumors: report from the Children's Oncology Group. J Clin Oncol 2014; 32: 465-70.
30. Park JY, Kim DY, Suh DS, Kim JH, Kim YM, Kim YT, et al. Outcomes of Surgery Alone and Surveillance Strategy in Young Women With Stage I Malignant Ovarian Germ Cell Tumors. Int J Gynecol Cancer. 2016; 26: 859-64.
31. Nicosia SV, Matus-Ridley M, Meadows AT. Gonadal effects of cancer therapy in girls. Cancer 1985; 55: 2364-72.
32. Schilsky RL, Lewis BJ, Sherins RJ, Young RC. Gonadal dysfunction in patients receiving chemotherapy for cancer. Ann Intern Med 1980; 93: 109-14.
33. Hackethal A, Brueggmann D, Bohlmann MK, Franke FE, Tinneberg HR, Munstedt K. Squamous-cell carcinoma in mature cystic teratoma of the ovary: systematic review and analysis of published data. Lancet Oncol 2008; 9: 1173-80.
34. Hannan A, Malik MA, Fasih S, Badar F, Siddiqui N. Malignant ovarian germ cell tumors at a tertiary care setting in Pakistan. J Ayub Med Coll Abbottabad 2015; 27: 624-7.
35. Lee RB, Kelly J, Elg SA, Benson WL. Pregnancy following conservative surgery and adjunctive chemotherapy for stage III immature teratoma of the ovary. Obstet Gynecol 1989; 73: 853-5.
36. Chen CH, Yang MJ, Cheng MH, Yen MS, Lai CR, Wang PH. Fertility preservation with treatment of immature teratoma of the ovary. J Chin Med Assoc 2007; 70: 218-21.

Pharmacokinetic, pharmacodynamic, and clinical aspects of ovulation induction agents: A review of the literature

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Abstract

Controlled ovarian hyperstimulation is a key step for successful outcomes of assisted reproductive technique cycle outcomes. Many medications are available, which are commonly useed solely or in combination to achieve multiple follicular development. Pharmacokinetic, pharmacodynamic, and clinical information of ovulation induction drugs deserve to be elucidated for every individual patient before commencing infertility treatment. New concepts and new treatment protocols are introduced as ovulation physiology is understood by infertility specialists. Increasing treatment success by minimizing aderse effects is a milestone of all ovarian stimulation protocols that use these novel interventions. Achievement of a satisfactory cycle outcome includes retrieval of sufficient oocytes, a single clinical pregnancy, and avoidance of ovarian hyperstimulation syndrome. In this review, we evaluate the current literature to determine the most reliable and relevant information about the most used ovulation induction drugs. (J Turk Ger Gynecol Assoc 2017; 18: 48-55)

Keywords: Pharmacokinetic, pharmacodynamic, ovulation induction, infertility

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Introduction

Pharmacokinetics is the study of drug metabolism in the body according to the rates of three processes: absorption, distribution, and elimination. Pharmacodynamics is the study of the mechanism of action by which drugs exert their pharmacologic effects; the binding of a drug to its target receptor or enzyme followed by a signal transduction pathway by which the receptor activates second messenger molecules, and finally the description of intracellular processes altered by the impact of the drug are components of the pharmacokinetics. Pharmacogenetics and pharmacogenomics are the study of the role of genetic inheritance in individual variation to drug response. Administration of a drug to different individuals can result with different clinical results based on the pharmacogenomic variability among individuals rather than pharmacokinetics. Individualisation of drug therapy can be tailored in the future by using pharmacogenomic information.

Recently, infertility became a relatively common public health problem because of the increased prevalance of advanced childbearing age of women. Ovulation induction treatment accompanied by artificial insemination or assisted reproduction are commonly used in infertile women. Pharmacokinetic, pharmacodynamic, and pharmacogenetic aspects of commonly used infertility drugs should be known to improve cycle outcomes. In this review, we aimed to discuss these clinical issues by evaluation of the current published literature regarding ovulation induction agents.

The ovulation induction agents that are commonly used during infertility treatment are shown in the Table 1.

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1. Antiestrogens (selective estrogen receptor modulators, aromatase inhibitors)

a. Selective estrogen receptor modulators

Estrogen reseptor modulators exert partial agonist and antagonist effects according to the tissue estrogen receptor content and estrogen availability level. Selective estrogen receptor modulators (SERMs) act by inhibiting the negative feedback effect of circulating estrogen on the hypothalamic pituitary unit (1). Clomiphene citrate (CC), tamoxifen, and raloxifene are three commonly used SERMs in women's health care.

CC is well absorbed from the gastrointestinal tract when administered orally. The commonly used daily dosage of CC is between 50-150 mg. Lower than 50 mg doses can be needed for the hyperresponder patient group, especially patients with polycystic ovary syndrome (PCOS). Although rarely needed, higher than 150 mg doses increase the antagonistic effect of CC on the endometrium and cervix, which is not warranted. CC is metabolized by hepatic transformation and excreted by feces which increases its bioavailability. High binding capacity to plasma proteins, entering enterohepatic cycle and accumulation in fatty tissues incerases the elimination half life of CC (5 days). Tamoxifen has a slightly higher elimination half life than CC (7 days). CC is exactly a weak estrogen agonist and a moderate estrogen antagonistic molecule. SERMs act on estrogen receptor containing tissues such as the hypothalamus, pituitary, ovary, endometrium, vagina, and cervix by competing with estrogen and decreasing the intracellulary estrogen receptor content. CC contains two isomeric forms, both of which include different clinical efficacy. Zuclomiphene is the less potent form with long elimination half time, which still exists in the body during early pregnancy achieved by utilization of CC for ovulation induction. Enclomiphene is the more potent form with short elimination half time which mainly exerts the clinical effect of CC following oral administration. CC is a category X drug but congenital anomaly rates are similar to the normal population. Enclomiphene is the more potent form with a short half life.

In two observational studies, ovulation and pregnancy rates seemed to be improved for patients with PCOS who were treated with tamoxifen following CC failure (2, 3). According to a Cochrane review conducted by Brown et al. (4), pregnancy rate, ovulation rate, miscarriage rate, live birth rate, and ongoing pregancy rate were similar between ovulation induction with CC and tamoxifen.

Table 1. Commonly used ovulation induction agents

Estrogen antagonists Clomiphene citrate Letrozole
Insulin sensitizing agents Metformin
Gonadotropins • Urinary FSH- recombinant FSH- long acting FSH (corifollitropin alfa) • Recombinant LH- 1 FSH+ 1 LH (hMG)- 2 FSH+ 1 LH • Urinary hCG- recombinant (rec) hCG • Urinary-hMG [1 LH (hCG derived) + 1 FSH]- highly purified (hp) hMG • Pure FSH-hpFSH • Urinary hCG- hphCG- rechCG
GnRH analogs GnRH agonists GnRH antagonists
FSH: follicle-stimulating hormone; LH: luteinizing hormone; hMG: human menopausal gonadotropin; hCG: human chorionic gonadotropin; GnRH: gonadotropin-releasing hormone

b. Aromatase inhibitors

Anastrazole and letrozole are nonsteroid competetive inhibitors of aromatase. These drugs have been developed for treatment of locally-advanced and metastatic breast cancer of postmenopausal women. Following oral administration, their elimination half time is 2 days. Hot flushes, nausea, headache, vaginal bleeding, and backache are adverse effects. The estrogen suppresion effect of aromatase inhibitors (AI) are dose dependant. The hypothalamo-pituitary-ovarian axis remains intact during ovulation induction treatment and this advantage results with monofollicular ovulation and lower multiple pregnancy rates. Absence of hostile antiestrogenic effect of CC on endometrium and cervix is another benefit of AIs. In a prospective randomized trial conducted by Diamond et al. (5), ovarian stimulation using letrozole resulted in a significantly lower rate of multiple pregnancy accompanied by a lower rate of live birth when compared with gonadotropin, but not when compared with CC treatment among women with unexplained infertility. Legro et al. (6) conducted another prospective randomized trial and they concluded that when compared with clomiphene, higher live birth and ovulation rates were achieved with ovulation induction using letrozole among infertile women with PCOS. Roque et al. (7) performed a systematic review based on randomized controlled trials comparing cycle outcomes of CC and letrozole among patients with PCOS. A statistically significant increase in the live birth and pregnancy rate was detected in the letrozole group when compared with CC use [relative risk (RR)=1.55 and RR=1.38, respectively]. Ovulation, miscarriage, and multiple pregnancy rates between the two groups were found similar. The authors concluded that regarding live birth and pregnancy rates, ovulation induction using letrozole results with better cycle outcomes when compared with CC in patients with PCOS (7). Letrozole's pharmacodynamic beneficial effects result with

higher pregnancy rates when compared with CC. Letrozole has shorter elimination half time (45 hours) than CC. Accumulation of CC within the body results with extended depletion of estrogen receptors accompanied by hostile effects on estrogen sensitive genital tissues. Letrozole increases the biosynthesis of endometrial receptivity markers such as integrins. In 2005, an oral presentation at an American Society of Reproductive Medicine meeting increased concerns regarding congenital malformation and teratogenicity risks of letrozole. This presentation has since been critisized because of the design of the study and the lack of publication in a peer-reviewed journal. Contrarily, cardiac and congenital abnormality rates of pregnancies achieved with CC have been found increased in some studies (8, 9). Tulandi et al. (10) performed a multicenter study comparing the neonatal outcome of 514 letrozole pregnancies with 297 CC pregnancies in 2006 and they concluded that congenital malformation and chromosomal abnormality rates of letrozole and CC were similar (2.4% vs. 4.8%, respectively). In addition, the cardiac anomaly rate of CC was significantly higher than letrozole (1.8% vs. 0.2%, respectively; p=0.02).

