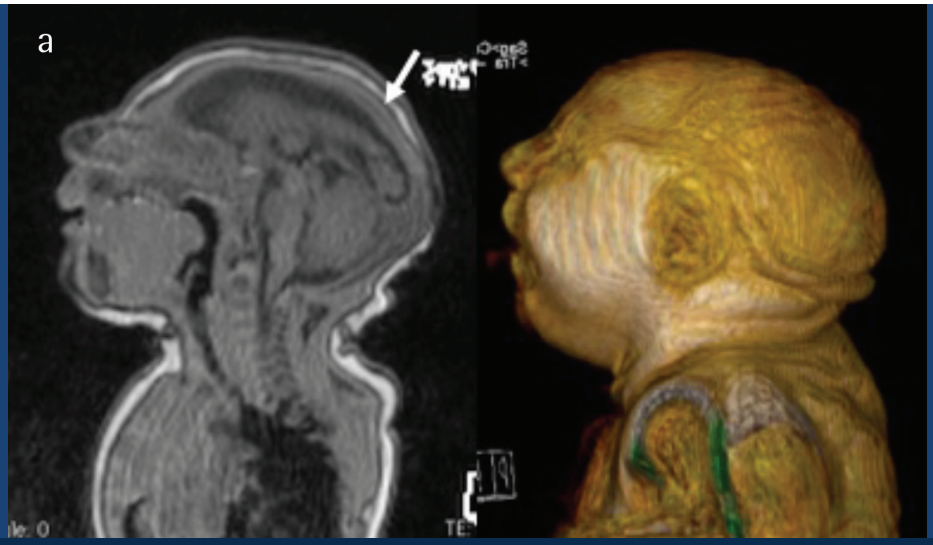
ISSN 1309-0399 • EISSN 1309-0380

TURKISH-GERMAN GYNECOLOGICAL EDUCATION and RESEARCH FOUNDATION

Journal of the Turkish-German Gynecological Association



Volume 19
Issue 1
March
2018

Cover Picture: Zika virus and pregnancy. Pereira et al. (Page 39)

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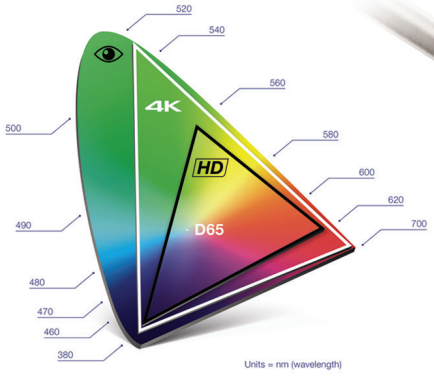

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Published by Turkish German Gynecology Education Research Foundation. / Türk Alman Jinekoloji Eğitim Araştırma ve Hizmet Vakfı tarafından yayınlanmaktadır.
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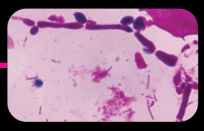
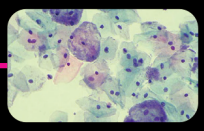
Printing at: Özgün Ofset Ticaret Ltd. Şti.
Yeşilce Mah. Aytekin Sk. No: 21 34418 4. Levent, İstanbul, Turkey
Phone: +90 (212) 280 00 09
Printing Date: March 2018
ISSN: 1309-0399 **E-ISSN:** 1309-0380
International scientific journal published quarterly.

Candida albicans



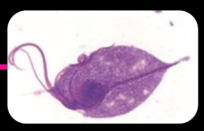
Candida albicans'ın oluşturduğu
- Kandidal vulvovajinit,

Gardnerella vaginalis



Gardnerella vaginalis ve anaerob bakterilerin oluşturduğu
- Bakteriyel vajinozis,

Trichomonas vaginalis



Trichomonas vaginalis'in oluşturduğu
- Trikomonal vajinit ve

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SEN D İYİ YAŞA!

TÜRKİYE'DE, HER 10 KADINDAN 7'SİNDE D VİTAMİNİ EKSİKLİĞİ VAR.[2]

- KADINLARDA YETERLİ DÜZEYDEKİ D VİTAMİNİ, SERUM AMH DÜZEYİNİ VE OVER RESERVLERİNİ OLUMLU YÖNDE ETKİLEMEKTEDİR.[1]
- ANOVULATUAR (YUMURTLAYAMAMA) İNFERTİLİTENİN %75'İ PCOS KAYNAKLIDIR.[4] NORMAL D VİTAMİNİ DÜZEYİ, PCOS SEMPTOMLARI ÜZERİNDE OLUMLU ETKİ YAPMAKTADIR.[3]

D VİTAMİNİ İHTİYACINIZ İÇİN HER GÜN

1 FIS YETER!

Referanslar

1- Association between serum 25-hydroxyvitamin D and ovarian reserve in premenopausal women.
Jukic AM, Steiner AZ, Baird DD.
2- Annede D vitamini eksikliği değerlendirilmesi, Cerrahpaşa Tıp Fakültesi, Taksim Eğitim Araştırma Hastanesi Çocuk Kliniği
3- Polistik Over Sendromu'nda D vitamini seviyesinin hormonal ve biyokimyasal parametrelerle ilişkisi
(Correlations of Vitamin D Level with Hormonal and Biochemical Parameters in Polycystic Ovary Syndrome)
4- Polikistik Over Sendromu Sezai Şahmay, Nil Atakul Türkiye Klinikleri J Fam Med-Special Topics 2013;4(6):56-64

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Journal of the
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Editorial

Dear Colleagues,

It is my great pleasure to meet with you again in the first issue of the Journal of the Turkish - German Gynecological Association (JTGGA) in the publishing year of 2018. I would like to remind you that the archive of our journal starting with September 2009 issue has been indexed in PubMed Central. Besides it is indexed in PubMed Central, EMBASE, Scopus, CINAHL, Gale/ Cengage Learning, EBSCO, DOAJ, ProQuest and Index Copernicus and Emerging Sources Citation Index (ESCI).

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

In this issue, we have many research articles and two good reviews. In this issue, you will read several good papers from all over the world from India to Brazil, Italy and USA. You will enjoy and educate with an interesting Quiz. One of the reviews is about Zika Virus. The recent epidemic of Zika virus (ZIKV) infection in Central and South America is one of the most serious global public health emergencies since the Ebola outbreak in West Africa. In Brazil, especially in the north, northeast, and southeast parts of the country, the ZIKV outbreak is a cause of concern for pregnant women because ZIKV intrauterine infection has been found to be associated with multiple brain malformations and microcephaly. In Brazil, the number of newborns with confirmed microcephaly per year recorded during the ZIKV outbreak, has been approximately 15 times greater than previously reported. Considering that the infection is self-limiting and symptomatic, it is usually diagnosed at the time of routine prenatal scan, especially in the third trimester. In other cases, the disease is detected after childbirth through neuroimaging. This review provides an insight into the history and evolution of ZIKV in Brazil, including current knowledge concerning the transmission, diagnosis, and pathogenesis of the infection. In addition, this review describes the pre- and postnatal neuroimaging findings obtained using ultrasound, magnetic resonance imaging, and computed tomography. Enjoy reading.

Dear Readers, Dear Colleagues,

Again, we would like to invite you to join us for our 12th Turkish - German Gynecology Congress to be held in Cyprus between April 27 and May 1, 2018. Please take note of the dates April 27th through May 1st 2018 in your calendars. Turkish - German Gynecological Education and Research Foundation has already kicked off its activities to organize its 12th Congress on these days at the Elexus Hotel and Convention Center in Kyrenia, Cyprus. On behalf of the Turkish-German Gynecological Association (TGGA), we owe a great dept of gratitude to all our colleagues who have contributed their efforts in the preparation of the congress, particularly to Congress Secretary (notably to Prof. Cem DEMİREL and Prof. Yaprak ÜSTÜN) and to the representatives of the Figure Company who have excellently undertaken the organization.

Our Congress will attract further attention and will once again provide a nice meeting occasion to all of us thanks to its recognition in the international platform of science after many years of devoted work. We kindly invite you, all our

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Editorial

distinguished colleagues to this unique scientific event. 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. The 12th congress is also being designed to host a rich scientific program and pre-congress courses, interactive surgery sessions as well as joint sessions with international societies. A pretty rich program awaits you at the congress. Now is a time for more collaboration rather than competition.

Dear Researchers,

The best 3 abstracts submitted to the Congress Scientific Committee within the scope of the XI. Turkish-German Gynaecology Congress will be rewarded with the Bayer Abstract Award. The best abstract will be rewarded with 5000 TL. As traditional, the best abstracts submitted in the field of Endoscopic Surgery will receive a 4.000 TL Cihat-Aysun ÜNLÜ reward. The aim of these awards is to appreciate the prolificacy of our colleagues in research projects and to encourage especially our young colleagues for the forthcoming years. Please do not forget to mark the congress on your calendars in order to not to miss this scientific festival.

Dear Colleagues,

The long-term success of our journal can be achieved by publications with high scientific quality. To reach this purpose, we need support from all our members and our scientists. Our Journal born in 2000 is doing very well, its already has left its childhood behind by becoming professional and reached puberty under the name of *J Turk Ger Gynecol Assoc*. On behalf of the office staff and the editorial board of the *J Turk Ger Gynecol Assoc*, we would like to extend our thanks to all of our reviewers during the past year for your outstanding contributions. I owe a great depth of gratitude to our national and international editorial board members for maintaining the continuity of our journal successfully and notably to Prof. Yaprak ÜSTÜN and Prof. Gazi YILDIRIM. The maintenance of our success will be possible by your support. We are waiting for your valuable and precious works for publishing.

On behalf of TGGF, we wish you a sunny Spring.

Sincerely,

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

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Impact of energy devices on the post-operative systemic immune response in women undergoing total laparoscopic hysterectomy for benign disease of the uterus

Kallol Kumar Roy[1], Netra GC[1], Seema Singhal[1], Juhi Bharti[1], Sunesh Kumar[1], Dipendra K. Mitra[2], Ruma Ray[3], Jyoti Meena[1], Perumal Vanamail[1]

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Abstract

Objective: Laparoscopic surgery is associated with reduced surgical stress response, lesser post- operative immune function, and consequent early recovery compared with conventional open surgery. There is a lack of evidence regarding the inflammatory stress response with the use of different energy devices. The present study was conducted to evaluate and compare the inflammatory response in total laparoscopic hysterectomy (TLH) using three different energy devices.

Material and Methods: A prospective randomized controlled study was conducted in 60 women with abnormal uterine bleeding undergoing TLH. They were divided into three groups based on the energy devices used, namely integrated bipolar and ultrasonic energy (Thunderbeat), ultrasonic (Harmonic) and electrothermal bipolar vessel sealing system (Ligasure). Cytokines and chemokines were measured in all three groups at different time points.

Results: Serum levels of interleukin (IL)-6 and tumor necrosis factor-alpha (TNF-α) increased postsurgery in all three groups and gradually declined by 72 hours. The geometric mean serum (IL)-6 levels was highest with Ligasure at 24 hours as compared with the other groups. Levels of TNF-α, macrophage inflammatory protein (MIP-1) α, MIP-1 β were also higher at 3 hours in the Ligasure group. When the differences between the groups were measured at different time points, there was a significantly greater increase in serum IL-6 levels in the Ligasure group at 24 hours ($p=0.010$). No significant difference was found in the post-operative course between the groups.

Conclusion: A greater inflammatory response was seen after the use of Ligasure indicating greater tissue damage. However, this response was not correlated with any difference in postoperative recovery. (J Turk Ger Gynecol Assoc 2018; 19: 1-6)

Keywords: Total laparoscopic hysterectomy, immune response, cytokines, chemokines

Received: 9 June, 2017 **Accepted:** 24 November, 2017

Introduction

Surgical trauma induces a stress response and leads to immunologic consequences. Surgical procedures stimulate a cascade of events that cause metabolic and inflammatory change. The extent and duration of the post-operative inflammatory response depends on the severity and type of intra-operative insult (1). Studies have reported that laparoscopic surgery was associated with reduced surgical stress response, lesser post-operative immune function, and consequent early recovery compared with conventional open surgery (2-4). Identification of markers of injury as a potential tool to predict perioperative outcomes remains an area of interest among researchers. Cytokines and chemokines are low-molecular-weight proteins produced by cells in the immune system in response to a variety of stimuli. They stimulate a cascade of

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Journal of the Turkish-German Gynecological Association published by Galenos Publishing House.
DOI: 10.4274/jtgga.2017.0076

events including the production and growth of lymphocytes, which in turn regulate the inflammatory response to surgical injury, affecting wound healing. Therefore, cytokines and chemokines serve as markers of operative stress response (5,6). It is imperative to understand the cytokine response to surgical trauma and the translation of this physiologic response into therapeutics is likely to optimize peri-operative care.

In endoscopic gynecologic surgery, the use of energy-based tissue sealing and cutting instruments has greatly advanced and facilitated complex laparoscopic procedures. Currently, various electrosurgical devices are commercially available such as advanced bipolar or ultrasonic devices (7,8). Due to the limited number of studies in the literature, there is insufficient evidence to recommend one energy source over another.

At present, there is lack of evidence regarding the inflammatory stress response with the use of different devices in cases of total laparoscopic hysterectomy (TLH). The present study was conducted to evaluate and compare the inflammatory response in terms of cytokines and chemokines in TLH using an integrated bipolar and ultrasonic energy device (Thunderbeat™, Olympus, Japan), ultrasonic energy device (Harmonic, Ethicon Endosurgery, USA), and electrothermal bipolar vessel sealing system (Ligasure™ V, Covidien, USA), which could explain a possible early recovery benefit of one energy source over another.