2. Metformin

Metformin is a biguanide oral antidiabetic medication that increases the sensitivity of insulin receptors in peripheral cells. Adding metformin to treatment cycle protocols for increasing pregnancy rates among patients with PCOS is a matter of debate. In a systematic review, Palomba et al. (11) concluded that infertile patients with PCOS treated with gonadotrophins for in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) cycles, implantation rates seemed improved but pregnancy or live birth rates did not increase by using metformin despite lower rates of ovarian hyperstimulation syndrome (OHSS) and miscarriage (11, 12). Specific phenotypes and features of patients with PCOS who will benefit from metformin should be defined before liberally advising metformin to all patients with PCOS. Longer than 3 weeks administration of metformin has been found to decrease miscarriage rates [odds ratio (OR) 0.41, 95% confidence interval: (0.21 to 0.78), p=0.0086]. In a Cochrane database review by Tso et al. (13) in which the clinical effects of metformin treatment before and during IVF or ICSI in women with PCOS were evaluated, the authors concluded that despite significantly beneficial effects for OHSS prevention, no conclusive evidence has been detected for improved live birth rates by using metformin treatment before or during assisted reproductive technique (ART) cycles. Unlike Palomba et al. (11), they emphasized that the use of this insulin-sensitising agent increased clinical pregnancy rates without exerting any beneficial effect on abortus rate, retrieved oocyte number, total gonadotropin dose, stimulation time, fertilization and cycle cancellation rate (13).

3. Gonadotropins

Follicle-stimulating hormone (FSH), luteinizing hormone (LH), human chorionic gonadotropin (hCG), and thyroid stimulating hormone are heterodimer glycoprotein hormones including alfa and beta subunits. Alfa subunits of these hormones are made up of same 92 aminoacides. The beta subunit is responsible for the biologic specificity of the hormone. The serum elimination half times of these hormones are relatively short except hCG. Although the beta subunits of LH and hCG have the same rate of 80%, the plasma elimination half time of hCG is 10 times higher than LH. C terminal peptides and sialic acid residues containing 31 amino acids cause this paharmacocinetic difference. The bioavailability of recombinant FSH (rFSH) and recombinant LH (rLH) is around 70% and 60% following subcutaneous administration, respectively (14-16). No pharmacokinetic interactions occur between rFSH and rLH when administered simultaneously. The serum elimination half time of rFSH and rLH is around 24 and 10-12 hours, respectively. Steady state plasma levels are achieved after 3-4 days following repeated rFSH injections. Among patients whose endogenous hypothalamopituitary axis have been suppressed, rFSH alone can efficiently achieve folliculogenesis and also steroidogenesis despite low serum LH levels. Different clinical responses to the same FSH medication doses are caused by FSH receptor polymorphism, also called pharmacogenetics, rather than pharmacokinetic actions of the drug (14-17).

Batch-to-batch inconsistency, foreign proteins, and unpredictable clinical eficiency are major drawbacks of urine-derived gonadotropins. In a prospective randomized multicenter study, Frydman et al. (18) compared rFSH with urinary FSH according to ART cycle outcomes. Achievement of a higher number of oocytes with lower total doses and shorter stimulation times, rFSH was found more potent than urinary FSH. However, the increased oocyte numbers were not reflected by increased pregnancy rates for rFSH against urinary FSH.

Stimulation of folliculogenesis in the treatment of infertility has been traditionally conducted by using gonadotropins extracted from the urine of postmenopausal women. Urine-derived products consist of a mixture of gonadotropins with unpredictable clinical efficiencies and biologically active mediators such as binding proteins, growth factors, and prion proteins. The variation of exact amount of gonadotropins in human menopausal gonadotropin (hMG) preparations results with diverse effects on gonads during ovulation induction. The hCG content and hMG product increases parallel to the increasing purity of the drug to standardise the biologic activity. hCG is secreted by the embryo and placenta and physiologically supports implantation and pregnancy. The receptor binding

affinity of hCG is 2 times higher than LH. LH has a shorter serum elimination half life than hCG (23 vs. 32-33 hours, respectively). hCG accumulates in the body significantly and causes LH receptor downregulation unlike LH itself. Six to 8 IU of LH is biologically equivalent to 1 IU of hCG, which demonstrates the potency of hCG over LH. Controversy has not been resolved as to whether r-hLH or hCG should be used for ART to increase cycle outcomes (19).

In a prospective observational study, Requena et al. (20) compared endocrine profile of oocyte donors stimulated with FSH plus rLH (2/1 in ratio) or hMG. Although retrieved oocyte numbers following treatment with recombinant gonadotropins were higher than urinary gonadotropins (hMG) (16.5 vs. 11.8; p=0.049), harvested metaphase II ooocyte numbers were higher by using urinary gonadotropins (hMG) (71.2% vs. 80.6%; p=0.003). When serum steroid hormone levels (estradiol, progesterone, testosterone and androstenedione) were evaluated on the day of triggering and cycle day 6, slightly elevated levels were detected in the recombinant gonadotropins when compared with the urinary gonadotropins. Comparison of intrafollicular levels of steroid hormones were found statistically insignificant between the two protocols and ongoing pregnancy rates were also similar (46.1% vs. 46.1%) (20).

In the Menopur in GnRH Antagonist Cycles with Single Embryo Transfer (MEGASET) trial, Devroey et al. (21) evaluated the safety and efficacy of rFSH and highly purified menotropin (hphMG) for controlled ovarian hyperstimulation in GnRH antagonist cycles with mandatory single blastocyst transfer. Although higher oocyte numbers were achieved with rFSH against hMG, similar MII oocyte numbers were harvested. The authors concluded that despite the significant discrepancy in pharmacodynamic effects, highly purified hMG was found have a similar effect as rFSH in GnRH antagonist cycles with mandatory single blastocyst transfer based on clinical pregnancy rates of both fresh and freeze thaw cycle transfers of day 5 embryos (21).

Recently, a gonadotropin preparation that includes rFSH and rLH 2/1 in ratio was commercially developed. Dosing studies performed on hypogonadotropic hypogonadism patients that evaluted the clinical eficacy of this new drug revealed that 75 IU of LH were sufficient for optimal folliculogenesis (22). Some studies in the literature have demonstrated the nourishing effects on cycle outcomes and ovarian response rates of addition of LH activity to stimulation regimens in certain groups of patients. Women aged older than 35 years, those with diminished ovarian reserve, and women with LH receptor polymorphisms are theoretical candidates for this approach. Adding LH to the treatment protocol activates theca cells to produce more androgens, which are eventually converted to estrogens in granulosa cells to increase the estrogenic milieu within the ovarian follicle and also oocyte quality (23).

Pacchiarotti et al. (24) conducted a prospective randomised trial to compare IVF outcomes in ovarian stimulation protocols with recombinant FSH plus recombinant LH (2/1 in ratio) versus hMG. Treatment with rFSH plus rLH or with hMG was found to produce the same results in terms of implantation rates, pregnancy rates, and embryo quality. Although a statistical difference in oocyte quality, with a better quality in the hMG group was detected, this difference was levelled because of the total number of oocytes retrieved, which was higher in the rFSH plus rLH group, thus the total number of MII oocytes was similar in both groups at the expense of higher OHSS rates for rFSH plus RLH group. The reduction of the amount of FSH used in the hMG group also led to lower cost of the IVF cycle (24).

Bosch (25) published a review article regarding the pharmacologic characteristics and clinical applications of rFSH plus rLH (2/1 in ratio). Although the 2:1 combination of r-hFSH and r-hLH seems to be an optimum ovulation induction regimen regarding safety and clinical efficacy in patients with hypogonatrophic hypogonadism, use of this drug combination in ovarian stimulation for IVF remains controversial because the target population that may receive a benefit from this combination therapy is not well defined. Patients needing >3000 IU rFSH during COH, patients showing plateau on follicular growth, and those with inadequate response after 7 days r-FSH have been suggested as candidates for adding rLH to stimulation regimens based on previous studies (25).