Material and Methods

A prospective randomized controlled study (RCT) was conducted in the Department of Obstetrics and Gynecology over a period of one year (March 2015-April 2016), at the All India Institute of Medical Sciences, New Delhi. Ethical clearance from the Institutional Review Board was taken prior to commencement of the study and informed consent was obtained from all the participants. Sixty patients with abnormal uterine bleeding due to benign uterine diseases that was not responsive to medical therapy were recruited. Patients who had body mass index (BMI) of 20-30 kg/m^2, uterine weight up to 300 grams, and uterine enlargement corresponding to 10 weeks gravid uterus were included in the study. Women with pelvic inflammatory disease, malignancy, more than one previous abdominal surgery, autoimmune diseases or history of anti-inflammatory drug use in the last one month were excluded. Sixty women who underwent TLH during study period were included and divided into three groups by adopting a randomization technique; Thunderbeat (group 1), Harmonic (group 2) and Ligasure (group 3) with 20 patients in each arm. To maintain an equal number of patients at any given time of the study, the block randomization technique was adopted. Accordingly, a total of 45 patients were recruited during a period of two months. These 45 patients were divided into 5 blocks, each containing 9 patients. In each block, randomization was performed in such a way that 3 patients were allocated randomly for each procedure. During the course of the study, 15 more patients were found to be eligible during a 3-month period. This block of 15 patients was randomized into three groups such that each group contained 5 patients. Block randomization was performed using Random Allocation Software version 1.0. All surgeries were performed by the same surgeon to avoid bias. A detailed examination of all patients was performed before the procedure, which included a hemogram, liver and renal function tests, blood sugar, chest X-ray, and electrocardiogram. Pre-operative endometrial aspirate was taken in all patients to rule out malignancy. Pre-operative ultrasound (transvaginal) was performed to estimate uterine weight based on length, width, and the anteroposterior diameter of the uterus. The inflammatory response in terms of both cytokines and chemokines induced by different energy devices was measured. The cytokines that were measured included interleukins (IL-6, IL-2, IL-17), tumor necrosis factor-alpha (TNF-α), interferon gamma (IFN-γ), and chemokines included chemokine ligand 5 [regulated on activation, normal T cell expressed and secreted (RANTES)] and macrophage inflammatory proteins (MIP-1 α, MIP-1 β). Venous blood samples were collected, serum was isolated, and stored at -70 °C. Serum cytokines and chemokines were then measured using a cytometric bead array assay. All patients underwent TLH under general anesthesia. Venous samples were collected pre-operatively (a) and post operatively at 3 hours (b), 24 hours (c) and 72 hours (d) in all patients to measure inflammatory mediators. A multiplexed cytometric bead array (BD, USA) was used to determine serum levels of pro-inflammatory cytokines and chemokines. This flow cytometric bead-based technology allowed simultaneous quantitation of multiple analytes. A constant one was added uniformly to all values before converting into log values to normalize the data log-transformation to avoid zero values because the data obtained were skewed. The geometric mean of log values was calculated as follows;

Geometric mean = Antilog (average)-1

In the post-operative period, all patients received slow intravenous analgesia in the form of tramadol 1.5 mg/kg body weight, eight-hourly for 24 hours, followed by oral tramadol as and when required. Patients were assessed for the return of gastro intestinal (GI) activity at 6 hours. Post-operative pain was assessed 24 hours after surgery using a visual analog scale (VAS) with scores of 0-10, where 0 represented no pain and 10 represented the worst pain possible. An assessment was also made for febrile episodes and other complications such as wound infection and vaginal bleeding.

Statistical analysis

Data analysis was performed using the SPSS software (IBM, version 19.0). Descriptive measures such as mean, median, and standard deviation were computed for all continuous variables. A comparison of mean values between the groups was tested using one-way analysis of variance (ANOVA). Differences in serum parameters from pre-treatment to post-treatment time points were compared across the groups using the ANOVA test. Post-hoc pairwise comparison was performed using the Bonferroni test. In the event of non-normally distributed data, median values were compared using the non-parametric Mann-Whitney U test for two groups, and the Wilcoxon test for more than two groups. Frequency data by categories were compared using the chi-square/Fisher's exact test as appropriate. For all statistical tests, p values <0.05 were considered as statistically significant.

Results

The baseline characteristics of three groups were comparable with respect to age, BMI, uterine weight, indication for surgery, history of previous surgery, mean surgical duration, and length of hospital stay (Table 1). The geometric mean values of the different cytokines and chemokines were measured at various time points (preoperative, 3 hours, 24 hours, and 72 hours) and a comparison was made between the three groups. It was observed that serum levels of IL-6 peaked at 3 hours postsurgery in both Harmonic and Thunderbeat techniques and gradually declined by 72 hours, whereas the geometric mean of serum IL-6 levels was highest in the Ligasure group at 24 hours (Figure 1). TNF-α levels and RANTES peaked at 3 hours and gradually declined by 72 hours with all three energy devices (Figure 2). The highest geometric mean value of TNF-α was observed with Ligasure at three hours.

Higher and sustained values of RANTES were observed with Thunderbeat as compared with the other two methods. The geometric mean values of MIP-1 α and MIP-1 β peaked at 3 hours and gradually declined by 72 hours. The highest geometric mean value of MIP-1 α and MIP-1 β was observed in the Ligasure method (Figure 3). This indicated greater tissue damage in the Ligasure group.

To compare between the groups, differences in the geometric mean values were taken at various time points. There was a significantly higher increase in serum IL-6 levels in the Ligasure group as compared with Thunderbeat and Harmonic groups at 24 hours (p=0.010). This indicated that there was greater tissue damage in the Ligasure group. However, there was no significant difference between the three groups in terms of inflammatory response as seen in levels of TNF-α, RANTES, MIP-1 α, and MIP-1 β levels. This shows that there was equivalent tissue trauma in all three groups (Table 2). The levels of other

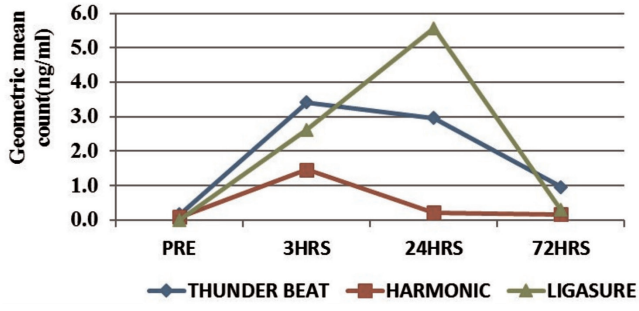

Figure 1. Geometric mean of serum interleukin-6 (ng/mL) among the three groups in the pre- and post-operative (3 hours, 24 hours, 72 hours) period

Table 1. Clinical characteristics of the patients

Variable	Group 1 (n=20)	Group 2 (n=20)	Group 3 (n=20)	p value
Age, years (mean ± SD)	42.00±7.36	44.00±8.09	42.50±5.69	0.654
BMI, kg/m^2 (mean ± SD)	25.50±2.06	24.95±2.74	25.00±2.83	0.756
Uterine weight, grams (mean ± SD)	148.50±62.09	153.50±63.60	144.50±64.60	0.904
Chief symptom, no (%)				
Menorrhagia Post-menopausal bleeding Abdominal pain Others	18 (90) 1 (5) 0 1 (5)	18 (90) 2 (10) 0 0	15 (75) 2 (10) 2 (10) 1 (5)	0.335
History of previous surgery, no (%)				
Laparotomy Laparoscopy Nil	2 (10) 2 (10) 16 (80)	2 (10) 1 (5) 17 (85)	2 (10) 3 (15) 15 (75)	0.335
Mean surgical duration (minimum)	49.50±15.97	48.80±13.84	46.50±14.05	0.785
Length of hospital stay (hours)	77.45±19.74	72.10±6.12	71.80±10.76	0.334
SD: Standard deviation; BMI: Body mass index				

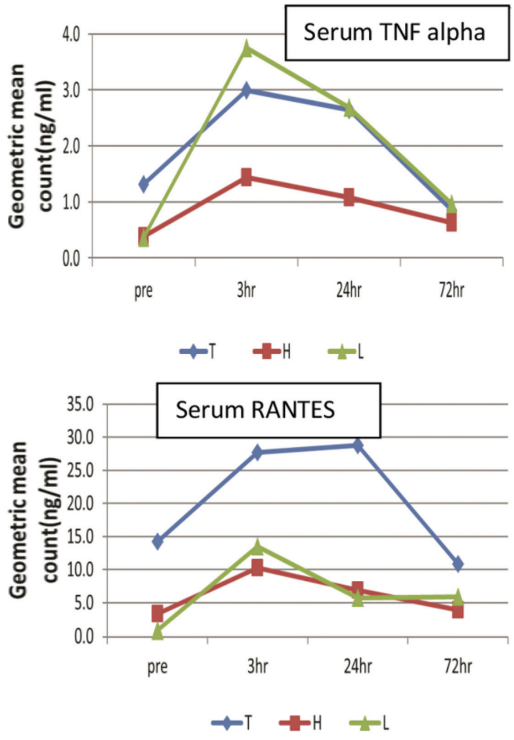

Figure 2. Geometric mean of serum tumor necrosis factor-α (ng/mL) and regulated on activation, normal T cell expressed and secreted (ng/mL) among the three groups in the pre- and post-operative (3 hours, 24 hours, 72 hours) period

T: Thunderbeat; H: Harmonic; L: Ligasure; TNF: Tumor necrosis factor; RANTES: Regulated on activation, normal T cell expressed and secreted

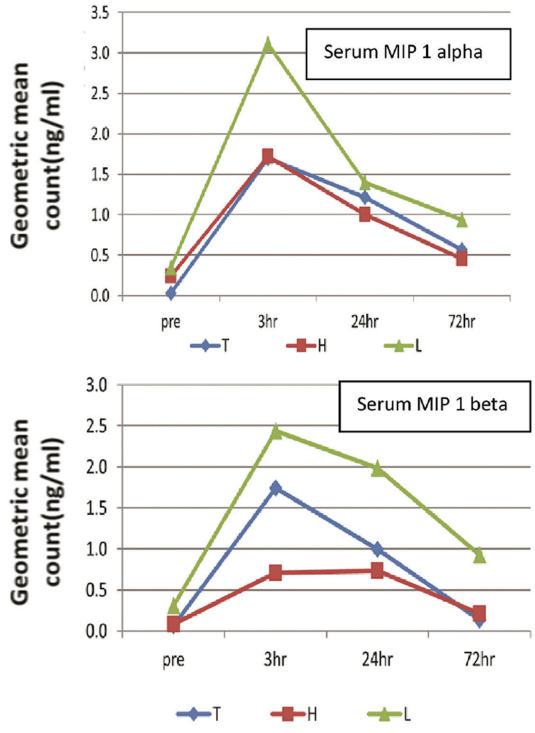

Figure 3. Geometric mean of serum macrophage inflammatory protein-1 α (ng/mL) and macrophage inflammatory protein-1 β (ng/mL) among the three groups in the pre- and post-operative (3 hours, 24 hours, 72 hours) period

T: Thunderbeat; H: Harmonic; L: Ligasure; MIP: Macrophage inflammatory protein

Table 2. Comparison of cytokines and chemokines between patients in the three groups

		Group 1	Group 2	Group 3	p value
Difference in IL-6 levels (ng/mL)	a-b	1.608	1.94	2.68	0.215
	a-c	0.985	0.925	3.10	0.010
	a-d	0.194	0.176	0.573	0.513
Difference in TNF-alpha levels (ng/mL)	a-b	0.236	0.245	0.544	0.231
	a-c	0.196	0.175	0.433	0.346
	a-d	0.098	0.070	0.162	0.443
Difference in RANTES levels (ng/mL)	a-b	0.274	0.411	0.886	0.274
	a-c	0.289	0.257	0.553	0.657
	a-d	0.109	0.530	0.565	0.485
Difference in MIP-1 alpha levels (ng/mL)	a-b	0.483	0.250	0.157	0.796
	a-c	0.341	0.208	0.069	0.778
	a-d	0.419	0.333	0.183	0.746
Difference in MIP-1 beta levels (ng/mL)	a-b	0.419	0.199	0.412	0.533
	a-c	0.358	0.205	0.274	0.648
	a-d	0.167	0.048	0.648	0.240

a: Preoperative serum levels; b: Serum values 3 hours post-operative; c: Serum values 24 hour postoperative; d: Serum values 72 hour postoperative; MIP: Macrophage inflammatory protein; IL: Interleukin; TNF: Tumor necrosis factor; RANTES: Regulated on activation, normal T cell expressed and secreted

markers (IL-2, IL-17 and IFN-γ) did not rise at the chosen time points postsurgery. Therefore, data could not be pooled for analysis.

The inflammatory response in the three groups was then analyzed in terms of clinical post-operative recovery. No significant difference was observed between the three groups in terms of return of GI activity at 6 hours [group 1: 17 (85%) vs. group 2: 18 (90%) vs. group 3: 16 (80%); p=0.676], fever [group 1: 0% vs. group 2: 1 (5%) vs. group 3: 3 (13%); p=0.153] and mean VAS score (group 1: 3.60±0.94 vs. group 2: 3.35±0.87 vs. group 3: 3.60±0.94; p=0.613).

To summarize, each device lead to some amount of inflammatory response in terms of a rise in cytokines and chemokines in the immediate post-operative period; however, Ligasure produced a more sustained and greater inflammatory response at 24 hours postsurgery. However, this rise was not associated with any difference in the post-operative course.

Discussion

Surgical trauma leads to an acute-phase response, mediated by cytokines, which are signaling peptides. Cytokines recruit reticuloendothelial cells (lymphocytes, monocytes and macrophages) and induce the production of chemokines to amplify the response. In turn, these mediators participate in the process of angiogenesis and wound repair (9,10). Cytokines balance the inflammatory and anti-inflammatory effects and an uncontrolled production of inflammatory cytokines can result in delayed recovery after surgery. Excessive cytokines also cause insulin resistance through a complex immunophysiologic response to surgery (5). Various cytokines such as IL-2, IL-6, IL-17, IFN-γ, and TNF-α, and chemokines such as RANTES, and MIP-1 α and MIP-1 β are released by activated macrophages during surgery. The hypothesis of enhancing postoperative recovery after surgery is partially based on limiting the release of cytokines from tissue injury (5,6).

Minimally invasive surgery is associated with reduced inflammatory response, and better preserved immune competence and recovery benefits in the post-operative period as compared with open surgery (2,3). Fretland et al. (4) compared the stress immune response in cases of laparoscopic and open resection of colorectal liver metastasis. The authors found significantly lower levels of inflammatory mediators [HMGB-1, cfDNA, IL-6, C-reactive protein (CRP), and MIP-1 β] (p<0.05) in the laparoscopy group compared with the open surgery group. Our previous randomized study compared surgical stress response after non-descent vaginal hysterectomy (NDVH) with laparoscopic-assisted vaginal hysterectomy (LAVH) in 20 women. The increment in levels of IL-6 from preoperative to 3-hour postoperative levels were analyzed and were significantly higher in the LAVH group (0.38 ng/mL) as compared with the NDVH group (0.06 ng/mL) (p=0.027). NDVH, which is associated with minimal surgical trauma and tissue handling, resulted in a lower inflammatory response (11).

The practice of endoscopy was revolutionized with the advent of modern multifunctional energy devices. Studies have evaluated the different available devices in terms of their safety, efficacy, extent of thermal injury, and versatility (7,8); however, none of the devices has been proven superior over another. In an attempt, Sietses et al. (12) compared an ultrasonic energy device with monopolar electrosurgery and analyzed the immune response evoked by each device. They measured changes in leukocyte count, CRP, and monocyte human leukocyte antigen – antigen D-related expression in cases of laparoscopic cholecystectomy. Both the ultrasonic energy device and monopolar diathermy resulted in a comparable stress immune response and therefore were found to be equally traumatic. However, it was speculated that surgical injury during lap cholecystectomy was insufficient to demonstrate a difference despite the expected advantage of the ultrasonic energy source over diathermy (12).

Our preliminary study shows novel observations regarding the differences in inflammatory response of IL-6, TNF-α, RANTES, MIP-1 α, and MIP-1 β for various energy sources and their impact on post-operative recovery in TLH. Serum levels of IL-6 at 24 hours were found to be significantly higher with Ligasure as compared with the Harmonic scalpel and Thunderbeat. This indicates that there is greater tissue trauma with Ligasure.

Of all the markers, IL-6 is the main acute-phase protein (5); its levels are an indicator of the extent of surgical trauma and thus can be considered as a predictor of morbidity after surgical intervention (4). IL-6 has been used as a parameter for the extent of inflamed and damaged tissues and higher levels have been found in cases of open surgery than in laparoscopy (6,10). Previous studies have also shown that IL-6 levels rise and peak between 3 and 24 hours after surgery and return to baseline in 3-4 days, similar to our findings (10,13,14).

Studies with other cytokines and chemokines are lacking. Although there was no significant difference for other cytokines and chemokines between the three groups, the various cytokines and chemokines rapidly peaked between 3 and 24 hours after the surgical trauma and this transient increase in soluble factors declined in blood by 72 hours.

All the patients received tramadol in the post-operative period for analgesia. It has a dual mode of action; besides being a weak opioid agonist, it inhibits uptake of serotonin and noradrenaline. It is clearly evident from previous studies that it does not influence IL levels in the post-operative period (15). Non-steroidal anti-inflammatory drugs were not used to remove the confounding factor.

We found no differences in post-operative outcomes with the use of any of the devices, despite the difference in levels of inflammatory markers. To the best of our knowledge, no prior randomized controlled trial has addressed this issue in cases of laparoscopic hysterectomy. Available studies on the comparison of post-operative outcomes with use of various energy sources in different surgeries (thyroidectomy, colectomy) have inconsistent results. The majority of studies show no difference in outcomes; however, small sample sizes and the heterogeneity of the studies are the main limitations (16).

To our knowledge, this is the first RCT to study the impact of these three energy devices on the immune system in humans. However, the small sample size remains the main constraint and any conclusion regarding the superiority of one energy source over another in terms of immune response cannot be drawn. Larger studies are required for more definitive results.

Ethics Committee Approval: *Ethics committee approval was received for this study from the ethics committee of All India Institute of Medical Sciences, New Delhi (No: RT-31/25.02.2015).*

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

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Concept - K.K.R., S.S., S.K., R.R., D.K.M.; Design - K.K.R., S.K., N.G.C., S.S., J.B., D.K.M., R.R.; Supervision - K.K.R., S.K., J.M., D.K.M., R.R.; Materials - K.K.R., S.S., N.G.C., R.R., D.K.M., J.M., P.V.; Writer - K.K.R., S.S., N.G.C., J.B., J.M.*

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

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

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Short-term results of the efficacy of percutaneous tibial nerve stimulation on urinary symptoms and its financial cost

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Abstract

Objective: Overactive bladder (OAB) affects 16.9% of women in the United States. Percutaneous tibial nerve stimulation (PTNS) is a third-line treatment for patients who are refractory to behavioral and pharmacologic therapies. We aimed to evaluate the effects of PTNS on urinary symptoms in patients diagnosed as having refractory OAB and investigate the cost of medications and clinical visits before and after PTNS treatment.

Material and Methods: We reviewed 60 women with refractory OAB treated with PTNS. Episodes of urinary frequency, leakage, urgency, and nocturia; number of follow-up visits; and medications were recorded. The mean quarterly drug, physician, nurse, and provider costs were calculated. The episodes of urinary symptoms, numbers of follow-up visits, and costs of medications and visits before and after PTNS were compared.

Results: Of the 60 patients with refractory OAB, 24 patients who completed 12 weekly sessions of initial PTNS were evaluated. The number of urinary symptoms and follow-up visits significantly decreased after PTNS ($p<0.05$). The average quarterly medication cost decreased from $656.36±292.45 to $375.51±331.79 after PTNS ($p=0.001$). After PTNS, quarterly physician and nurse visit costs decreased from $81.73±70.39 to $25.89±54.40 and from $55.23±38.32 to $15.53±19.58, respectively ($p<0.05$). The quarterly total provider cost was similar before and after PTNS.

Conclusion: PTNS treatment significantly improved urinary symptoms of patients with refractory OAB and reduced the costs of medications and physician and nurse visits. (J Turk Ger Gynecol Assoc 2018; 19: 7-10)

Keywords: Transcutaneous electric nerve stimulation, tibial nerve, urinary bladder, overactive, costs and cost analysis, lower urinary tract symptoms, office visits

Received: 27 September, 2017 **Accepted:** 20 December, 2017

Introduction

Overactive bladder (OAB) syndrome is defined as "urgency, with or without urge incontinence, usually with frequency and nocturia in the absence of proven infection or other obvious pathology" by the International Continence Society (1). The prevalence of OAB varies between 12.8% and 17.4% and increases with age for both sexes (2,3). In the United States (US), its prevalence is 16.0% in men and 16.9% in women, and 29.8 million adults aged ≥40 years are estimated to have OAB symptoms (4,5).

The American Urological Association and the Society of Urodynamics, Female Pelvic Medicine and Urogenital Reconstruction suggest that the first-line treatment should be composed of diet and behavioral therapies such as reducing caffeine, alcohol, and fluid intake; weight loss; pelvic floor physical therapy; timed voiding; and bladder training. Antimuscarinic therapies may be added to behavioral therapies at the same time. Second-line treatment should include pharmacologic agents, antimuscarinics or oral β3-adrenoceptor agonists for a minimum of 3 months. Adverse effects of medication or inadequate symptom relief may lead

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Journal of the Turkish-German Gynecological Association published by Galenos Publishing House.
DOI: 10.4274/jtgga.2017.0115

to discontinuation of pharmacologic therapy. Intradetrusor botulinum toxin A, percutaneous tibial nerve stimulation (PTNS), and sacral nerve stimulation (SNS) may be recommended as third-line therapies to patients who are refractory to behavioral and pharmacologic therapies (6).

PTNS is the least invasive form of neuromodulation, which uses electrical stimulation to afferent fibers of the posterior tibial nerve (L4-S3) through a needle electrode inserted 3-5 cm cephalad to the medial malleolus. The needle is connected to a device that provides 0.5-9 mA electrical stimulation at 20 Hz. A grounding pad is placed on the bottom of the foot. Nerve stimulation is confirmed by flexion of the big toe and sensory stimulation on the bottom of the foot is created. The exact mechanism of PTNS is unknown. However, it seems that afferent nerve stimulation leads to activation of inhibitory sympathetic neurons through a direct sacral route and modulates the efferent outflow to the lower urinary tract. Treatment is composed of 12 weekly stimulation sessions lasting 30 minutes each. After this initial treatment, sessions may be tapered or maintained according to symptom improvement (7).

The aim of the present study was to evaluate the effects of PTNS on urinary symptoms in patients diagnosed as having refractory OAB and to analyze the cost of medications and clinical visits before and after PTNS treatment.

Material and Methods

In this retrospective study, we reviewed patients who were diagnosed as having refractory OAB and treated with PTNS in the Urogynecology Clinic of the University of Texas Medical Branch, a tertiary-care center in Galveston, Texas. Sixty patients who were diagnosed as having OAB and who were resistant to treatment with anticholinergic medications and behavioral modification participated in this study. Forty-six of the 60 patients had undergone at least 1 PTNS session. Twenty-four had completed 12 weekly PTNS sessions. The weekly stimulation sessions lasted 30 minutes and were performed in an outpatient setting. We included women who were aged 21-85 years, who were diagnosed with refractory OAB, and who had completed 12 weekly PTNS sessions. Pregnant women, those with cancer, pacemaker users, Texas Department of Criminal Justice inmates, women who had urinary retention or urinary tract obstruction, and those whose symptoms were suspected to be neurologic or inflammatory in origin were excluded. The study was approved by the Institutional Review Board (IRB: 16-0219).

First, all patients undergoing PTNS treatment were identified using the current procedural terminology code 64566, which is a medical procedural code under the category - neurostimulator procedures on peripheral nerves. As maintained by the American Medical Association, the procedure was billed under this code, with the descriptor "posterior tibial neurostimulation, percutaneous needle electrode, single treatment, including programming". Then age; body mass index (BMI); episodes of urinary frequency, urgency, nocturia, and incontinence; and numbers of physician and nurse visits were obtained from electronic medical records. Medications used and clinic visits were reviewed within the year before and the year after PTNS treatment. Financial data included the costs of physicians, nurses, and medications. We calculated the costs of visits for physicians and nurses by using plans of Medicare, a US national social insurance program.

Finally, we compared the episodes of urinary symptoms and numbers of follow-up visits and analyzed the difference in the cost of the medications and visits before and after PTNS treatment.

Statistical analysis

Descriptive statistics for continuous variables are presented as mean ± standard deviation, and categorical variables are presented as numbers and percentages. We performed statistical analysis by applying the Wilcoxon signed-rank test and paired samples t-test for nonparametric and parametric sample comparison for treatments before and after PTNS, respectively. The results were analyzed with a 95% confidence interval and $p<0.05$ was regarded as statistically significant. For the statistical analysis, we used SPSS version 16.0 (SPSS Inc., Chicago, IL, USA).

Results

A total of 60 patients with a diagnosis of refractory OAB according to the International Continence Society were reviewed. Of the 60 patients, 6 were treated with intradetrusor botulinum toxin A (Botox®) injection, 7 were treated with SNS, 46 underwent at least 1 PTNS session, and 1 patient declined the third-line treatment. Of the 46 patients, 24 met the inclusion criteria of the study (Figure 1).

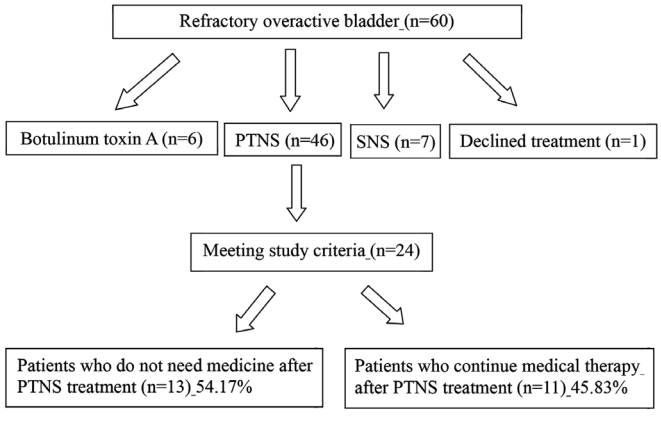

Figure 1. Flow of patients through the trial
PTNS: Percutaneous tibial nerve stimulation; SNS: Sacral nerve stimulation

The mean age of the patients was 70.25±11.14 years. The mean BMI was 30.46±6.86 kg/m^2. After initial PTNS therapy, 54.17% of the patients required no pharmacotherapy. The number of urgency episodes decreased from 3.12 to 1.79 (p=0.001), frequency episodes from 7.29 to 5.58 (p=0.002), leakage episodes from 2.62 to 1.33 (p=0.002), and nocturia episodes from 3.33 to 2.17 (p=0.01) (Table 1). The mean number of follow-up visits decreased from 1.34±0.78 to 0.40±0.63 after PTNS (p=0.001). After PTNS treatment, quarterly medication, nurse, and physician costs significantly decreased (Table 2). The quarterly total provider cost was calculated by adding the cost of follow-up visits to the cost of PTNS maintenance visits. The quarterly total provider cost was similar before and after PTNS treatment (p=0.143). However, the total provider cost for the last quarter decreased from $169.04±103.69 to $55.14±94.62 (p=0.005).

Table 1. Changes in urinary symptom episodes of patients with refractory overactive bladder

	Before PTNS*	After PTNS**	p
Urgency	3.12±1.19 4 (1-4)	1.79±1.21 2 (0-4)	0.001
Frequency	7.29±2.15 7 (3-12)	5.58±1.90 5 (2-9)	0.002
Leakage	2.62±2.41 2 (0-8)	1.33±1.61 1 (0-5)	0.002
Nocturia	3.33±1.81 3 (0-7)	2.17±2.06 2 (0-10)	0.01

PTNS: Percutaneous tibial nerve stimulation; *: At last visit just before PTNS treatment; **: At completion of 12 weekly sessions of initial PTNS treatment
Results are expressed as mean ± standard deviation, median (minimum-maximum)

Table 2. Comparison of quarterly costs before and after percutaneous tibial nerve stimulation treatment

	Before PTNS	After PTNS	p
Drug ($)	656.36±292.45	375.51±331.79	0.001
Physician visit ($)	81.73±70.39	25.89±54.40	0.004
Nurse visit ($)	55.23±38.32	15.53±19.58	0.001
Provider ($)	136.96±81.50	181.04±146.34*	0.143

PTNS: Percutaneous tibial nerve stimulation; *: With cost of maintenance PTNS session
Results are expressed as mean ± standard deviation

Discussion

The results of our study showed that urinary symptoms were improved and the costs of medications and physician and nurse visits were reduced significantly in patients with refractory OAB who were treated with PTNS.

OAB syndrome is composed of symptoms that have significant effects on quality of life. Although urinary frequency, urgency, and nocturia are common symptoms, the main symptom is urgency (8). Patients are in fear of needing to urinate or urinary incontinence. From the patient's perspective, OAB is a chronic and feared condition due to these symptoms; they feel boxed in and embarrassed (9).

PTNS is a third-line treatment for patients with refractory OAB. This therapy is effective in the alleviation of lower urinary tract symptoms such as incontinence, urgency, and frequency by electrical stimulation to afferent fibers of the posterior tibial nerve to the sacral center of micturition (6). Congregado Ruiz et al. (10) showed that leakage and daytime and nighttime frequency episodes were significantly improved after PTNS treatment (10). van Balken et al. (11) also found a positive response with PTNS therapy in terms of leakage episodes, voiding frequency, and nocturia. The Study of Urgent PC vs Sham Effectiveness in Treatment of Overactive Bladder Symptoms (SUmiT) trial, which was a double-blind, sham-controlled randomized trial, demonstrated the superiority of PTNS compared with sham groups. It reported moderate or marked improvement in bladder symptoms (54.5% in the PTNS-treated patients and 20.9% in the sham group). The study showed that PTNS reduced the number of voids, urgency, and incontinence episodes per day (12). We also found that number of urgency, frequency, leakage, and nocturia episodes significantly improved after PTNS treatment, similar to the literature.

The estimated health care cost of OAB in the USA is more than $65 billion per year (13). The total medical cost of patients with OAB receiving antimuscarinic therapy is estimated to be approximately $2000 per year (14). In our study, quarterly drug costs fell by almost half (from $656.36±292.45 to $375.51±331.79) after PTNS treatment. We also found a significant reduction in physician and nurse visit costs as a result of the decrease in the number of follow-up visits. Although quarterly total provider costs, including cost of follow-up visits and PTNS maintenance visits, were statistically similar before and after PTNS (p=0.143), the last quarter cost was significantly decreased from $169.04±103.69 to $55.14±94.62 (p=0.005). We concluded that after the initial 12 weeks of PTNS treatment, diminishing requirements for ongoing therapy towards the end of treatment led to the decrease in the last quarter cost. To the best of knowledge, there are no pure cost analysis studies of PTNS in patients with refractory OAB syndrome in the literature. Staskin et al. (15) reviewed the costs and effectiveness of PTNS in the current treatment of OAB. The authors recommended PTNS as an efficacious, less costly, and less invasive therapy compared with SNS for patients with OAB and refractory to antimuscarinic therapy. Although our study is

also not an exact cost analysis of PTNS, we noted a reduction in the costs of medications and physician and nurse visits after PTNS treatment.

Our study has some limitations due to its retrospective design. However, the recall bias risk is low because all retrospective data related to the patient treatments and costs were objective data obtained from electronic patient files with no need for recall. Secondly, the size of the study is too small and follow-up times of the patients are relatively short for the assessment of long-term outcomes of PTNS treatment in patients with refractory OAB. On the other hand, the strength of our study is that it adds new results to the current literature on the changes in urinary symptoms and various costs of PTNS treatment in these patients.