In a systematic review and meta-analysis, Lehert et al. (26) suggested that in poor responders, r-hLH supplementation of r-hFSH compared with rhFSH alone may result in significantly higher oocyte numbers, clinical pregnancy rates, and ongoing pregnancy rates. Based on this entity, Humaidan et al. (27) are currently conducting a randomized controlled multicenter trial to explore the possible advantages of a fixed-dose combination of r-FSH plus r-LH over r-FSH monotherapy in patients with poor ovarian response (POR) according to the definition determined in the European Society of Human Reproduction and Embryology (ESHRE) Bologna criteria.

Long-acting gonadotropins

Corifollitropin alfa is a long acting recombinant FSH, which acts for 7 days following administration to support folliculogenesis. Although pharmacodynamic actions of long-acting rFSH is the same as with rFSH, the serum elimination half time of long-acting rFSH is 65 hours, which is twice that of rFSH. Dose finding studies revealed that patients weighing above and below 60 kg, 100 μg and 150 μg long-acting rFSH are recommended for clinical efficiency (28). In a Cochrane database meta-analysis, Pouwer et al. (29) revealed that although the use of a medium

dose (150 to 180 μg) of long-acting rFSH seemed to be a safe and equally effective treatment option when compared with daily rFSH in women with unexplained subfertility, reduced live birth rate in women receiving a low dose (60 to 120 μg) of long-acting rFSH compared with daily rFSH was also observed.

The safety and effectiveness of long-acting FSH for use in hyper- or poor responders and in women with all causes of subfertility is an area of current research. In a systematic review and meta-analysis including 4 randomized trials, Mahmoud Youssef et al. (30) concluded that corifollitropin alfa in combination with daily GnRH antagonist seemed to be an alternative for daily rFSH injections in view of efficiency and safety profile among normoresponder patients undergoing controlled ovarian hyperstimulation in IVF/ICSI treatment cycles.

4. Recombinant human chorionic gonadotropin versus urinary human chorionic gonadotropin

hCG is used for final maturation of oocytes during ART cycles. Urine-derived hCG has some disadvantages compared with recombinant hCG (rhCG) such as batch-to-batch inconsistency, uncontrolled source, and unpredictable biologic activity. Chang et al. (31) compared the efficacy and safety of 250 μg and 500 μg of rhCG with 10 000 IU of urinary hCG (uhCG) in ART in a randomized controlled prospective study. As the primary end point of the study, total harvested oocyte numbers were similar for both groups. Based on the results of this study, rhCG was found effective and tolerable in terms of induction of final follicular maturation and luteinization for women undergoing ART procedures. Youssef et al. (32, 33) performed two consecutive Cochrane metaanalyses to assess the safety and efficacy of subcutaneous rhCG and high-dose rLH compared with intramuscular uhCG for inducing final oocyte maturation triggering in IVF and ICSI cycles and they concluded that equivalent pregnancy rates and OHSS incidences were found between rhCG or rhLH and uhCG when used for final follicular maturation in IVF. According to these findings, the authors recommended using uhCG as the best selection for final oocyte maturation triggering in IVF and ICSI treatment cycles.

5. Gonadotropin-releasing hormone agonist trigger for final oocyte maturation

hCG has been used as a surrogate for midcycle LH peak to induce final oocte maturation before oocyte retrieval in ART. The relatively long elimination half time of hCG obtains a luteotrophic effect during the luteal phase, but also increases the OHSS risk. Despite obtaining a stimulus for final oocyte maturation, ovulation triggering with hCG has no beneficial effect on endometrial receptivity and oocyte quality when compared with spontaneous ovulation (34). The FSH surge accompanies the LH surge during physiologic ovulation that triggers natural cycles. This midcycle surge of FSH is thought to promote nuclear maturation of the oocyte, cumulus cell accumulation, and LH receptor formation on granulosa cells. When GnRH antagonists were introduced to the market, the use of GnRH agonists for final oocyte maturation came into consideration again.

Pioneer studies in this field resulted with disappointment regarding low pregnancy rates and high abortion rates of IVF-ET cycles triggered with GnRH agonists (35). Modifications of luteal phase support solved this clinical problem and nowadays GnRh agonists are more frequently used for final maturation, especially for patients with increased OHSS risk. Although GnRH agonist trigger strategy seems to decrease OHSS risk with satisfactory pregnancy rates by using modified luteal phase support; early OHSS can still occur even when embryo transfer is deferred (36-38). Oocyte donors, high responser patients, patients who demand fertility preservation, and also normal responder patients are suggested as the target groups for GnRH agonist trigger. During the luteal phase of ART cycles triggered with GnRH agonists, the relatively shortstanding LH surge and central inhibition of gonadotropin secretion due to supraphysiological serum estradiol levels causes depletion of LH support, which is needed by the corpora lutea to enhance implantation by secretion of progesterone and also many other implantation favoring mediators. Although luteal estradiol supplementation is not needed for ART cycles triggered with hCG, this intervention is strongly recommended until the 7th gestational week during ART cycles triggered with GnRH agonists (38). Humaidan et al. (36) suggested administering 1500 IU hCG intramuscularly during oocyte retrieval procedures when GnRH agonists have been used for final oocyte maturation of GnRH antagonist cycles. This intervention has dramatically lowered abortion rates and boosted the pregnancy rates for this group of patients.

Different doses of different GnRh agonists have been successfully used for final oocyte maturation in the literature. Youssef et al. (39) performed a Cochrane metaanalysis to evaluate the differences between GnRH agonists and HCG in terms of safety and effectiveness for triggering final oocyte maturation in IVF-ICSI among women undergoing a GnRH antagonist protocol. Unlike the Humaidan group, the authors concluded that when GnRH agonists were used for final oocyte maturation in fresh autologous cycles, lower live birth rates, lower ongoing pregnancy rates, and a higher rates of early miscarriage were achieved. Youssef et al. (39) recommended the use of GnRH agonists as an oocyte maturation trigger for women who are spared for fresh transfers, who are oocyte donors, and who demand to freeze autologous oocytes for fertility preservation. Recently, Engmann et al. (40)

reviewed the advantages and potential drawbacks of GnRH agonist triggering by performing a strengths, weaknesses, opportunities and threats (SWOT) analysis. Based on this analysis modality, the authors recommended intensive luteal support with transdermal oestradiol and intramuscular progesterone alone if peak serum oestradiol is 4000 or more pg/mL after GnRHa triggering or dual triggering with GnRH agonist and hCG 1000 IU if peak serum oestradiol is less than 4000 pg/mL. The recommendations of the same group based on the follicle number were as follows: administration of hCG 1500 IU 35 h after GnRH agonist trigger if there are less than 25 follicles ≥11 mm on the day of ovulation trigger, or freeze all oocytes or embryos if there are over 25 follicles (40).

6. Gonadotropin-releasing hormone antagonist (short) versus gonadotropin-releasing hormone agonist (long) protocol

Al-Inany et al. (41) conducted a Cochrane metaanalysis for comparing these mostly used COH protocols. They investigated the safety and effectiveness of GnRH antagonists by comparison with the long protocol of GnRH agonists for ovarian stimulation in ART cycles. In this review, the authors concluded that when compared with long GnRH agonist protocols, the antagonist protocol was associated with a wide decrease in OHSS rates and similar live birth rates (41, 42). The same group recently conducted a Cochrane systematic review and similar live birth rates were observed between GnRH antagonist and long GnRH agonist protocols. When compared with GnRH agonists, GnRH antagonist-based protocols lowered the incidence of all OHSS severity grades (OR 0.61). The miscarriage rates were found similar between these two protocols. The cycle cancellation rate following POR to ovulation induction was higher in women who received GnRH antagonist protocols compared with GnRH agonist protocols (OR 1.32) (43). Based on these results, GnRH antagonist protocols seem to be the best and safest protocol for patients with high baseline OHSS risk. Contrarily, GnRH agonist protocols result with higher oocyte yield than GnRH antagonist protocols among poor responder patients. Sunkara et al. (44) performed a randomized controlled study among poor responders undergoing IVF treatment. The number of oocytes retrieved was significantly higher with long GnRH agonists compared with short agonist regimens (4.42±3.06 vs. 2.71±1.60) and similar between long agonist and antagonist regimens (4.42±3.06 vs. 3.30±2.91). Total gonadotropin dose and duration of stimulation were significantly higher using long agonist regimens compared with short agonist and antagonist regimens. The ongoing pregnancy rates were 16.2% with antagonist protocols and 8.1% with long and short agonist protocols (p=0.48). Based on these results, the authors concluded that long GnRH agonist and antagonist regimens can be a better selection as ovulation induction regimens for poor responders, whereas the short agonist regimen seems to be a less effective treatment strategy because fewer oocytes are retrieved (44). Al Inany et al. conducted a Cochrane systematic review including 73 RCTs, with 12 212 participants, comparing GnRH antagonist to long-course GnRH agonist protocols. Although the quality of the selected studies for this systematic review was moderate, the use of GnRH antagonist was found associated with a substantial reduction in OHSS without reducing the likelihood of achieving live birth when compared with long-course GnRH agonist protocols (43).