In conclusion, PTNS therapy improves urgency, frequency, nocturia, and leakage episodes in patients with refractory OAB in the short term. This treatment also reduces the costs of medications and physician and nurse visits in the follow-up of these patients.

Ethics Committee Approval: *Ethics committee approval was received for this study from the Ethics Committee of University of Texas Medical Branch (IRB: 16-0219).*

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

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Concept - Z.K., G.S.K.; Design - Z.K., S.Ü., G.S.K..; Supervision - S.Ü., G.S.K.; Materials - D.C., J.H., Writer - Z.K.*

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

Financial Disclosure: *TGGF has provided financial support for this study.*

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The comparison of the degree of apoptosis in ovaries and fallopian tubes between two different surgical interventions for tubal ligation: A rat model

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Abstract

Objective: To compare the degree of apoptosis in ovaries and tubal epithelium observed secondary to tubal ligation either by Pomeroy's method or bipolar electrocauterization in a rat model.

Material and Methods: A total of 24 female Sprague-Dawley rats were randomly assigned into 3 study groups: control (n=8), Pomeroy (n=8), and the electrocauterization group (n=8). Apoptotic cells were detected on the primary, secondary, tertiary follicles of the ovaries, and on the tubal epithelium using terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end-labeling. The apoptotic index was calculated for each group by the percentage of the stained cells.

Results: The apoptotic index of tubal epithelium was significantly higher in the bipolar electrocauterization group compared with the control and Pomeroy groups (3.1±0.8 vs. 1.4±1.0, p=0.018 and 2.0±1.2, p=0.03, respectively) whereas there was no significant difference between Pomeroy's method and the control group. The apoptotic index of primary follicles was higher in the bipolar electrocauterization group compared with the control and Pomeroy's method groups (3.4±0.5 vs. 1.2±0.4, p<0.001 and 1.8±0.8, p=0.005, respectively), but there was no significant difference between Pomeroy's method and the control group. The apoptotic index of secondary and tertiary follicles was similar for each group.

Conclusion: Pomeroy's technique, as a permanent contraception method, is associated with lower apoptotic index on ovary and fallopian tube when compared with bipolar electrocauterization. (J Turk Ger Gynecol Assoc 2018; 19: 11-6)

Keywords: Tubal ligation, bipolar electrocauterization, Pomeroy, ovarian reserve, fallopian tube

Received: 5 July, 2017 **Accepted:** 7 December, 2017

Introduction

Tubal sterilization is a widely used contraceptive procedure with a high efficacy. More than 190 million women around the globe have elected to undergo surgical sterilization as a safe and reliable method of permanent contraception, and it is the second most preferred method in the United States (1).

Despite the advantages of permanent contraception, some concerns have been raised about its complications. Tubal sterilization has been accused of several adverse effects such as irregular menstrual cycles, dysmenorrhea, and climacteric symptoms (2).

The possible explanation of these complications was initially hypothesized as the disturbed vascularization of the ovaries, which subsequently interfered with the ovarian cycle and metabolism (3). There are several studies in the literature reporting the effect of tubal sterilization on ovarian function, follicular development, hormonal levels, and ovarian blood supply (4,5). However, there is still no consensus as to whether changes in ovarian blood flow due to tubal ligation cause

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Journal of the Turkish-German Gynecological Association published by Galenos Publishing House.
DOI: 10.4274/jtgga.2016.0192

damage in ovaries and fallopian tubes. There is a lack of evidence at this time to understand whether women undergoing sterilization will experience an earlier onset of menopause. In addition to these uncertainties about the effect of tubal ligation on ovarian and tubal blood flow, it is also unknown whether the type of surgical technique per se plays role in disturbed ovarian vascularization and ovarian reserve.

The aims of the current study were two-fold; i) to compare the apoptotic index of ovarian follicles between Pomeroy's technique and bipolar electrocauterization, ii) to compare the apoptotic index of the tubal epithelium between these techniques.

Material and Methods

Ethical statement

The Animal Research Ethics Committee of Hacettepe University approved the study protocol by (Approval no: 2012/16). The "Principles of laboratory animal care" (NIH publication no. 86-23, revised 1985) and specific national laws were followed throughout the study.

Study design and surgical procedure

A total of 24 female 16-week-old adult, non-pregnant Sprague-Dawley rats, weighing between 250 and 300 g were included in this experimental study. All animals were provided by Hacettepe University, Animal Research Center, and they were randomly divided into 3 experimental groups; control (n=8), Pomeroy's method (n=8), and the bipolar electrocauterization group (n=8). All rats were kept in a positive pressure room, which was equipped with high-efficiency particulate air filter, in filter-topped cages. Unlimited access to food and water was provided during the 12-hour light-dark conditions.

Anesthesia was obtained via intraperitoneal administration of ketamine hydrochloride 75 mg/kg (Ketalar; Eczacıbaşı, İstanbul, Turkey) and xylazine hydrochloride 10 mg/kg (Rompun; Bayer Türk İlaç Ltd., İstanbul, Turkey). The abdominal wall was cleaned with povidone-iodine (Baticon; Drogsan, Turkey) after being shaved. Surgery was performed on a 37 °C heated warming plate after covering the surgical site with a sterile towel, and laparotomies were performed through a midline incision.

No intervention was made on the control group. In the Pomeroy's method group, rats received bilateral tubal ligation with Pomeroy's technique; after elevating the tube, the loop was ligated with a 2/0 Vicryl suture (Johnson & Johnson, USA) 2 cm away from the ovary (6). In the bipolar electrocauterization group, the tube was cauterized by bipolar electrocoagulation 2 cm away from the ovary and interrupted via surgical scissors. After the procedures, the abdominal wall was closed layer-to-layer with 2/0 Vicryl suture (Johnson & Johnson, USA) in each group.

During the recovery period after the first surgery, all animals were kept under the above-mentioned conditions in the same animal research laboratory at Hacettepe University. Fifteen days after the first surgery, a second laparotomy was performed to each rat under intraperitoneal anesthesia and bilateral oophorectomy and salpingectomy was performed within a mean surgical time of 10 minutes. After completing the second surgical procedure, all rats were sacrificed via cervical dislocation. In each group, apoptotic cells on the primary, secondary, and tertiary follicles of ovaries and on the tubal epithelium were stained using terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine triphosphate nick end-labeling (TUNEL).

Histology and TUNEL assay

Standard paraffin wax embedding was used. Specimens were fixed in 10% buffered formaldehyde, dehydrated in a graded ethanol series, cleared in xylene, and embedded in paraffin wax. The paraffin blocks were sectioned using a sledge microtome (Leica Microsystems, Germany) with 5-μm thickness.

Apoptosis of follicular cells and tubal epithelial cells was assessed using enzymatic labeling of DNA-strand breaks with a TdT-mediated TUNEL assay with a Cell Death Detection Kit (Roche, cat no: 11 684 817910) in accordance with the manufacturer's instructions. In this technique, the TdT binds to the 3'-OH end and synthesizes a polynucleotide at the nick end. The biotinylated nucleotides then interact with avidin-peroxidase, which can be detected histochemically.

All slides were examined under a Leica DM6000B microscope and photographed using a Leica DC490 digital camera.

A positive control and a negative control for the detection of DNA fragmentation were also performed as previously described (7).

Apoptotic index

All preparates were evaluated by a blinded, experienced histologist (LKS). Epithelium cells in the fallopian tube sections and only the granulosa cells in the ovarian sections were counted. As a counting procedure: i) five random areas in the fallopian tube sections were photographed under x40 magnification and 100 epithelium cells were counted in total; ii) in the ovarian tissue sections, 100 granulosa cells were counted for each type of follicle (primary, secondary, and tertiary). The apoptotic index was calculated by the percentage of the positively-stained cells under microscopic examination of the ovarian tissue and fallopian tube following staining with TdT. According to the apoptotic index, the absence of stained cells corresponded to 0 points; stained cells of 1-25% corresponded to 1 point; 26-50% to 2 points; 51-75% to 3 points, and 76-100% to 4 points. The apoptotic index was compared between the three groups.

Statistical analysis

All statistical analysis were performed using SPSS, version 16.0 (SPSS, Chicago, IL, USA). The comparison of scores between groups was performed using the Kruskal-Wallis test. Post-hoc analysis was performed using Tukey's procedure to calculate the difference between groups. Values are given as means, standard deviation, and $p<0.05$ was accepted as statistically significant.

Results

There was a statistically significant difference between groups regarding the apoptotic index in the tubal epithelium (Figure 1). In binary comparisons, the bipolar electrocauterization group had a significantly higher apoptotic index compared with the control and Pomeroy's method groups (3.1±0.8 vs. 1.4±1.0, $p=0.018$ and 2.0±1.2, $p=0.03$, respectively), whereas there was no significant difference between the Pomeroy's method and control group (2.0±1.2 vs. 1.4±1.0, $p>0.05$) (Table 1).

Similarly, the apoptotic index detected on the primary follicles of the ovaries indicated a significant difference between groups (Figure 2). In binary comparisons, the bipolar electrocauterization group had significantly increased apoptotic index compared with the control and Pomeroy's method

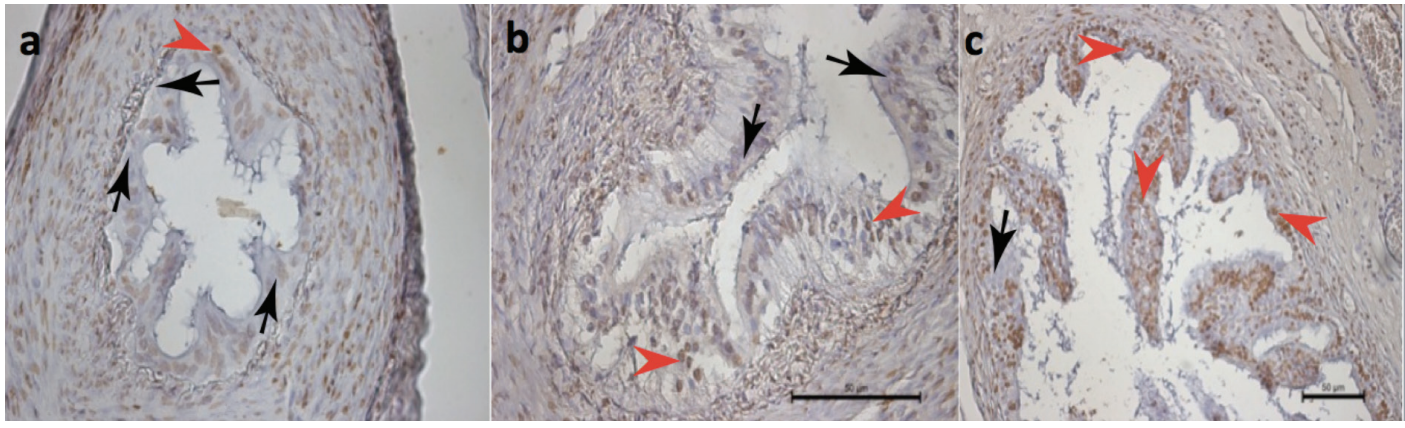

Figure 1. " ➢ " denotes apoptotic epithelial cells in histologic section of fallopian tubes of the rats stained with ApopTag (brown stain), "➢" denotes non-stained normal epithelial cells; a) control group, b) Pomeroy group, c) bipolar electrocauterization group (TUNEL assay x400)

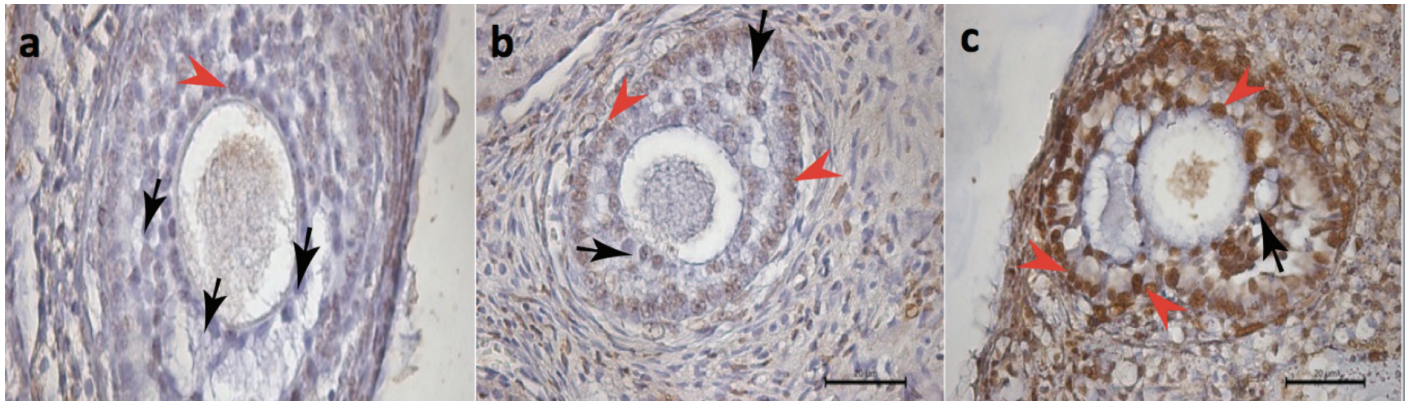

Figure 2. " ➢ " denotes apoptotic granulosa cells of primary follicles in histologic sections of the rat ovaries stained with ApopTag (brown stain), "➢" denotes non-stained granulosa cells of the primary follicles; a) control group, b) Pomeroy group, c) bipolar electrocauterization group (TUNEL assay x630)

Table 1. The numbers of follicle types in each group and the mean apoptotic index of each follicle type

	Control (n=8) Mean ± SD	n (%)	Pomeroy (n=8) Mean ± SD	n (%)	Bipolar cautery (n=8) Mean ± SD	n (%)
Tubal epithelium	1.4±1.0[a]	8 (100)	2.0±1.2[b]	8 (100)	3.1±0.8[a,b]	8 (100)
Primary follicles	1.2±0.4[c]	6 (75)	1.8±0.8[d]	5 (63)	3.4±0.5[c,d]	5 (63)
Secondary follicles	1.3±0.6	3 (38)	1.6±0.9	5 (63)	2.3±1.0	6 (75)
Tertiary follicles	1.3±0.8	6 (75)	1.5±0.7	5 (63)	2.4±0.5	2 (25)

[a]: $p=0.018$, [b]: $p=0.03$, [c]: $p>0.001$, [d]: $p=0.005$; SD: Standard deviation

groups (3.4±0.5 vs. 1.2±0.4, p<0.001 and 1.8±0.8, p=0.005, respectively), whereas there was no significant difference between the Pomeroy's method and control group regarding apoptotic index (1.8±0.8 vs. 1.2±0.4, p>0.05) (Table 1).

On the other hand, the apoptotic indexes in secondary (Figure 3) and tertiary (Figure 4) follicles were similar in each study group (p=0.237 and p=0.069, respectively, Table 1).

Discussion

Menstrual disorders such as prolonged or frequent bleeding, spotting, and dysmenorrhea have been observed after tubal sterilization procedures and these symptoms have been evaluated under the heading *"Post-tubal ligation syndrome"*. The effects of surgical female sterilization and whether the method of surgical sterilization per se has an effect on ovarian function have been investigated for years, yet there is still no consensus on this topic and study results are conflicting. We found that bipolar electrocauterization was associated with an increased apoptotic index of tubal epithelium and primary follicles, whereas Pomeroy's technique had no effect on the apoptotic index of any follicle types or tubal epithelium.

There are two theories for the potential effects of surgery on ovarian function. First, tubal surgery may damage the vascular and neuronal structures within the mesosalpinx, which in turn may lead to cessation of paracrine/endocrine factors or nervous stimuli from the uterus to the ovaries (5). Second, the surgical procedure may cause impaired ovarian blood flow and diminished ovarian reserve (3). Based upon these theories, studies have been designed and several conflicting results have been reported (8).

Following tubal sterilization, a decrease in ovarian function and reserve has been demonstrated by measuring mid-luteal plasma progesterone, (4) follicle-stimulating hormone (FSH), luteinizing hormone (LH), and estradiol (5). In some other

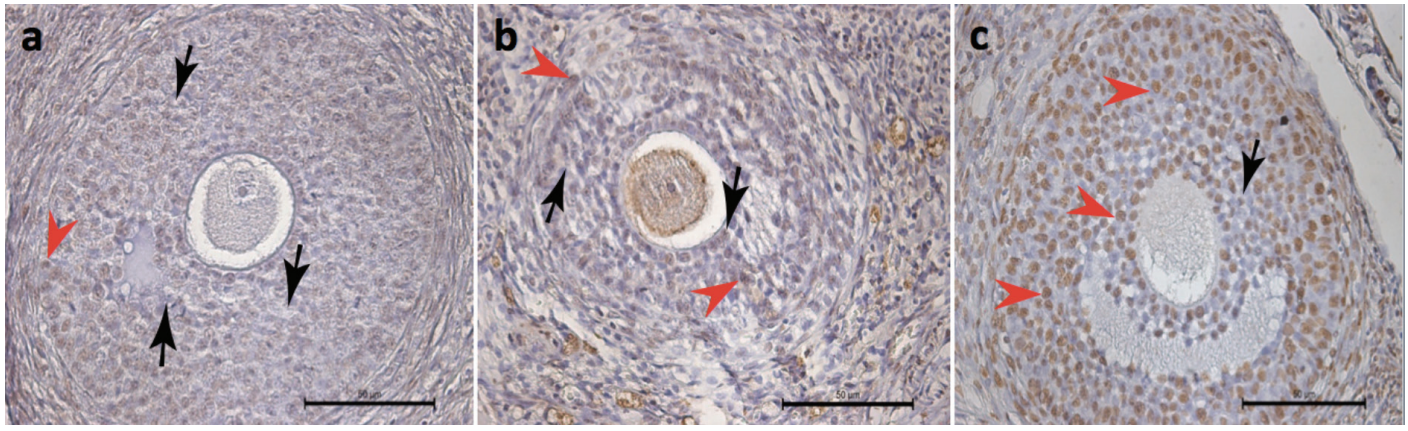

Figure 3. " ➢" denotes apoptotic granulosa cells of secondary follicles in histologic sections of the rat ovaries stained with ApopTag (brown stain), "➢" denotes non-stained granulosa cells of the secondary follicles; a) control group, b) Pomeroy group, c) bipolar electrocauterization group (TUNEL assay x400)

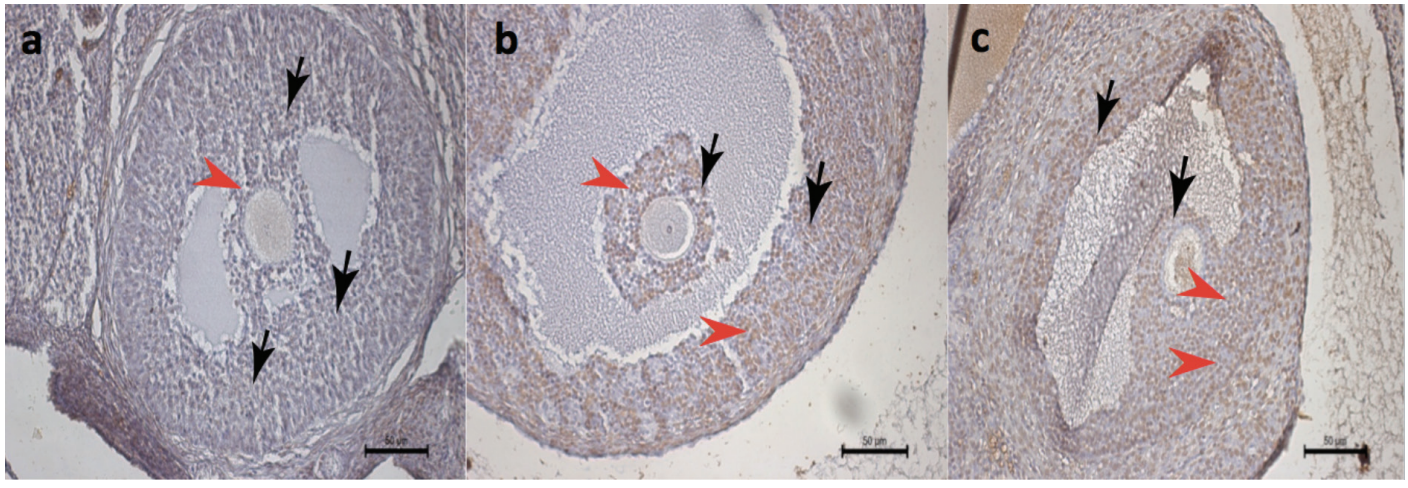

Figure 4. " ➢" denotes apoptotic granulosa cells of tertiary follicles in histologic sections of the rat ovaries stained with ApopTag (brown stain), "➢" denotes non-stained granulosa cells of the tertiary follicles; a) control group, b) Pomeroy group, c) bipolar electrocauterization group (TUNEL assay x200)

studies, it was reported that the decreasing ovarian function was associated with disturbed utero-ovarian blood flow/ hypoxia subsequent to the surgical procedure (3,9-11). In contrast to these studies, it has also been reported that tubal sterilization and/or the method of sterilization per se had no effect on ovarian function. Garza-Flores et al. (12) reported no difference in the levels of mid-luteal estradiol and progesterone before and after tubal sterilization. Wu et al. (13) compared FSH, LH, prolactin, estradiol, and progesterone levels between women with tubal sterilization and controls and they found no difference between the two groups. There are also two Doppler studies reporting no alteration in utero-ovarian blood flow after tubal sterilization (8,14).

In addition to human studies, there are few animal studies regarding the effects of surgical sterilization on ovarian function and morphology. Riedel et al. (15) reported that the type of sterilization was related to ovarian function in rabbits, and animals sterilized with unipolar electrocauterization had significantly lower levels of progesterone compared with the bipolar electrocauterization group and control group. Zhao et al. (16) performed a study on monkeys and reported no difference in progesterone levels after salpingectomy. In addition to hormonal assays, there are also studies assessing the effect of surgical sterilization on the histologic findings of the ovaries (11,14,17). Ovarian morphology of rabbits was evaluated and a significant reduction of tertiary follicle numbers per ovary in the postoperative 3rd month was reported, irrespective of the sterilization procedure (17). Kuscu et al. (18) investigated the late effects of sterilization on ovarian histology in rats. They concluded that the median number of healthy tertiary follicles per rat had been significantly reduced when compared with controls, regardless of the method used for sterilization. Nevertheless, we found no difference between the apoptotic index of the secondary and tertiary follicles regardless of the tubal sterilization method.

In the present study, we found that the apoptotic indexes in the tubal epithelium and primary follicles of ovaries were increased in the bipolar electrocauterization group compared with the control and Pomeroy's technique groups. In human studies, it has been previously reported that tubal sterilization with the modified Pomeroy's technique was neither associated with a decrease in ovarian reserve nor with an adverse effect on the blood supply of ovarian stroma when compared with healthy women without surgery (19,20) and that tubal sterilization through bipolar electrocoagulation was most likely to have an adverse effect on the ovarian reserve in the postoperative period compared with mechanical clips (21). However, to the best of our knowledge, this is the first study to compare the apoptotic index in the fallopian tubes and ovaries vis-à-vis two surgical contraception methods.

The apoptotic index detected in both surgical methods may have an impact on long-term functions of ovaries and tubes. It is an important issue when we take into account patient requests for tubal reversal. There are only a few studies have investigated the predictive factors of tubal reversal success (22,23), and it is yet not possible to draw exact conclusions from the available data (24). From this point of view, one might speculate that the higher apoptotic index of the tubal epithelium in bipolar electrocauterization might be related with a lower success rate of tubal reversal.

There is an ongoing debate regarding ovarian cancer prevention with tubal ligation. Although a recent meta-analysis showed a 34% reduction in the risk of epithelial ovarian cancer with tubal ligation (25), in the general population, it is recommended to perform a bilateral salpingectomy instead of tubal ligation for patients who would like to have a tubal sterilization with the belief of better cancer prevention and contraception (26). In this context, further studies are warranted to compare the effect of salpingectomy and tubal ligation only on the ovary.

There are several limitations to the current study. The main limitation is the confounding effect of a natural ovarian cycle. Follicles that would not progress to ovulation would naturally undergo apoptosis, so this fact should be kept in mind while interpreting the results of the study. However, we designed the study with a control group in order to eliminate this possible confounder. The other limitations of the current study are the lack of monitoring of anti-Müllerian hormone levels to investigate the ovarian reserve and lack of a long-term follow-up period to compare the menopausal onset time of the rats. One should bear in mind that even the findings of current study were against using bipolar electrocauterization in tubal ligation; the study results should be confirmed by randomized, controlled, long-term human studies to determine whether there is clinical relevance to our significantly different findings.

In conclusion, tubal sterilization with Pomeroy's technique, as a permanent contraception method, revealed a lower apoptotic index when compared with tubal sterilization with bipolar electrocauterization.

Ethics Committee Approval: *Ethics committee approval was received for this study from the Ethics Committee of Hacettepe University School of Medicine (Approval no: 2012/16).*

Informed Consent: *N/A.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Concept - S.H., S.M., L.K.S.; Design - S.H., S.M., R.K., L.K.S.; Supervision - S.H., L.K.S., F.K.; Materials - A.S., L.K.S., F.K., R.K.; Writer - S.M., A.S., S.H.*

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

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

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Relationships of nuclear, architectural and International Federation of Gynecology and Obstetrics grading systems in endometrial cancer

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Abstract

Objective: To examine correlations among nuclear, architectural, and International Federation of Gynecology and Obstetrics (FIGO) grading systems, and their relationships with lymph node (LN) involvement in endometrioid endometrial cancer.

Material and Methods: Histopathology slides of 135 consecutive patients were reviewed with respect to tumor grade and LN metastasis. Notable nuclear atypia was defined as grade 3 nuclei. FIGO grade was established by raising the architectural grade (AG) by one grade when the tumor was composed of cells with nuclear grade (NG) 3. Correlations between the grading systems were analyzed using Spearman's rank correlation coefficients, and relationships of grading systems with LN involvement were assessed using logistic regression analysis.

Results: Correlation analysis revealed a significant and strongly positive relationship between FIGO and architectural grading systems (r=0.885, p=0.001); however, correlations of nuclear grading with the architectural (r=0.535, p=0.165) and FIGO grading systems (r=0.589, p=0.082) were moderate and statistically non-significant. Twenty-five (18.5%) patients had LN metastasis. LN involvement rates differed significantly between tumors with AG 1 and those with AG 2, and tumors with FIGO grade 1 and those with FIGO grade 2. In contrast, although the difference in LN involvement rates failed to reach statistical significance between tumors with NG 1 and those with NG 2, it was significant between NG 2 and NG 3 (p=0.042). Although all three grading systems were associated with LN involvement in univariate analyses, an independent relationship could not be established after adjustment for other confounders in multivariate analysis.

Conclusion: Nuclear grading is significantly correlated with neither architectural nor FIGO grading systems. The differences in LN involvement rates in the nuclear grading system reach significance only in the setting of tumor cells with NG 3; however, none of the grading systems was an independent predictor of LN involvement. (J Turk Ger Gynecol Assoc 2018; 19: 17-22)

Keywords: Endometrial cancer, grade, lymph node involvement

Received: 9 January, 2017 **Accepted:** 24 October, 2017

Introduction

Endometrioid-type endometrial cancer (EC) is graded histologically according to the criteria set forth by the International Federation of Gynecology and Obstetrics (FIGO) (1). This grading system consists of a combination of two different grading systems, architectural grading and nuclear grading. In the FIGO grading system, features for architectural grading have been adopted from well-defined criteria of the Gynecologic Oncology Group (GOG) pathology committee (2). FIGO stated that in tumors with notable nuclear atypia that is inappropriate for the architectural grade (AG), the final grade should be established by raising the AG by one grade (3). However, FIGO did not define any criteria to determine "notable nuclear atypia", which led to confusion both for pathologists and physicians.

The abstract of this study was accepted as a poster presentation at the 20th International Meeting of the European Society of Gynaecological Oncology (ESGO 2017); November 4-7, 2017; Vienna, Austria.

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Journal of the Turkish-German Gynecological Association published by Galenos Publishing House.
DOI: 10.4274/jtgga.2017.0004

Lymph node (LN) involvement is one of the main prognostic factors for patients with EC. The five-year overall survival rate exceeds 80% in patients with negative LNs, but in cases of LN metastasis, it decreases to approximately 50% (3). Several primary tumor characteristics have been demonstrated to be related with the risk of LN metastasis, of which tumor grade is one of the most consistently reported.

In the present study, by using strict diagnostic criteria, we aimed to examine correlations among the nuclear, architectural, and FIGO grading systems, and their relationships with LN involvement in endometrioid-type EC.

Material and Methods

The clinicopathologic records of patients with EC, who underwent total hysterectomy and systematic pelvic lymphadenectomy with or without paraaortic LN dissection at a single institution between January 2010 and January 2015, were reviewed retrospectively. Patients with non-endometrioid histotype, primary synchronous malignancy, no residual disease in the hysterectomy specimen, or who had not undergone LN dissection were excluded.

As a routine strategy at our institution, all patients with newly diagnosed EC were offered treatment with total hysterectomy with systematic pelvic lymphadenectomy if they were medically operable and did not desire fertility preservation. Paraaortic LN dissection was added to pelvic lymphadenectomy in the presence of at least one of the following risk factors: a) non-endometrioid histotype, b) FIGO grade 2 or 3 endometrioid carcinoma, c) deep (≥50%) myometrial invasion on frozen-section examination.

The study was performed in accordance with the ethical standards described in an appropriate version of the 1975 Declaration of Helsinki, as revised in 2013. Written informed consent was not required for this type of retrospective study. Ethical approval was obtained from the institutional local ethics committee.

All available histopathology slides were reviewed in each case by two sub-specialized gynecologic pathologists with respect to primary tumor characteristics including histotype, AG, nuclear grade (NG), FIGO grade, and LN involvement.