In conclusion, rational use and administration of ovulation induction drugs necessitate evaluation of pharmacokinetic, pharmacodynamic, and clinical aspects of each individual medication based on pharmacologic and clinical evidence. This clinical practice will eventually increase the success of ovulation induction protocols performed for infertility treatment and decrease the health threatening risks that arise from the treatment burden.

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References

1. Brenner GM, Stevens C. Pharmacokinetics. Pharmacology; Chapter 2: 925: 2012.
2. Gulekli B, Ozaksit G, Turhan NO, Senoz S, Oral H, Gokmen O. Tamoxifen: an alternative approach in clomiphene resistant polycystic ovarian syndrome patients. J Pak Med Assoc 1993; 43: 89-90.
3. Dhaliwal LK, Suri V, Gupta KR, Sahdev S. Tamoxifen: An alternative to clomiphene in women with polycystic ovary syndrome. J Hum Reprod Sci 2011; 4: 76-9.
4. Brown J, Farquhar C, Beck J, Boothroyd C, Hughes E. Clomiphene and anti-oestrogens for ovulation induction in PCOS. Cochrane Database Syst Rev 2009: CD002249.
5. Diamond MP, Legro RS, Coutifaris C, Alvero R, Robinson RD, Casson P, et al. Letrozole, Gonadotropin, or Clomiphene for Unexplained Infertility. N Engl J Med 2015; 373: 1230-40.
6. Legro RS, Brzyski RG, Diamond MP, Coutifaris C, Schlaff WD, Casson P, et al. Letrozole versus clomiphene for infertility in the polycystic ovary syndrome. N Engl J Med 2014; 371: 119-29.

7. Roque M, Tostes AC, Valle M, Sampaio M, Geber S. Letrozole versus clomiphene citrate in polycystic ovary syndrome: systematic review and meta-analysis. Gynecol Endocrinol 2015; 31: 917-21.
8. Reefhuis J, Honein MA, Schieve LA, Rasmussen SA; National Birth Defects Prevention Study. Use of clomiphene citrate and birth defects, National birth defects prevention study, 1997-2005. Hum Reprod 2011; 26: 451-7.
9. Davies MJ, Moore VM, Willson KJ, Van Essen P, Priest K, Scott H, et al. Reproductive technologies and the risk of birth defects. N Engl J Med 2012; 366: 1803-13.
10. Tulandi T, Martin J, Al-Fadhli R, Kabli N, Forman R, Hitkari J, et al. Congenital malformations among 911 newborns conceived after infertility treatment with letrozole or clomiphene citrate. Fertil Steril 2006; 85: 1761-5.
11. Palomba S, Falbo A, La Sala GB. Effects of metformin in women with polycystic ovary syndrome treated with gonadotrophins for in vitro fertilisation and intracytoplasmic sperm injection cycles: a systematic review and meta-analysis of randomised controlled trials. BJOG 2013; 120: 267-76.
12. Uysal S, Zeki Isik A, Eris S, Yigit S, Yalcin Y, Ozun Ozbay P. Correlation of endometrial glycodelin expression and pregnancy outcome in cases with polycystic ovary syndrome treated with clomiphene citrate plus metformin: a controlled study. Obstet Gynecol Int 2015; 2015: 278591.
13. Tso LO, Costello MF, Albuquerque LE, Andriolo RB, Macedo CR. Metformin treatment before and during IVF or ICSI in women with polycystic ovary syndrome. Cochrane Database Syst Rev 2014: CD006105.
14. Porchet HC, Le Cotonnec JY, Loumaye E. Clinical pharmacology of recombinant human follicle-stimulating hormone. III. Pharmacokinetic-pharmacodynamic modeling after repeated subcutaneous administration. Fertil Steril 1994; 61: 687-95.
15. le Cotonnec JY, Porchet HC, Beltrami V, Munafo A. Clinical pharmacology of recombinant human luteinizing hormone: Part I. Pharmacokinetics after intravenous administration to healthy female volunteers and comparison with urinary human luteinizing hormone. Fertil Steril 1998; 69: 189-94.
16. Le Cotonnec JY, Loumaye E, Porchet HC, Beltrami V, Munafo A. Pharmacokinetic and pharmacodynamic interactions between recombinant human luteinizing hormone and recombinant human follicle-stimulating hormone. Fertil Steril 1998; 69: 201-9.
17. Yilmaz B, Kelekci S, Savan K, Oral H, Mollamahmutoglu L. Addition of human chorionic gonadotropin to clomiphene citrate ovulation induction therapy does not improve pregnancy outcomes and luteal function. Fertil Steril 2006; 85: 783-6.
18. Frydman R, Howles CM, Truong F. A double-blind, randomized study to compare recombinant human follicle stimulating hormone (FSH; Gonal-F) with highly purified urinary FSH (Metrodin) HP) in women undergoing assisted reproductive techniques including intracytoplasmic sperm injection. The French Multicentre Trialists. Hum Reprod 2000; 15: 520-5.
19. Ezcurra D, Humaidan P. A review of luteinising hormone and human chorionic gonadotropin when used in assisted reproductive technology. Reprod Biol Endocrinol 2014; 12: 95.
20. Requena A, Cruz M, Ruiz FJ, García-Velasco JA. Endocrine profile following stimulation with recombinant follicle stimulating hormone and luteinizing hormone versus highly purified human menopausal gonadotropin. Reprod Biol Endocrinol 2014; 12: 10.
21. Devroey P, Pellicer A, Nyboe Andersen A, Arce JC; Menopur in GnRH Antagonist Cycles with Single Embryo Transfer Trial Group. A randomized assessor-blind trial comparing highly purified hMG and recombinant FSH in a GnRH antagonist cycle with compulsory single-blastocyst transfer. Fertil Steril 2012; 97: 561-71.
22. http://www.emea.europa. eu/humandocs/PDFs/EPAR/pergoveris/H-714-PI-en.pdf
23. Bosch E, Pau E, Albert C. Impact of LH administration on oocyte quality and embryo morphology. Fertil Steril 2006;86: (Suppl 2): S425.
24. Pacchiarotti A, Sbracia M, Frega A, Selman H, Rinaldi L, Pacchiarotti A. Urinary hMG (Meropur) versus recombinant FSH plus recombinant LH (Pergoveris) in IVF: a multicenter, prospective, randomized controlled trial. Fertil Steril 2010; 94: 2467-9.
25. Bosch E. Recombinant human follicular stimulating hormone and recombinant human luteinizing hormone in a 2:1 ratio combination. Pharmacological characteristics and clinical applications. Expert Opin Biol Ther 2010; 10: 1001-9.
26. Lehert P, Kolibianakis EM, Venetis CA, Schertz J, Saunders H, Arriagada P, et al. Recombinant human follicle-stimulating hormone (r-hFSH) plus recombinant luteinizing hormone versus r-hFSH alone for ovarian stimulation during assisted reproductive technology: systematic review and meta-analysis. Reprod Biol Endocrinol 2014; 12: 17.
27. Humaidan P, Schertz J, Fischer R. Efficacy and Safety of Pergoveris in Assisted Reproductive Technology--ESPART: rationale and design of a randomised controlled trial in poor ovarian responders undergoing IVF/ICSI treatment. BMJ Open 2015; 5: e008297.
28. Fauser BC, Alper MM, Ledger W, Schoolcraft WB, Zandvliet A, Mannaerts BM, et al. Pharmacokinetics and follicular dynamics of corifollitropin alfa versus recombinant FSH during ovarian stimulation for IVF. Reprod Biomed Online 2010; 21: 593-601.
29. Pouwer AW, Farquhar C, Kremer JA. Long-acting FSH versus daily FSH for women undergoing assisted reproduction. Cochrane Database Syst Rev 2015: CD009577.
30. Mahmoud Youssef MA, van Wely M, Aboulfoutouh I, El-Khyat W, van der Veen F, Al-Inany H. Is there a place for corifollitropin alfa in IVF/ICSI cycles? A systematic review and meta-analysis. Fertil Steril 2012; 97: 876-85.
31. Chang P, Kenley S, Burns T, Denton G, Currie K, DeVane G, et al. Recombinant human chorionic gonadotropin (rhCG) in assisted reproductive technology: results of a clinical trial comparing two doses of rhCG (Ovidrel) to urinary hCG (Profasi) for induction of final follicular maturation in in vitro fertilization-embryo transfer. Fertil Steril 2001; 76: 67-74.
32. Youssef MA, Al-Inany HG, Aboulghar M, Mansour R, Abou-Setta AM. Recombinant versus urinary human chorionic gonadotrophin for final oocyte maturation triggering in IVF and ICSI cycles. Cochrane Database Syst Rev 2011: CD003719.
33. Youssef MA, Abou-Setta AM, Lam WS. Recombinant versus urinary human chorionic gonadotrophin for final oocyte maturation triggering in IVF and ICSI cycles. Cochrane Database Syst Rev 2016: CD003719.
34. George K, Kamath MS, Nair R, Tharyan P. Ovulation triggers in anovulatory women undergoing ovulation induction. Cochrane Database Syst Rev 2014: CD006900.
35. Kolibianakis EM, Schultze-Mosgau A, Schroer A, van Steirteghem A, Devroey P, Diedrich K, et al. A lower ongoing pregnancy rate can be expected when GnRH agonist is used for triggering final oocyte maturation instead of HCG in patients undergoing IVF with GnRH antagonists. Hum Reprod 2005; 20: 2887-92.
36. Humaidan P, Kol S, Papanikolaou EG; Copenhagen GnRH Agonist Triggering Workshop Group. GnRH agonist for triggering of final oocyte maturation: time for a change of practice? Hum Reprod Update 2011; 17: 510-24.
37. Gülekli B, Göde F, Sertkaya Z, Isık AZ. Gonadotropin-releasing hormone agonist triggering is effective, even at a low dose, for final oocyte maturation in ART cycles: Case series. J Turk Ger Gynecol Assoc 2015; 16: 35-40.