Architectural grading was performed using the criteria of the GOG pathology committee (2): AG 1, tumors with well-preserved glandular morphology in which solid nests of neoplastic cells comprise ≤5% of the lesion; AG 2, tumors in which the solid areas comprise 5 to 50% of the lesion; and AG 3, tumors in which >50% of the lesion is arranged in solid sheets of neoplastic cells. AG was based upon assessment of glandular and solid areas, excluding areas of squamous differentiation.

Nuclear grading was performed using the criteria defined by Zaino et al. (4): NG 1, uniform round-to-oval nuclei, with even distribution of chromatin and inconspicuous nucleoli; NG 2, irregular oval nuclei, with chromatin clumping and moderate size nucleoli; and NG 3, large, pleomorphic nuclei, with coarse chromatin, and large irregular nucleoli. NG of a tumor was assigned based on the features displayed by the majority of tumor cells.

In the present study, the "notable nuclear atypia" was defined as NG 3, and the FIGO grade was established by raising the AG by one grade when the tumor was composed of cells with NG 3. Figure 1 shows microscopy views of the samples from each grading system.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics 20 (SPSS/IBM, Chicago, IL, USA) software. Correlations between the grading systems were analyzed using Spearman's rank correlation coefficient. The relationships of primary tumor characteristics with LN involvement were assessed using logistic regression analysis. Variables with a p value <0.05 in univariate analysis were included into multivariate analysis. The effects of variables on LN involvement were reported as adjusted odds ratios and 95% confidential intervals.

Results

A total of 135 patients were enrolled in the analysis. The majority of patients had AG 1 (56.3%), NG 2 (45.9%), and FIGO grade 1 (54.1%) tumors. Eighty (59.3%) patients had pelvic lymphadenectomy alone, and 55 (40.7%) had combined pelvic and paraaortic lymphadenectomy. LN involvement was identified in 25 (18.5%) patients (Table 1).

Correlation analysis revealed that there was a significant and very strongly positive relationship between the FIGO and architectural grading systems (r=0.885, p=0.001); however, correlations of nuclear grading with the architectural (r=0.535, p=0.165) and FIGO grading systems (r=0.589, p=0.082) were moderate and statistically non-significant (Table 2).

The rates of LN involvement according to each grading system are summarized in Table 3. LN involvement was detected in 7.9% of tumors with AG 1, 25.0% of tumors with AG 2, and 47.3% of tumors with AG 3. LN involvement rates according to FIGO grades were as follows: 5.4% for grade 1, 31.6% for grade 2, and 37.5% for grade 3. LN involvement rates differed significantly between tumors with AG 1 and AG 2 (p=0.045), and between tumors with FIGO grades 1 and 2 (p=0.031), whereas there were no significant differences between AG 2 and AG 3 (p=0.069), and between FIGO grades 2 and 3 (p=0.327).

The rates of LN involvement based on nuclear grading system were as follows: 6.2% for NG 1, 20.9% for NG 2, and 36.0% for NG 3. In contrast to architectural and FIGO grading systems,

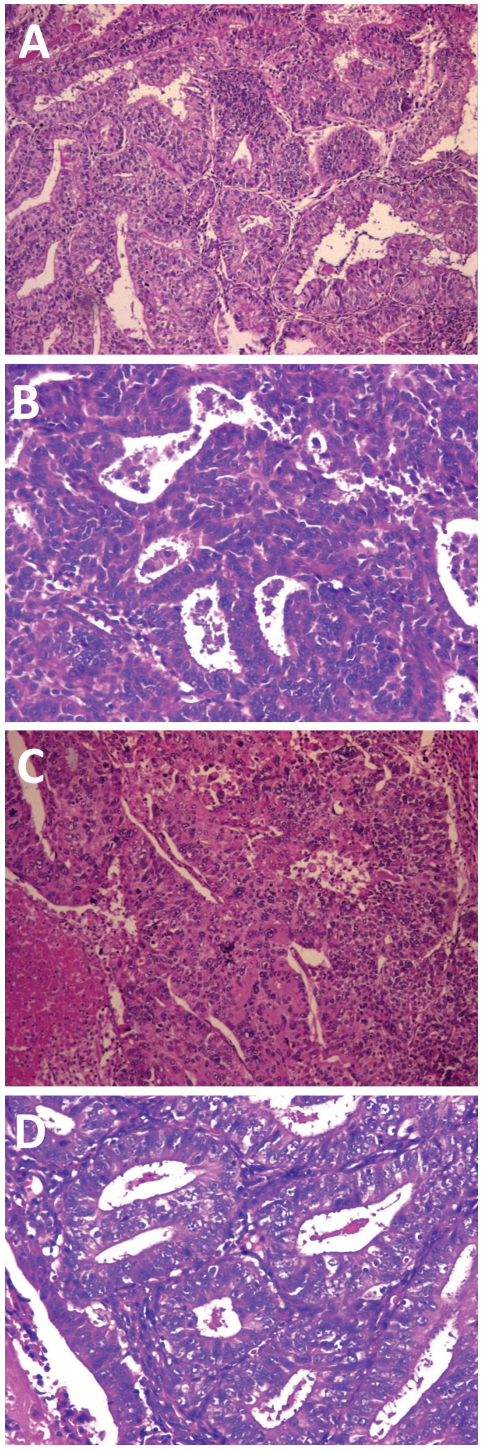

Figure 1. Microscopic views of samples from each grading systems; a) FIGO grade 1: composed of AG 1 and NG 1 (Hematoxylin & Eosin, x100), b) FIGO grade 2: composed of AG 2 and NG 2 (Hematoxylin & Eosin, x200), c) FIGO grade 3: composed of AG 3 and NG 3 (Hematoxylin & Eosin, x100), d) FIGO grade 2: consisting of cells with "notable nuclear atypia (NG 3)" inappropriate for the architectural grade (AG 1) (Hematoxylin & Eosin, x200)
NG: Nuclear grade; AG: Architectural grade; FIGO: International Federation of Gynecology and Obstetrics

the difference in LN involvement rates failed to reach statistical significance between tumors with NG 1 and those with NG 2 (p=0.115), but it was significant between NG 2 and NG 3 (p=0.042) (Table 3).

Table 1. Characteristics of the patients

Variables	Values
Age, median (range), years	57 (32-77)
Lymphadenectomy, n (%)	
Pelvic alone	80 (59.3)
Combined pelvic and paraaortic	55 (40.7)
Number of pelvic LNs removed, median (range)	20 (6-38)
Number of paraaortic LNs removed, median (range)	19 (10-45)
Number of total LNs removed (pelvic and/or paraaortic), median (range)	35 (6-73)
Nuclear grade, n (%)	
NG 1	48 (35.6)
NG 2	62 (45.9)
NG 3	25 (18.5)
Architectural grade, n (%)	
AG 1	76 (56.3)
AG 2	40 (29.6)
AG 3	19 (14.1)
FIGO grade, n (%)	
Grade 1	73 (54.1)
Grade 2	38 (28.1)
Grade 3	24 (17.8)
Tumor size, median (range), cm	3.4 (0.1-9.5)
Myometrial invasion	
<1/2	75 (55.6)
≥1/2	60 (44.4)
Lymphovascular space involvement, n (%)	26 (19.3)
LN involvement, (pelvic and/or paraaortic), n (%)	25 (18.5)
Pelvic	21 (15.6)
Paraaortic	12 (8.9)
Isolated paraaortic (in the setting of negative pelvic nodes)	4 (3.0)
$FIGO_{2008}$ stage, n (%)	
IA	53 (39.3)
IB	36 (26.7)
II	11 (8.1)
IIIA	7 (5.2)
$IIIC_1$	14 (10.4)
$IIIC_2$	10 (7.4)
IVB	4 (3.0)

LN: Denotes lymph node; NG: Nuclear grade; AG: Architectural grade; FIGO: International Federation of Gynecology and Obstetrics

In order to assess independent relationships between grading systems and LN metastasis, two different logistic regression models were developed because a strong correlation between FIGO grade and AG would confound the possible associations (Table 4). NG and AG were assigned to the first model, and the FIGO grade was separately evaluated in the second model. Both models also included deep myometrial invasion and lymphovascular space involvement (LVSI) as potential covariates. Although all three grading systems were associated with LN involvement in univariate analyses, an independent relationship could not be established after adjustment for other confounders in multivariate analyses. LVSI was consistently the sole independent predictor of LN metastasis in multivariate analyses (p=0.001).

Table 2. Distribution of nuclear grades among each architectural and International Federation of Gynecology and Obstetrics grade, and correlations among grading systems

	Nuclear grade			*r*	p
	NG 1 (n=48)	NG 2 (n=62)	NG 3 (n=25)		
Architectural grade				0.535	0.165
AG 1	48	25	3		
AG 2	0	35	5		
AG 3	0	2	17		
FIGO grade				0.589	0.082
Grade 1	48	25	0		
Grade 2	0	35	3		
Grade 3	0	2	22		
FIGO grade vs. AG				0.885	0.001

NG: Nuclear grade; AG: Architectural grade; FIGO: International Federation of Gynecology and Obstetrics; *r*: Spearman's rank correlation coefficient

Discussion

After FIGO's equivocal statement regarding nuclear atypia, some researchers attempted to develop more objective definitions in nuclear as well as final grading of EC. First, Zaino et al. (4) reported that if the "notable nuclear atypia" was defined as grade 3 nuclei, and the final FIGO grade was established by raising the AG by one grade only when the majority of the neoplasm was composed of cells with NG 3,

Table 3. Lymph node involvement according to each grading system

Variables	n	Lymph node involvement, n (%)	p
Nuclear grade			
NG 1	48	3 (6.2)	0.038
NG 2	62	13 (20.9)	
NG 3	25	9 (36.0)	
NG 1 vs. NG 2			0.115
NG 2 vs. NG 3			0.042
Architectural grade			
AG 1	76	6 (7.9)	0.016
AG 2	40	10 (25.0)	
AG 3	19	9 (47.3)	
AG 1 vs. AG 2			0.045
AG 2 vs. AG 3			0.069
FIGO grade			
Grade 1	73	4 (5.4)	0.001
Grade 2	38	12 (31.6)	
Grade 3	24	9 (37.5)	
Grade 1 vs. grade 2			0.031
Grade 2 vs. grade 3			0.327

NG: Nuclear grade; AG: Architectural grade; FIGO: International Federation of Gynecology and Obstetrics

Table 4. Relationships of grading systems with lymph node involvement

Variables	Univariate			Multivariate		
	OR	95% CI	p	OR	95% CI	p
Model 1						
Nuclear grade 3	3.87	1.16-8.75	0.041	1.85	1.37-2.11	0.115
Architectural grade 2-3	4.75	1.51-10.59	0.016	3.14	1.75-2.88	0.072
Myometrial invasion ≥50%	4.59	1.33-9.90	0.025	2.40	1.16-6.24	0.084
LVSI	7.53	2.81-13.14	0.001	8.02	1.90-16.41	0.001
Model 2						
FIGO grade 2-3	6.74	1.59-12.31	0.001	4.55	1.75-12.66	0.063
Myometrial invasion ≥50%	5.64	1.68-10.27	0.026	2.73	0.98-8.02	0.076
LVSI	8.49	2.13-15.35	0.001	6.94	1.90-15.54	0.001

OR: Odds ratio; CI: Confidential interval; LVSI: Lymphovascular space involvement; FIGO: International Federation of Gynecology and Obstetrics

the FIGO grading system showed prognostic utility. Following the analysis of the clinicopathologic data obtained from 715 patients with endometrioid EC, the authors found that tumors upgraded using this criterion had a relative risk of progression 1.9 times higher than that of the group from which they were moved. In contrast, if the notable atypia was considered as both NG 2 and NG 3, the relative risk was almost identical to that of the group from which they were moved. Later, in a study of 476 patients with endometrioid EC, Takeshima et al. (5) suggested that upgrading of AG should be performed when more than 25% of the neoplastic cells showed grade 3 nuclei. The authors reported that tumors that had 26% to 50% of neoplastic cells with grade 3 nuclei showed a similar risk of recurrence as did tumors that had more than 50%. In a large single institutional analysis that investigated a convenient method for the modification of AG by nuclear features, Ayhan et al. (6) reported that in determining the FIGO grade, upgrading of AG 1 or AG 2 tumors by grade 3 nuclei was the most reliable method. The authors also noted that all three grading systems significantly predicted poor disease outcome, but only the FIGO grade, stage, and cervical involvement remained independent predictors of survival in multivariate analysis.

Our data indicated that tumors with grade 3 nuclei significantly differed from tumors with NG 1 and from NG 2 in terms of LN involvement. On the contrary, such a significant difference was not evident between tumors with NG 1 and those with NG 2. These findings support previous studies (4-6) that suggested that "notable nuclear atypia" should be defined as NG 3.

The lack of an objective definition for "notable nuclear atypia" and the moderate inter-observer agreement in distinction of squamous from non-squamous solid growth in the FIGO grading system led to the proposal of alternative binary grading systems by some researchers over the past two decades (7-9). Lax et al. (7) described a binary grading system that uses a low magnification evaluation of the presence of necrosis, pattern of invasion, and amount of solid growth to divide endometrioid ECs into low- and high-grade tumors. The authors suggested that a tumor should be considered as high-grade when it exhibits at least two of the following features: i) more than 50% solid growth (without distinction of squamous from non-squamous epithelium); ii) a diffusely infiltrative, rather than expansive, growth pattern; and iii) tumor cell necrosis. The authors reported that both inter- and intraobserver agreements using the binary grading system were superior compared with the FIGO and nuclear grading systems. Scholten et al. (8) conducted a study to compare the reproducibility of FIGO grading system with the novel binary grading system proposed by Lax et al. (7); however, they found that the inter-observer agreement for both systems was moderate, with 70% and 73% agreement rates for the FIGO and binary grading systems, respectively. The authors proposed that if a simple architectural binary grading system that divides tumors into low- and high-grade based solely on the proportion of solid tumor growth (≤50% or >50%) was used in the grading of ECs, a much better agreement rate (85%) could be achieved. In another alternative binary grading system (low-grade vs. high-grade), Alkushi et al. (9) suggested that tumors should be considered high-grade in the presence of at least two of the following criteria: i) predominantly papillary or solid growth pattern, ii) mitotic index ≥6/10 high power fields, and iii) severe nuclear atypia. The authors reported that this system had more prognostic power than the three-tiered FIGO and binary system of Lax et al. (7) when applied to all tumors regardless of tumor histotype; however, the FIGO grading system was superior for prognostication when only endometrioid type ECs were considered.

Currently, none of these alternative systems has become widespread because it is not clear whether they would significantly improve the prognostic utility of the current method (10). Moreover, in a recent study comparing new binary systems with the existing three-tiered FIGO grading system, Guan et al. (11) demonstrated that the FIGO grading system using the nuclear criteria of Zaino et al. (4) was prognostically superior to the other systems, particularly in patients with endometrioid-type EC.

There are also some studies in the literature reporting that NG is more useful than FIGO grade in terms of predicting poor disease outcome (12,13). However, these trials are heterogeneous regarding tumor histotype. Non-endometrioid tumors including serous and clear cell histotypes are graded principally by nuclear features alone (3). Therefore, the association of NG with poor disease outcome in these trials, may in fact reflect the poor outcome of non-endometrioid tumors. On the other hand, in studies examining the tumors with endometrioid histology alone, architectural and FIGO grades have been mostly demonstrated to be prognostically superior to NG (4,6,11).