38. Gurbuz AS, Gode F, Ozcimen N, Isik AZ. Gonadotrophin-releasing hormone agonist trigger and freeze-all strategy does not prevent severe ovarian hyperstimulation syndrome: a report of three cases. Reprod Biomed Online 2014; 29: 541-4.
39. Youssef MA, Van der Veen F, Al-Inany HG, Mochtar MH, Griesinger G, Nagi Mohesen M, et al. Gonadotropin-releasing hormone agonist versus HCG for oocyte triggering in antagonist-assisted reproductive technology. Cochrane Database Syst Rev 2014: CD008046.
40. Engmann L, Benadiva C, Humaidan P. GnRH agonist trigger for the induction of oocyte maturation in GnRH antagonist IVF cycles: a SWOT analysis. Reprod Biomed Online 2016; 32: 274-85.
41. Al-Inany HG, Youssef MA, Aboulghar M, Broekmans F, Sterrenburg M, Smit J, et al. Gonadotrophin-releasing hormone antagonists for assisted reproductive technology. Cochrane Database Syst Rev 2011: CD001750.
42. Cicek MN, Kahyaoglu I, Kahyaoglu S. The comparison of microdose flare-up and multiple dose antagonist protocols based on hCG day estradiol (E2), progesterone (P) and P/E2 ratio among poor responder patients in ICSI-ET cycles. Eur Rev Med Pharmacol Sci 2015; 19: 539-44.
43. Al-Inany HG, Youssef MA, Ayeleke RO, Brown J, Lam WS, Broekmans FJ. Gonadotrophin-releasing hormone antagonists for assisted reproductive technology. Cochrane Database Syst Rev 2016: CD001750.
44. Sunkara SK, Coomarasamy A, Faris R, Braude P, Khalaf Y. Long gonadotropin-releasing hormone agonist versus short agonist versus antagonist regimens in poor responders undergoing in vitro fertilization: a randomized controlled trial. Fertil Steril 2014; 101: 147-53.

Retrospective evaluation of pregnant women with celiac disease

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Abstract

Objective: To show celiac disease (CD) and its poor pregnancy outcome relationship, and to demonstrate the importance of a gluten-free diet together with low-dose low-molecular-weight heparin (LMWH) and low-dose corticosteroid (LDC) in the management of pregnancies with CD.

Material and Methods: This study consisted of 2 groups of patients. Six patients with CD (control group) on a gluten-free diet were monitored during their first pregnancies within the framework of antenatal care program and their pregnancy outcomes were compared with eight poorly-treated pregnant patients with CD (study group) who were referred from other medical institutions. LMWH (enoxaparine 1x2000 Anti-XA IU/0.2 mL/day), and LDC (methylprednisolone 1x4 mg p.o/day) were used in the control group. Their obstetric histories and outcomes of their last pregnancies were compared. The patients' obstetric risk levels were evaluated using the "Beksac Obstetrics Index" (BOI).

Results: There were miscarriages in 50% of the study group. There were also 50% and 75% preterm deliveries in the control and study groups, respectively. The BOI of the study group was significantly worse than the control group (1.31 vs. 0.31±0.21, $p<0.01$). There were no statistically significant differences between age (24±4.7 vs 31.7±6 years, $p=0.448$), gestational day of birth (259.3±8.5 vs 246.6±24.3), birthweight (2691±698 vs 2262±359 g, $p=0.394$), and cesarean section rates ($p=0.118$).

Conclusion: CD is a risk factor for adverse pregnancy outcome. Miscarriage and preterm labor are critical complications in pregnancies complicated by CD. A gluten-free diet is important in the treatment. LMWH and LDC seem to be helpful in the management of pregnant women with CD. (J Turk Ger Gynecol Assoc 2017; 18: 56-9)

Keywords: Celiac disease, gluten-free diet, pregnancy, perinatal morbidity, low-molecular- weight heparin, low-dose corticosteroids

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Introduction

Celiac disease (CD) is a small intestinal enteropathy, which is activated by gluten ingestion in patients with a genetic background (1). The prevalence of the disease is around 1% (2, 3). "Gluten, gluten-specific T-cells, the major histocompatibility complex antigen HLA-DQ, and transglutaminase type 2 (TG2)" are the main actors of this disorder. It has been reported that repeated miscarriages and obstetric complications are more frequent in patients with CD (4, 5).

CD is an autoimmune disorder characterized by circulating anti-TG2 autoantibodies (6, 7). It has been reported that anti-TG2 antibodies act negatively on endometrial receptivity and impair decidual angiogenesis together with interstitial trophoblast migration along with various mechanisms (8). It has also been reported that the chorionic villus (materno-fetal interface) is one of the main targets of anti-TG2 autoantibodies and these antibodies directly attack syncytiotrophoblasts (9). In other words, impaired endometrial receptivity and disturbed syncytiotrophoblastic apoptosis might be the main causes of impaired fetal perfusion (intrauterine hypoxia) and poor obstetric outcome.

In this report, we compared the pregnancy outcomes of 6 primigravida patients at complete remission on a gluten-free diet and 8 referred patients with CD who had various gestational symptoms in terms of obstetric outcome.

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Material and Methods

This retrospective study was conducted at the Department of Obstetrics and Gynecology, Hacettepe University, between March 2014 and March 2016. There were two groups of patients. The control group comprised six patients with CD on gluten-free diet who were monitored during their first pregnancies. Their pregnancy outcomes were compared with eight pregnant patients (study group) with CD from other medical institutions who had been referred because they had not adhered to their recommended gluten-free diet. All patients were diagnosed with CD before their pregnancies.

We used the Beksac Obstetrics Index (BOI), which is an obstetrics index for the assessment of risk levels of high-risk pregnancy groups [(number of alive children + π/10)/Gravida], in order to compare these two groups (π=3.14) (10).

Patients in the control group were followed up under the "autoimmune disorders in pregnancy" protocol within the special antenatal care program of the Division of Perinatal Medicine. Laboratory tests were performed (complete blood count, liver function enzymes, antithrombin-III and activated protein-C activities, complement 3 and 4, blood glucose level, hereditary thrombophilia-related polymorphisms, antibodies such as antinuclear antibodies, antiphospholipid antibodies, anti-smooth muscle antibodies, anti-double stranded DNA, and others according to individual differences), and necessary precautions were undertaken. Low-dose low-molecular-weight heparin (LMWH) (enoxaparine 1x2000 Anti-XA IU/0.2 mL/day), and low-dose-corticosteroid (methylprednisolone 1x4 mg p.o/day) were used together with CD-specific treatment in the control group. In one patient, low-dose corticosteroid (LDC) was used alone without LMWH. Study group patients who did not have an abortion received standard CD treatment at the outpatient clinic.

The Statistical Package for the Social Sciences version 17 (IBM SPSS Statistics, Chicago, IL, USA) was used for data analysis. Pearson's Chi-square and Fisher's exact test were used for categorical variables and the t-test was used for continuous variables.

This study was performed in compliance with the ethics principles of the university board and those of the national committee. All patients were informed about the study and signed informed consent. The non-interventional clinical research ethics board approval number is GO 16/100 (2016).

Results

The demographics of the patients are given in Table 1 and 2. The mean age of the control group patients was 24±4.7 years and the mean age of patients in the study group was

Table 1. Demographic findings of patients in their last pregnancies (under medical treatment)

Patient	Age (years)	Gravida	Parity	Abortion	Living children	Gestational day	Birthweight (gram)	Labor type	BOI
1	18	1	1	0	1	260	2850	CS	1.31
2	21	1	1	0	1	214	1950	CS	1.31
3	24	1	1	0	1	234	2350	V	1.31
4	22	1	1	0	1	269	3100	V	1.31
5	28	1	1	0	1	274	3800	CS	1.31
6	31	1	1	0	1	229	2100	CS	1.31
CS: cesarean section; V: vaginal delivery; BOI: Beksac Obstetric Index									

Table 2. Demographic findings of patients in their last pregnancies (without any treatment)

Patient	Age (years)	Gravida	Parity	Abortion	Living children	Gestational day	Birth-weight (gram)	Labor type	BOI
1	26	3	1	2	1	Abortion	-	-	0.44
2	31	2	0	2	0	Abortion	-	-	0.16
3	26	4	1	3	1	269	2400	CS	0.33
4	36	4	1	3	1	Abortion	-	-	0.33
5	41	6	2	4	1	256	2650	CS	0.22
6	28	2	0	2	0	Abortion	-	-	0.16
7	27	3	2	1	2	253	2200	CS	0.77
8	39	3	1	2	0	190	1800*	V	0.11
CS: cesarean section; V: vaginal delivery; BOI: Beksac Obstetrics Index									

31.7±6 years (p=0.448). The control group consisted solely of primigravid patients. They were known to have CD before their pregnancies, referred to obstetricians early in their pregnancies, and precautions were taken early to ensure a successful outcome. All patients referred from other clinics were multigravida patients and almost all of them had previous abortions. Half of this group's pregnancies ended with abortion. Half of the pregnancies referred from other medical institutions ended with abortion. The mean gestational day of birth was 259.3±8.5 in the study group and 246.6±24.3 in the control group. There were no statistically significant differences (p=0.697).

Birthweights were similar in both groups. Mean birthweight in control group was 2691±698 and 2262±359 in study group (p=0.394).

Four of six patients in the control group and three of four patients in non-treatment group gave birth via cesarean section. There were no statistically significant difference regarding their delivery routes (p=0.118).

The patients' obstetrics risk levels were evaluated using the BOI. The BOIs of the entire control group was 1.31 because they were all primigravida patients. The mean BOI value of the non-treatment group patients was 0.31±0.21. This difference was statistically significant (p=0.001).

Discussion

CD is an autoimmune small intestinal enteropathy, which is activated by dietary gluten (cereal prolamins) and its incidence is about 1% (1, 3). Increased risk of pregnancy failure and obstetric complications has been reported in patients with CD (1, 4, 5). It has been reported that up to 50% of women with untreated CD have a history of miscarriage and other unfavorable pregnancy outcomes, which is similar to our findings (11). Untreated patients with CD also have a higher risk of developing intrauterine growth retardation, low birthweight, stillbirth, pre-term birth, and small-for-gestational-age babies compared with pregnancies with treated CD (11, 12). In our study, there were 50% and 75% preterm deliveries in the control and study groups, respectively.

Anti-TG2 autoantibodies were reported to be the main source of placenta-specific inflammatory process in patients with CD, which resulted in intrauterine hypoxia and impaired fetal perfusion (6, 7). Impaired apoptosis of syncytiotrophoblasts and disturbed endometrial receptivity by circulating anti-TG2 antibodies seems to be the reason for these implantation and placentation disorders (1, 8, 9).

In our small series, we demonstrated that patients with active CD who were not on a proper gluten-free diet experienced poor pregnancy outcomes, and their BOIs were statistically significantly lower than patients with CD on a gluten-free diet. It has been reported that anti-TG2 plasma levels decreased when CD went into complete remission with a gluten-free diet (13). Control of circulating anti-TG2 antibodies should be the goal during perinatal surveillance. This may be a rationale in for prophylactic use of LDCs in certain cases, especially when the complement system is activated.

On the other hand, the rising prevalence of venous thromboembolism among patients with inflammatory bowel disease and autoimmune diseases should be our concern during the management of these pathologies (14). The importance of prophylactic low-dose low- molecular-weight heparin use in diseases such as CD in necessary cases lies in the following: anti-TG2 antibodies bind to human endometrial endothelial cells and impair endometrial angiogenesis by inhibiting the activation of matrix metalloprotease-2 (MMP-2) activity (1, 8). Thus, these biologic changes may be responsible for the induction of venous thromboembolic events. The other possible mechanism for the endothelial injury of vascular structures around the materno-fetal interface (chorionic villae) is the direct attack of anti-TG2 antibodies on endothelial cells of spiral veins, together with endovascular trophoblasts covering and occluding the tip of spiral arteries, which are the opening to the intervillous space, and the syncytiotrophoblasts that cover the outer surface of chorionic villae. Autoimmune antibody positivity should be taken into consideration as a risk factor for poor pregnancy outcome (15).

The final goal should be the elimination of anti-TG2 antibodies through dietary precautions and/or suppression of antibodies by the preventive use of LDCs in necessary cases, especially when the complement system is activated. Endothelial injury of vascular structures should be eliminated. Low-dose LMWH might be critical in cases with antithrombin III and activated protein-C activity changes. Elimination of thrombus formation is also critical to prevent secondary activation of the complement system, which itself may also give harm to surrounding tissues. In our series, we used low-dose LMWH in 5 of the 6 CD patients in the control group (patients with CD under long-term follow-up) due to active protein-C and antithrombin III activity changes. All of these patients delivered successfully without perinatal mortality and severe morbidity. Three of these patients had preterm deliveries without important neonatal complications.

We must also remember that the destruction of chorionic villae by these toxic materials (anti-TG2 antibodies, cell degregades of endothelial cells of spiral veins and complement system proteins) will result in the release of fetal cell degregades (syncytiotrophoblasts) into the maternal circulation and cause a graft-versus-host-like inflammatory process in the placenta. All these patho-biologic events are most probably the reason of impaired fetal perfusion and hypoxia, and these might be the reason of increased obstetric complications such

as miscarriage, intrauterine growth retardations, preterm deliveries, and possibly preeclampsia in patients with CD, as observed in the uncontrolled patients with CD of our clinical series. We believe that patient-specific individualized management is essential in pregnancies with CD and dietary control is necessary to provide better pregnancy outcomes. LMWH and LDC seem to be helpful in the management of pregnancies with CD.

Further studies are necessary in this field to understand CD in pregnancy. This study is limited by the low number of patients.

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References

1. Di Simone N, De Spirito M, Di Nicuolo F, Tersigni C, Castellani R, Silano M, et al. Potential new mechanisms of placental damage in celiac disease: anti-transglutaminase antibodies impair human endometrial angiogenesis. Biol Reprod 2013; 89: 88.
2. West J, Logan RF, Hill PG, Lloyd A, Lewis S, Hubbard R, et al. Seroprevalence, correlates, and characteristics of undetected coeliac disease in England. Gut 2003; 52: 960-5.
3. Tatar G, Elsurer R, Simsek H, Balaban YH, Hascelik G, Ozcebe OI, et al. Screening of tissue transglutaminase antibody in healthy blood donors for celiac disease screening in the Turkish population. Dig Dis Sci 2004; 49: 1479-84.
4. Khashan AS, Henriksen TB, Mortensen PB, McNamee R, McCarthy FP, Pedersen MG, et al. The impact of maternal celiac disease on birthweight and preterm birth: a Danish population-based cohort study. Hum Reprod 2010; 25: 528-34.
5. Ciacci C, Cirillo M, Auriemma G, Di Dato G, Sabbatini F, Mazzacca G. Celiac disease and pregnancy outcome. Am J Gastroenterol 1996; 91: 718-22.
6. Sollid LM, Jabri B. Is celiac disease an autoimmune disorder? Current opinion in immunol 2005; 17: 595-600.
7. Di Simone N, Silano M, Castellani R, Di Nicuolo F, D'Alessio MC, Franceschi F, et al. Anti-tissue transglutaminase antibodies from celiac patients are responsible for trophoblast damage via apoptosis in vitro. Am J Gastroenterol 2010; 105: 2254-61.
8. Myrsky E, Kaukinen K, Syrjanen M, Korponay-Szabo IR, Maki M, Lindfors K. Coeliac disease-specific autoantibodies targeted against transglutaminase 2 disturb angiogenesis. Clin Exp Immunol 2008; 152: 111-9.
9. Anjum N, Baker PN, Robinson NJ, Aplin JD. Maternal celiac disease autoantibodies bind directly to syncytiotrophoblast and inhibit placental tissue transglutaminase activity. Reprod Biol Endocrinol 2009; 7: 16.
10. Beksac MS, Aydın E, Turgal M, Karaagaoglu E. An Obstetrics Index for the Assessment of Risk Levels of "High Risk Pregnancy" Groups. Gynecol Obstet Reprod Med 2015; 21: 10-3.
11. Casella G, Orfanotti G, Giacomantonio L, Bella CD, Crisafulli V, Villanacci V, et al. Celiac disease and obstetrical-gynecological contribution. Gastroenterol Hepatol Bed Bench 2016; 9: 241-9.
12. Saccone G, Berghella V, Sarno L, Maruotti GM, Cetin I, Greco L, et al. Celiac disease and obstetric complications: a systematic review and metaanalysis. Am J Obstet Gynecol 2016; 214: 225-34.
13. Green PH, Cellier C. Celiac disease. N Engl J Med 2007; 357: 1731-43.
14. Nguyen GC, Sam J. Rising prevalence of venous thromboembolism and its impact on mortality among hospitalized inflammatory bowel disease patients. Am J Gastroenterol 2008; 103: 2272-80.
15. Mumusoglu S, Beksac MS, Ekiz A, Ozdemir P, Hascelik G. Does the presence of autoantibodies without autoimmune diseases and hereditary thrombophilia have an effect on recurrent pregnancy loss? J Matern Fetal Neonatal Med 2016; 29: 2352-7.