In spite of sufficient data indicating prognostic validity of the current grading methods, there appears to be little exclusive data available on relationships of different grading systems specifically with LN involvement. Most of the data have focused on the risk of LN metastasis based on stratification of FIGO grade by myometrial invasion and/or tumor size, and usually demonstrated a dependent association (3,14-16). However, trials examining independent predictors of LN involvement by controlling the potential confounding factors such as myometrial invasion, tumor size, LVSI, and cervical involvement, generally failed to demonstrate a direct relationship between tumor grade and LN involvement (17,18). Consistent with previous studies, an independent relationship between any

of the three grading systems and LN metastasis could not be demonstrated in our study. However, our findings should be cautiously interpreted because our study and its design have limitations including the relatively small sample size and single institutional nature, which bring inherent problems of selection and referral bias. The small sample size of our study might have caused a sampling error, limiting the power in detecting associations.

In conclusion, based on our results, nuclear grading is correlated with neither the architectural nor the FIGO grading systems. As opposed to architectural and FIGO grading systems, in which the LN involvement rates significantly differ in grade 2 level (AG 2 and FIGO grade 2), the differences in LN involvement rates in the nuclear grading system reach statistical significance only in the setting of tumor cells with grade 3 features. Therefore, "notable nuclear atypia" should be defined as NG 3. However, none of the grading systems is an independent predictor of LN metastasis.

Ethics Committee Approval: *Ethics committee approval was received for this study from the Ethics Committee of Akdeniz University School of Medicine (No: 0676, Date: 08/12/2013).*

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

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Concept - T.T., E.P., T.Ş.; Design - T.T., E.P., G.E.; Supervision - E.P., T.Ş.; Materials - T.T., E.P., G.E.; Writer - T.T., E.P., S.B.*

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

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

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Clinicopathologic and survival results in serous endometrium carcinoma and subgroup analysis for mixed serous and pure serous histology

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Abstract

Objective: To review the clinicopathologic and survival outcomes of patients with serous endometrial cancer (EC) and to investigate subgroup analysis based on pure serous and mixed serous EC subtypes.

Material and Methods: Patients who underwent EC surgery between 2002 and 2014 and who were reported as serous EC were enrolled in the study. All patients were diagnosed as having serous EC or mixed serous EC with serous component higher than 10% based on the postoperative pathology report.

Results: A total of 93 patients were analyzed. The median disease-free and overall survival (OS) durations were 49.6 and 32.2 months, respectively. Forty-three patients (46.2%) relapsed and 35 patients (37.6%) died. The histologic type was pure serous EC in 52 (55.9%) and mixed EC in 41 (44.9%) patients. There was no statistical difference between the pure serous and mixed serous groups in terms of age, International Federation of Gynecology and Obstetrics stage, lymphadenectomy, lymph node metastasis or adjuvant therapy combinations. Twenty-nine (55.8%) patients in the pure serous group and 14 (34.1%) in the mixed serous group hade recurrence (p=0.038). Twenty-five (48.1%) patients in the pure serous group and 10 (24.4%) in the mixed serous group died (p=0.034). In the pure serous group, the mean disease-free and OS durations were shorter than in the mixed serous group (59 vs. 81 months and 73 vs. 95 months, log-rank p=0.055 and 0.041, respectively). Histologic type was a significant prognostic factor on recurrence and OS in the univariate analysis (Hazard ratio: 2.404, 95% Confidence interval: 1.01-5.71; 2.027, respectively), but not in the multivariate analysis, which included disease stage and age of the patients.

Conclusion: Compared with pure serous and mixed serous endometrium cancer groups, primary surgical treatments, clinicopathologic features and adjuvant treatments were similar, but there was a survival difference. Patients with pure serous cancer had a worse prognosis. However histology was not an independent factor for survival. (J Turk Ger Gynecol Assoc 2018; 19: 23-8)

Keywords: Endometrium cancer, histologic type, serous component

Received: 12 May, 2017 **Accepted:** 24 October, 2017

Introduction

Among the endometrial cancer (EC) subtypes, serous endometrial carcinomas are known as high-risk carcinomas. They account for nearly 10% of all ECs; however, the highest mortality due to EC is seen with serous carcinoma among all subtypes (1,2). Serous EC may either be seen as pure serous carcinoma or together with endometrioid (most common), clear cell or sarcomatous components (3). In this group known as mixed ECs, any component higher than 10% but less than 90% is considered widespread (4). The most frequent is the combination of serous and endometrioid components, which account for less than 1% of all patients with EC (5).

In general, pure serous and mixed serous EC are considered similar in terms of clinical features and survival, and they are treated in similar ways (6). A limited number of studies have investigated whether there was any difference between the two subtypes in terms of clinicopathologic features and survival rates. Survival appears to be similar in a few studies with small sample sizes, especially from pathology departments (3,6). The English literature comprises only a single study with a large sample size comparing the two subtypes one-to-one; in that

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Journal of the Turkish-German Gynecological Association published by Galenos Publishing House.
DOI: 10.4274/jtgga.2017.0065

study, Roelofsen et al. (7) demonstrated poorer survival in pure serous EC as compared with mixed serous EC and proposed that they might be two different entities. These tumors represent an infrequent subset of patients with EC; hence, most studies are retrospective and included a limited number of patients.

Our purpose in planning this study was to review the clinical features and survival results of serous endometrium cancers and to compare them with simple pure serous and mixed serous subgroups based on clinicopathologic features, surgery treatments, adjuvant treatments, recurrence rates, overall survival (OS) durations, and disease-free survival (DFS) durations.

Material and Methods

Study data were derived from the hospital records of patients who underwent surgery for EC between 2002 and 2014 at the same center. A total of 103 patients who were diagnosed as having pure serous EC or mixed serous EC based on the postoperative pathology report were recruited. Endometrioid type EC with serous component between 10-90% was considered mixed serous EC. The amount of serous component, which was between 10 and 80%, was not taken into account (no data are demonstrated). All patients who fulfilled the definition of mixed serous carcinoma were allocated to the same group. Mixed carcinomas without serous component were not included in the study. All pathologic examinations were performed by the same team of gynecopathologists.

Four patients with unavailable follow-up information, four patients who died of other reasons (2 cardiovascular reasons, 1 pulmonary embolism after femur fracture, 1 during postoperative chemotherapy), and two patients who underwent surgical procedures after neoadjuvant chemotherapy were excluded from the study. Demographic, clinical, surgical and pathological outcomes and data from adjuvant therapy of the remaining 93 patients were recorded on Excel software. Pure serous EC and mixed serous EC groups were compared in terms of clinical features, pathologic features, OS, and DFS.

Permission of the local ethics committee was not sought because this study was planned as a retrospective review. However, all patients signed an informed consent form, which allowed our center to use their clinical data for scientific trials.

Statistical analysis

Number Cruncher Statistical System 2007 (Kaysville, Utah, USA) program was used for statistical analyses. In addition to the descriptive statistics (mean, standard deviation, median, frequency, ratio, minimum and maximum) used to evaluate the study data, paired group comparison of the quantitative data was made using Student's t-test for parameters showing normal distribution and the Mann-Whitney U test for parameters not showing normal distribution. Qualitative data were compared using Pearson's chi-square test, the Fisher-Freeman-Halton test, Fisher's exact test, and Yates's continuity correction test (Yates's corrected chi-square). Kaplan-Meier survival analysis and the log-rank test were used to assess survival. Univariate and multivariate cox analysis were used in the analysis of OS and recurrence-related factors.

Results

The total number of patients was 93. The demographic and clinicopathologic characteristics of the 93 patients are presented in Table 1. The median OS was 49.6 months (range, 3-120 months), and the median DFS was 32.45 months (range, 2-120 months). Disease recurred in 43 patients (46.2%), and 35 patients (37.6%) died. The histology of tumor was pure serous in 52 (55.9%) patients and mixed serous in 41 (44.9%). Early-stage disease according to the International Federation of Obstetrics and Gynecology (FIGO) (FIGO 1-2) was found in 69.9% of patients and advanced-stage (FIGO 3-4) disease in 30.1% of patients.

Comparative analysis between pure serous and mixed serous groups (Table 2): There was no statistically significant difference between the two groups in terms of age, parity, chronic disease (diabetes or hypertension), positive peritoneal lavage fluid, endocervical invasion, lymphovascular invasion, depth myometrial invasion, tumor size (cm), and FIGO stage. The groups were not different in terms of the tumor spread, such as presence of tumor in the perimetrium, ovaries, fallopian tubes, omentum, and colon. There was also no statistically significant difference between the groups in terms of the number of patients who underwent lymphadenectomy,

Table 1. Stage, histology and survival outcomes of ninety-three patients

Characteristics	n (%)
Age (year) minimum-maximum (median); mean ± SD	37-82 (65); 64.1±9.9
Stage	
FIGO 1-2	65 (69.9)
FIGO 3-4	28 (30.1)
Pure serous histology	52 (55.9)
Mixed serous histology	41 (44.1)
OS (month) minimum-maximum (median); mean ± SD	3-120 (49.6); 56.9±35.7
DFS (month) minimum-maximum (median); mean ± SD	2-120 (32.4); 46.2±36.9

DFS: Disease-free survival; OS: Overall survival; SD: Standard deviation; FIGO: International Federation of Gynecology and Obstetrics

the number of lymph nodes removed, the rate of lymph node metastasis, or adjuvant therapy combinations (Table 2, 3). Recurrence was observed in 29 (55.8%) patients in the pure serous group and in 14 (34.1%) in the mixed serous group; the difference was statistically significant (Yates's continuity test p=0.038). Twenty-five (48.1%) patients in the pure serous group and 10 (24.4%) in the mixed serous group died; the difference was statistically significant (Yates' continuity test p=0.034). In the serous group, the mean DFS and OS durations were shorter than in the mixed serous group (59 vs. 81 months and 73 vs. 95 months, log-rank p=0.055 and 0.041, respectively) (Figure 1, 2).

Univariate and multivariate analysis of overall survival and recurrence-related factors (Table 4, 5): In the univariate analysis, subtype and stage were significant factors for recurrence and OS but not lymphovascular invasion. Significant factors were included in the age-adjusted multivariate analysis. Age and FIGO stage were independent

Table 2. Comparison of clinicopathologic and survival features between the serous and mixed carcinoma groups

Characteristic	Pure serous (n=52) n (%)	Mixed serous (n=41) n (%)	p value
Age, mean ± SD	65.5±10.4	62.4±9.2	[a]0.14
Parity, minimum-maximum (median)	0-9 (2)	0-10 (3)	[b]0.96
Chronic disease**	30 (57.7)	23 (56.1)	[c]1.00
Positive peritoneal lavage fluid	8 (15.4)	3 (7.3)	[e]0.33
Tumor in the perimetrium	5 (9.6)	3 (7.3)	[e]1.00
Ovary metastasis	10 (19.2)	3 (7.3)	[c]0.17
Fallopian tube metastasis	8 (15.4)	4 (9.8)	[c]0.62
Omentum metastasis	6 (11.5)	1 (2.4)	[e]0.12
Colon metastasis	2 (3.8)	0 (0)	[e]0.50
Lymphadenectomy	43 (80.8)	29 (70.7)	[c]0.37
Lymph node metastasis	6 (14.3)	6 (20.0)	[e]0.53
Removed no of lymph nodes, Minimum-maximum (median) total	2-30 (10) 591	1-44 (15) 430	[b]0.38
Lymphovascular invasion	24 (61.5)	28 (73.7)	[c]0.37
Cervical invasion Stroma Mucosa Non	 13 (25.0) 5 (9.6) 34 (65.4)	 11 (26.8) 4 (9.8) 26 (63.4)	[d]1.00
Depth myometrial invasion ≤50% >50%	 30 (58.8) 21 (41.2)	 20 (48.8) 21 (51.2)	[c]0.45
Tumor size (cm) Median (minimum-maximum) Mean ± SD	 11 (0.3-11) 4.2±2.5	 10 (0.5-11) 4.0±2.2	[b]0.68
Stage FIGO 1-2 FIGO 3-4	 33 (63.5) 19 (36.5)	 32 (78.0) 9 (22.0)	[c]0.19
Recurrence	29 (55.8)	14 (34.1)	**[c]0.03**
Mortality	25 (48.1)	10 (24.4)	**[c]0.03**
Overall survival (months) Mean ± SD (95% CI)	 73.3±6.2 61.1-85.6	 95.1±6.6 82.0-108.2	**[f]0.04**
Disease-free survival (months) Mean ± SD (95% CI)	 59.3±6.9 45.78-7.8	 81.6±8.1 65.7-97.5	**[f]0.05**

[a]Student's t-test; [b]Mann-Whitney U test; [c]Yates's continuity test; [d]Fisher-Freeman-Halton test; [e]Fisher's exact test; [f]Log-rank test; **Diabetes or hypertension; SD: Standard deviation; FIGO: International Federation of Gynecology and Obstetrics; CI: Confidence interval

Table 3. Adjuvant therapy combinations

Adjuvant therapy	Serous; n (%)	Mixed; n (%)	p value
Chemotherapy only	15 (28.8)	4 (9.8)	**0.037**
Chemotherapy + brachytherapy	14 (26.9)	10 (24.4)	0.816
Chemotherapy + EBRT	7 (13.5)	8 (19.5)	0.614
Chemo + EBRT + brachytherapy	4 (7.7)	7 (17.1)	0.204
EBRT+ brachytherapy	3 (5.8)	3 (7.3)	0.999
Brachytherapy	0	3 (7.3)	0.082
EBRT	0	2 (4.9)	0.192
No adjuvant treatment	5 (9.6)	2 (4.9)	0.459
Not available	4 (7.7)	2 (4.9)	0.691
EBRT: External beam radiation therapy			

Table 4. Univariate and multivariate analysis of effective factors for overall survival duration of the ninety-three patients

	Univariate analysis				Multivariate analysis			
	95% CI		HR	p	95% CI		HR	p
Age	-	-	-	-	1.022	1.097	1.059	**0.001**
Stage	1.302	4.686	2.470	**0.006**	1.281	4.718	2.459	**0.007**
LVSI	0.792	3.794	1.734	0.169	NS	NS	NS	NS
Subtype	1.022	4.021	2.027	**0.043**	0.724	2.943	1.460	0.291
HR: Hazard ratio; CI: Confidence interval; NS: Not selected; LVSI: Lymphovascular invasion								

Table 5. Factors associated with recurrence by univariate and multivariate analysis

	Univariate analysis				Multivariate analysis			
	95% CI		HR	p	95% CI		HR	p
Age	-	-	-	-	0.973	1.065	1.018	0.450
Stage	1.717	10.858	4.318	**0.002**	1.614	10.851	4.185	**0.003**
LVSI	0.855	5.839	2.234	0.101	NS	NS	NS	NS
Subtype	1.011	5.714	2.404	**0.047**	0.788	4.982	1.981	0.146
HR: Hazard ratio; CI: Confidence interval; NS: Not selected; LVSI: Lymphovascular invasion								

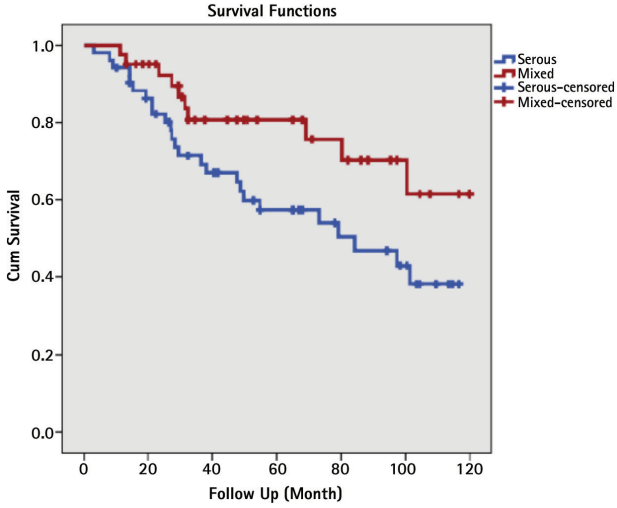

Figure 1. Overall survival graphs of pure serous and mixed serous groups

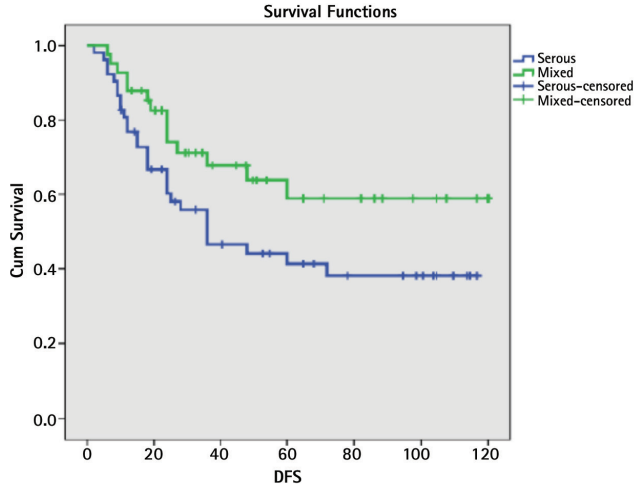

Figure 2. Disease-free survival in the pure serous and mixed serous groups

prognostic factors but not subtype in multivariate analyses.