What is your diagnosis?

A gravid 2 para 1 woman aged 20 years, presented at the 35th week of her pregnancy. Her medical history was unremarkable. Prenatal evaluation was performed using a Voluson E6 with a convex volumetric transducer (RAB 6-D 2-7 MHz) probe (GE, Zipf, Austria). During an ultrasound evaluation of the fetus, a hyperechogenic mass was detected in the right adrenal gland region. The mass was 30x26x15 mm in size (Figure 1). Doppler investigation showed no vascularization in the mass. Middle cerebral artery peak systolic velocimetry measurement did not show fetal anemia. Fetal anatomic structures, echocardiography, and biometry were otherwise normal. The mass showed a minimal decrease in size in subsequent ultrasound examinations; however, the center of the mass gradually became hypoechogenic and cystic over time. What is your diagnosis?

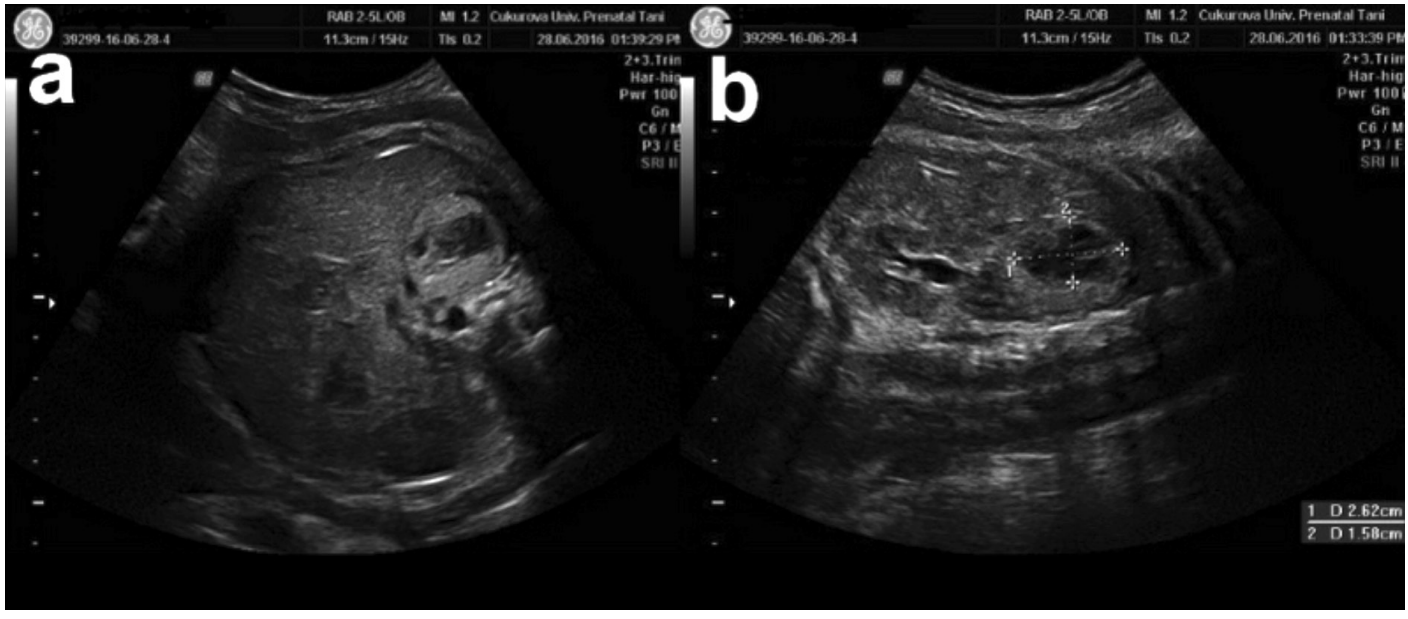

Figure 1. a, b: Axial (a) and coronal (b) view of fetal right adrenal gland is depicted. Central necrosis and hyperechogenic contour signifies bleeding into the surrenal tissue. 67x29 mm

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Answer

At 40 weeks of gestation, the woman delivered a male infant vaginally. Postnatal ultrasonographic evaluation showed a mass on the right adrenal gland. The mass was confirmed to be an adrenal hematoma during serial ultrasonographic evaluations. The newborn was otherwise noted to be healthy. The lesion spontaneously and completely regressed at the fourth month of life. During this time, no complications occurred. The newborn had no thrombocytopenia, anemia or any endocrine disorders. Adrenal gland hormone levels were normal.

The incidence of adrenal hemorrhage has been estimated to be 1.9/1000 in live births (1). The causes of fetal adrenal hemorrhage are poorly understood. The pathophysiology could be related to a sudden increase in intravascular pressure. The fetal adrenal glands are responsive to trauma and hemorrhage because of their relatively large size and vascularization (2). It has been described in association with fetal renal vein thrombosis, Beckwith-Wiedemann syndrome, and Galen vein aneurysm (3, 4). The right adrenal gland is involved in 75% of cases; the reason of this is likely due to the relatively shorter adrenal vein (5).

Prenatal ultrasound should be able to differentiate abdominal masses from adrenal masses. The differential diagnoses of fetal adrenal masses include neuroblastoma, extra lobular pulmonary sequestration, bronchogenic cysts, and adrenal cysts other than adrenal hemorrhage (6, 7). If hemorrhage becomes more complex sonographically, differentiation from neuroblastoma may be difficult. This is due to the fact that they have many similar ultrasonographic findings and also because adrenal hemorrhage could be a complication of neuroblastoma. Color flow Doppler evaluation can show blood flow in a neuroblastoma, which is always absent in a hematoma (5). A combination of ultrasound and magnetic resonance imaging (MRI) gives more definitive information to allow correct diagnosis (8). Bilateral adrenal lesions are most likely adrenal hemorrhage because bilateral adrenal tumors are very rare. In our case, we did not consider MRI because of the advanced gestational age.

The key in the diagnosis of hemorrhage is its change in sonographic appearance over time. At first, it is a solid and echogenic lesion, and then gradually the center of lesion becomes hypoechogenic and cystic, respectively. Finally, the whole lesion decreases in size and looks anechogenic. Dystrophic calcifications may also occur at the final stage (5). Most hemorrhages are reported to occur at birth or during the early neonatal period. Despite these ultrasonographic findings, the prenatal detection rate is much lower than the postnatal life (9). This is probably because adrenal glands are often omitted during prenatal evaluation. Postnatal hormone levels should be evaluated because adrenal hemorrhage may affect adrenal hormone activities (10).

When adrenal hematomas are diagnosed prenatally, patients should be counseled that these lesions can regress spontaneously and regression can even begin prenatally. Vaginal delivery is not contraindicated in these patients. However, potential complications of this situation should also be kept in mind and these patients should be encouraged to deliver in a tertiary healthcare center.

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References

1. Felc Z. Ultrasound in screening for neonatal adrenal hemorrhage. Am J Perinatol 1995; 12: 363-6.
2. Smith JA Jr, Middleton RG. Neonatal adrenal haemorrhage. J Urol 1979; 122: 674-7.
3. McCauley RG, Beckwith JB, Elias ER, Faerber EN, Prewitt LH Jr, Berdon WE. Benign hemorrhagic adrenocortical macrocysts in Beckwith-Wiedemann syndrome. AJR Am Roentgenol 1991; 157: 549-52.
4. Kütük MS, Doğanay S, Özdemir A, Görkem SB, Öztürk A. Sonographic diagnosis of fetal adrenal hemorrhage complicating a vein of Galen aneurysmal malformation. Balkan Med J 2016; 33: 112-4.
5. Houlihan C, Jampolsky M, Shilad A, Prinicipe D. Prenatal diagnosis of neuroblastoma with sonography and magnetic resonance imaging. J Ultrasound Med 2004; 23: 547-50.
6. Fang SB, Lee HC, Sheu JC, Lo ZJ, Wu BL. Prenatal sonographic detection of adrenal hemorrhage confirmed by postnatal surgery. J Clin Ultrasound 1999; 27: 206-9.
7. Izbizky G, Elias D, Gallo A, Farias P, Sod R. Prenatal diagnosis of fetal bilateral adrenal carcinoma. Ultrasound Obstet Gynecol 2005; 26: 669-71.
8. Flanagan SM, Rubesova E, Jaramillo D, Barth RA. Fetal supra renal masses- assessing the complementary role of magnetic resonance and ultrasound for diagnosis. Pediatr Radiol 2016; 46: 246-54.
9. Schrauder MG, Hammersen G, Siemer J, Goecke TW, Meurer B, Hart N, et al. Fetal adrenal haemorrhage-two-dimensional and three-dimensional imaging. Fetal Diagn Ther 2008; 23: 72-5.
10. Shin SI, Yoo JG, Park IY, Cheon JY. Prenatal diagnosis of fetal adrenal hemorrhage and endocrinologic evaluation. Obstet Gynecol Sci 2016; 59: 238-4.

CONGRESS CALENDER

INTERNATIONAL MEETINGS

(for detailed International Meeting please go website: http://www.medical.theconferencewebsite.com/conferences/obstetrics-and-gynaecology)

March 1-3, 2017	**Endo Dubai-Middle East Endoscopy Society Meeting** http://endo-dubai.com
May 4-6, 2017	**IPPS Congress, İstanbul, Turkey** www.ipps2017.org
July 2-5, 2017	**The 33rd Annual Meeting of ESHRE, Geneva, Switzerland** https://www.eshre.eu/annual-meeting
September 21-24, 2017	**The 19th World Congress on Gestational Trophoblastic Diseases, İstanbul, Turkey** www.worldcongressgtd2017istanbul.com
October 4-8, 2017	**IVF 19th World Congress on In Vitro Fertilization in conjunction with VI. Society of Reproductive Medicine and Surgery Congress, Antalya, Turkey** http://www.isivf2017.com
October 18-21, 2017	**26th ESGE/7. Ulusal JED Kongresi, Antalya, Turkey** http://jed2017.org
October 26-29, 2017	**13rd World Congress of Perinatal Medicine, Belgrade, Serbia** http://www.wcpm2017.com
October 28-November 1, 2017	**3rd Annual Meeting of the ASRM, San Antonio, USA** http://scientific.asrmcongress.org
April 27-May 01, 2018	**XII. Turkish German Gynecologic Congress** www.tajev2018.org

NATIONAL MEETINGS

March 2-5, 2017	**13. Uludağ Jinekoloji ve Obstetri Kış Kongresi, Bursa, Turkey** http://www.uludagkadindogum.org
March 15-17, 2017	**Ulusal Tıp Eğitimi Sempozyumu 2017-UTES2017, Antalya** http://kongre.teged.org/UTES2017
March 19-22, 2017	**5. Fetal Hayattan Çocukluğa "ilk 1000 gün" Gebe ve Çocuk Beslenmesi Kongresi, Ankara** http://www.annecocukbeslenmesi.org/Giris/Anasayfa
March 31-April 1, 2017	**2. Çukurova Pelvik Taban Hastalıkları Multidisipliner Yaklaşımlar Sempozyumu** http://www.pelviktaban.org
April 7-9, 2017	**5. Acıbadem Kadın Doğum Günleri** http://acibademkdg2017.org
May 11-14, 2017	**4. Klinik Embriyoloji Derneği Kongresi** http://kedkongresi2017.org
May 17-21, 2017	**15. TJOD ve 25. EBCOG Kongresi** http://www.ebcog2017.org / http://tjod2017.org
May 19-21, 2017	**CİSED 2. Ulusal Cinsel Sağlık Kongresi, Ankara** http://cised.org.tr/kongre2017/
September 28-October 1, 2017	**16. Perinatoloji Kongresi** http://www.perinatoloji2017.org
October 5-8, 2017	**5. Ulusal İşlevsel Ürolojik ve Kadın Ürolojisi Kongresi, Antalya** http://islevseluroloji2017.org/
November 2-4, 2017	**Türkiye Maternal Fetal Tıp Derneği Ultrasonografi Kursu, İstanbul** http://www.tmftpultrason2017.org

JTGGA CME/CPD CREDITING

Answer form for the article titled *"Pharmacokinetic, pharmacodynamic, and clinical aspects of ovulation induction agents: A review of the literature"* within the scope of CME/CPD

1. **Which of the following statements regarding selective estrogen receptor modulators is incorrect?**
   a) Increasing the dose of clomiphene citrate (CC) up to 250 mg daily does not have any detrimental effect on endometrial receptivity.
   b) Tamoxifen may also be utilized for ovulation induction in lie of CC.
   c) Enclomiphene is the more potent form of CC with shorter half life.
   d) Tamoxifen has a slightly higher elimination half life than CC.
   e) Raloxifene is primarily administered for treatment of osteoporosis.

2. **Choose the incorrect advantage of aromatase inhibitors over selective estrogen receptor modulators during ovulation induction.**
   a) Following oral administration their elimination half time is 2 days.
   b) Hypotalamopituitaryovarian axis remains intact during ovulation induction treatment and this advantage results with monofollicular ovulation and lower multiple pregnancy rates.
   c) Absence of hostile antiestrogenic effect of CC on endometrium and cervix is another benefit of aromatase inhibitors.
   d) When compared with CC, higher live birth and ovulation rates have been achieved with ovulation induction by using letrozole among infertile women with the polycystic ovary syndrome.
   e) When compared with CC, higher live birth and ovulation rates have been achieved with ovulation induction by using letrozole among unexplained infertile women.

3. **Which of the following statements about injectable gonadotropins is the weakest one regarding evidence based medicine?**
   a) Achievement of higher number of oocytes with lower total doses and shorter stimulation time, recombinant follicle-stimulating hormone (rFSH) has been found to be more potent than urinary FSH.
   b) In poor responders, r-hLH supplementation of r-hFSH compared with rhFSH alone may result in significantly higher oocyte number, clinical pregnancy rate and ongoing pregnancy rate.
   c) Equivalent pregnancy rates and ovarian hyperstimulation syndrome (OHSS) incidences have been found between rhCG or rhLH and urinary human chorionic gonadotropin (uhCG) when used for final follicular maturation in in vitro fertilization.
   d) Recombinant gonadotropins seem to be more advantageous regarding pregnancy rates when compared with urinary derived gonadotropins.
   e) Urinary derived products consist a mixture of the gonadotropins with unpredictable clinical efficiencies and biologically active mediators like binding proteins, growth factors and prion proteins.

4. **Select the inconvenient clinical situation for administration of gonadotropin-releasing hormone (GnRH) agonists to trigger ovulation.**
   a) Expectation of >25 mature follicles during ovulation induction.
   b) Oocyte donors.
   c) Women demanding to freeze autolog oocytes for fertility preservation.
   d) Women proceeding with fresh embryo transfer accompanied by conventional luteal phase support.
   e) Women with extremely high serum estradiol levels during ovulation induction.

5. **Which of the following statements is not an advantage of antagonist protocol over long agonist protocol during ovulation induction?**
   a) When compared with long GnRH agonist protocols, the antagonist protocol was associated with a wide decrease in OHSS rates.
   b) Total gonadotropin dose and duration of stimulation are significantly lower by using antagonist regimens when compared with long agonist regimen.
   c) Antagonist regimens seem to be more successful for poor responder patients when compared with long agonist regimens.
   d) Unlike long agonist protocol, possibility for utilization of GnRH agonist trigger strategy during antagonist protocol lowers OHSS risk.
   e) Antagonist regimens exert their effect on blocking premature luteinizing hormone (LH) surge more rapidly than agonist regimens.

6. **Choose the incorrect statement for different clinical conditions regarding selection of ovulation induction drugs.**
   a) Selective estrogen receptor modulators should be the first drug of choice for polycystic ovary syndrome patients.
   b) Gonadotropin regimens including LH activity seem to be more successful regarding achieved oocyte number for women older than 35 years.
   c) The clinical indications for utilization of commercially developed fixed dose (2/1) (FSH/human menopausal gonadotropin) including drugs have been exactly defined.
   d) Modification of luteal phase support following GnRH agonist triggering increases the pregnancy rates.
   e) Aromatase inhibitors should be the drug of choice for women who have a history of any cancer stimulated by estrogen.

JTGGA CME/CPD CREDITING

Questions on the article titled *"Pharmacokinetic, pharmacodynamic, and clinical aspects of ovulation induction agents: A review of the literature"* within the scope of CME/CPD

1st Question				
A	*B*	*C*	*D*	*E*

2nd Question				
A	*B*	*C*	*D*	*E*

3rd Question				
A	*B*	*C*	*D*	*E*

4th Question				
A	*B*	*C*	*D*	*E*

5th Question				
A	*B*	*C*	*D*	*E*

6th Question				
A	*B*	*C*	*D*	*E*

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

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JTGGA MANUSCRIPT 2017/1

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