Discussion

Serous endometrial carcinoma was first described by Hendrickson et al. (8) as tumors histologically similar to ovarian serous tumors and as a different and aggressive subtype of ECs. Patients with serous carcinoma represent 10% of ECs but are responsible for 39% of EC deaths (1). In the literature, data about survival in serous endometrial carcinomas are quite different. In a review, recurrence rates in stage I were reported as 7-50% (9). In the present study, 46.2% of patients had disease recurrence.

We investigated patients with serous endometrial carcinoma (serous component ratio at least 10%). In fact, this study focused on whether there was a difference in the clinicopathology and survival in patients with mixed serous and pure serous carcinoma. Mixed serous-type ECs are relatively less understood; the literature comprises fewer publications, and they are less prevalent. In a study conducted by the Gynecologic Oncology Group, Brinton et al. (5) detected mixed serous/endometrioid histology in 26 (0.6%) of 3828 patients with EC.

In the present study, we investigated patients with pure serous endometrial carcinoma and patients with mixed serous endometrial carcinoma with serous component of more than 10%. The clinicopathologic and survival parameters were statistically compared between these two subtypes.

The FIGO stage, which is considered as the most critical factor determining survival, was statistically similar in both groups (p=0.195). In more detail, we examined the spread of the disease via detailed analyses and determined no significant difference between the groups in terms of involvement of the perimetrium, fallopian tubes, intraperitoneal fluid, intestine or omentum (Table 2). Likewise, the number of patients who underwent lymphadenectomy, the number of patients with lymph node metastasis, and the number of lymph nodes removed were similar between the groups (Table 2). The groups were also similar in terms of the depth of myometrial invasion, endocervical involvement, tumor size, and lymphovascular space invasion, which are given as poor prognostic factors (Table 2).

Reviewing adjuvant therapy options in detail, we see seven different combinations of chemotherapy, external beam radiation therapy, and brachytherapy. Therapy options were statistically similar in the groups (Table 3).

Despite the absence of a difference between the groups in terms of either clinicopathologic findings or stage or treatment approach, the pure serous group seems to have had a worse prognosis. However, in the multivariate analysis, histologic subtype was not an independent factor for recurrence or survival (Table 4, 5). In a study on this topic, Roelofsen et al. (7) compared these two subtypes and found a 2.9-fold greater risk for recurrence and a 2.6-fold higher risk of death in the pure serous subtype. The authors suspected the two subtypes might be two different entities. To the best of our knowledge, no other clinical studies have compared these two groups.

The primary and adjuvant treatment of mixed serous and pure serous histology is not different. The European Society for Radiotherapy & Oncology, the European Society of Gynaecological Oncology, and the European Society of Medical Oncology in the first meeting reported that hysterectomy and bilateral salpingo-oophorectomy was the mainstay of therapy in apparent stage I disease and that radical hysterectomy was not recommended in stage II disease, whereas complete cytoreduction was required in advanced disease stages (10). However, there is no documentation on ovarian preservation. Bilateral salpingo-oophorectomy is mandatory (10). Differently, in the multidisciplinary panel, the authors stated staging omentectomy should be considered in serous carcinoma but not in clear cell or undifferentiated endometrial carcinoma and carcinosarcoma. No specific recommendations for mixed serous types have been provided. In adjuvant treatment issue, the largest retrospective study conducted to date suggested a survival benefit for the combination of chemotherapy and radiotherapy in uterine serous cancer (11). In the present study, adjuvant treatment combinations were similar for pure serous and mixed serous groups. In our clinic, an adjuvant treatment was given to all patients with non-ECC and the adjuvant treatment approach is not different for mixed serous or pure serous histologies.

The molecular biology and cellular origins of mixed-type endometrial carcinomas are poorly understood. In a study, molecular analysis of these two subtypes revealed that they were close in terms of molecular features and suggested that they could be treated similarly (12). In the present study, the groups were treated in similar ways in terms of both primary surgical therapy and adjuvant therapy. The pathologic outcomes of the groups were also similar (Table 2, 3). However, relapse and death rates were not similar.

It is arguable that planning more aggressive adjuvant therapy in a patient group where recurrence is considered to be more frequent — or contrarily, avoiding aggressive adjuvant therapy in a group with better prognosis — may be more convenient. However, randomized controlled studies are required to recommend different treatment to these groups.

Why survival is better in the togetherness of serous and endometrioid components, what kind of interaction influencing survival exists between two components, which of the components exists first in the endometrial cavity and why the other is included thereafter and whether this is important, and detailed molecular biology and cellular origins of these

two subtypes will be clarified in future studies.

This study has several limitations; the retrospective design and relatively small sample size for comparing the effect of adjuvant treatment options are the main limitations of the study. Also, it might be better to evaluate clinical outcomes according to serous component rates, but dividing the mixed histology into subgroups will reduce sample size and the serous component ratio of some patients was not clear. However, mixed serous histology (serous plus endometrioid) is not a common clinical entity (5).

In conclusion, this trial supported that mixed serous carcinomas have significantly better prognoses than pure serous carcinomas. However, histologic subtype was not an independent prognostic factor. Studies with large numbers of patients or multicenter studies that support our results may help in making a clearer picture.

Ethics Committee Approval: *No ethical approval has been sought because this was a retrospective study.*

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

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Concept - A.K., S.T., H.S., Y.M., Y.S.; Design - A.K., S.T., H.S., Y.M., Y.S.; Supervision - A.K., S.T., H.S., Y.M., Y.S.; Materials - A.K., S.T., H.S., Y.M., Y.S.; Writer - A.K.*

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

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

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The effect of progesterone use in the first trimester on fetal nuchal translucency

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Abstract

Objective: To evaluate the possible association between progesterone use in the first trimester of pregnancy and fetal nuchal translucency (NT).

Material and Methods: This is an observational case-control study, which was conducted with patients who underwent nuchal scans between March 2015 and February 2016 and consequently delivered live and healthy babies. The study group was composed of assisted reproductive technology pregnancies and used intravaginal progesterone 180 mg/day until gestational week 12. The control group comprised pregnant women who became pregnant spontaneously without using any progesterone preparation in the first trimester.

Results: One hundred sixty-four (57.5%) of 285 patients were in the control group and 121 (42.5%) were in the progesterone group. Age, bodyweight, gravidity, and parity number of previous births and abortus, gestational week, crown-rump lengths, free β-human chorionic gonadotropin, pregnancy-associated plasma protein A, and NT values of the progesterone and control groups were recorded and we investigated whether there was a statistically significant difference between the two groups in terms of these parameters; maternal weight was found to be higher in the progesterone group than in the control group and the difference between the groups was statistically significant ($p=0.019$ and $p=0.025$). Whether the difference in NT was caused by the effect of maternal weight was investigated using the covariance analysis test and maternal weight was not found to be statistically significant in the model ($p=0.284$).

Conclusion: Fetal NT was increased in the progesterone group compared with the untreated group in healthy pregnancies. (J Turk Ger Gynecol Assoc 2018; 19: 29-33)

Keywords: Progesterone, nuchal translucency, maternal weight, assisted reproductive technology pregnancies, prenatal screening test

Received: 3 May, 2017 **Accepted:** 20 December, 2017

Introduction

The first trimester combined test, which was first introduced in the 1990s, is a current test for the evaluation of fetal chromosomal anomaly (1-3). Data used to calculate risk in this test are fetal nuchal translucency (NT), maternal serum free β-human chorionic gonadotropin (β-hCG), and pregnancy-associated plasma protein-A (PAPP-A) between weeks 11 and 14 of pregnancy, in addition to maternal age (4,5). Giorlandino et al. (6), in their study published in 2015, hypothesized that progesterone could lead to abnormal blood flow patterns, and thus to increased NT. The authors, however, concluded that the results of the screening test were not affected.

NT, a sensitive marker in screening Down syndrome, is still considered controversial due to high false-positive rates (7-9). In addition, progesterone has been used widely for prophylaxis and treatment of abortus in cases of threatened miscarriage in the first trimester and in pregnancies conceived after assisted reproductive technology (ART) treatment (10-13). In this case, if the thickness of NT changes in patients using progesterone, the question is raised as to whether this condition increases the false positivity rate when screening for Down syndrome in the first trimester.

The aim of this study was to evaluate the possible association between progesterone use in the first trimester of pregnancy and fetal NT in healthy pregnancies without any known risk factors.

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Journal of the Turkish-German Gynecological Association published by Galenos Publishing House.
DOI: 10.4274/jtgga.2017.0056

Material and Methods

This is an observational case-control study, which was conducted with patients who underwent nuchal scans between March 2015 and February 2016 and consequently delivered live and healthy babies. All patients who participated in the study were between 11.0 and 13.6 weeks' gestation, which are the suitable gestational weeks for the first-trimester Down syndrome screening test, and their fetal crown-rump lengths (CRL) were between 41 mm and 84 mm. The study group was composed of patients who became pregnant with ART in a private *in vitro* fertilization (IVF) clinic and whose pregnancies were monitored; these patients used intravaginal progesterone 180 mg/day (Crinone gel; Serono, İstanbul, Turkey) until gestational week 12, as used in the monitoring of all ART pregnancies. The control group comprised pregnant women who became pregnant spontaneously without using any progesterone preparation in the first trimester. All the patients used folic acid, iron or multivitamin preparations, and those who were using other medications were not included in the study. Patients who were found to have high risk (cut-off 1/300) in the nuchal scan, whose NT values were above 2.5 mm and in whom any congenital or chromosomal anomalies were detected in amniocentesis or in the monitoring, and women who had systemic disorders such as diabetes and hypertension were excluded. Patients who had plural pregnancy or bleeding in the first trimester were also not included in the study.

In all patients, age, body weight, gravidity and parity, number of previous births and abortus, gestational week, CRL, PAPP-A, free β-hCG and NT values, presence/lack of nasal bone, and whether the patient was a smoker were recorded. For the patients' obstetric ultrasonographic (USG) evaluations, and CRL and NT measurements, a General Electric Voluson 730 Expert (GE Medical Systems, Kretztechnik, Zipf, Austria) with a 3D/4D transabdominal multifrequency probe was used. Two expert physicians performed the USG evaluations.

Statistical analysis

All statistical analyses were performed using the SPSS for Windows 11.5 software program (SPSS Inc., Chicago, IL). Compatibility of data with normal distribution was examined graphically and with the Kolmogorov-Smirnov test. For the quantitative variables, mean ± standard deviation and median (minimum-maximum) were used, and for the categorical variables, numbers (percentage) were used as descriptors in the study. When determining whether there was a statistically significant difference between the categories using qualitative variables with two categories in terms of quantitative variables, Student's t-test was used if the assumption of normal distribution was met; if not, the Mann-Whitney U test was used. The chi-square test was used to examine the relationship between the two categorical variables. Spearman's rank correlation coefficient was used to see if there was a statistically significant relationship between two quantitative variables because at least one of the variables did not meet the assumption of normal distribution. Covariance analysis (ANCOVA) was used to establish whether one or more continuous independent parameters had any impact on the dependent parameter. ROC analysis was used to find the discriminative factors between the groups. The significance level was set at $p=0.05$.

Results

One hundred sixty-four (57.5%) of the 285 patients were in the non-progesterone group and 121 (42.5%) were in the progesterone group. The age, body weight, gravidity and parity, number of previous births and abortus, gestational week, CRL, free β-hCG, PAPP-A and NT values of the progesterone and non-progesterone groups are shown in Table 1. Whether there was a statistically significant difference between the two groups regarding these parameters was examined. NT values were found to be higher in the progesterone group than in the non-progesterone group, and the difference between the groups were found to be statistically significant ($p=0.019$). Whether the difference in NT was caused by the effect of maternal weight was investigated using the ANCOVA test and it was not found to be statistically significant ($p=0.284$); it can be concluded that maternal weight does not affect NT. CRL values were found to be higher in the non-progesterone group than in the progesterone group, and the difference between the groups was found to be statistically significant ($p=0.026$). The parameter of gestational week was found to be higher in the non-progesterone group than in the progesterone group, and the difference between the groups was statistically significant ($p=0.006$).

The number of previous abortus was found higher in the progesterone group than in the non-progesterone group, and the difference between the groups was statistically significant ($p=0.019$). Maternal weight was found to be higher in the progesterone group than in the non-progesterone group, and the difference between the groups was statistically significant ($p=0.025$) (Table 1).

No statistically significant difference was found between the two groups regarding smoking status ($p=0.558$). The rate of non-smokers was 95.7% in the non-progesterone group and 94.2% in the progesterone group.

No statistically significant difference was found between the two groups with regards the presence/lack of nasal bone ($p=0.463$). The rate of presence of nasal bone was 93.9% in the non-progesterone group and 95.9% in the progesterone group.

We investigated whether fetal NT measurement was related to

maternal and fetal parameters and no statistically significant relationship was found between NT and these parameters (Table 2), and no statistically significant relationship was found between NT, smoking status of the mother, and the presence/lack of nasal bone (p=0.579 and p=0.950, respectively).

Discussion

The effect of first-trimester progesterone use on NT measurement was investigated in our study and it was seen that NT values were statistically and significantly higher in the progesterone group than in the non-progesterone group.

Giorlandino et al. (6) (2015) revealed that exogenous progesterone intake in the first trimester had an enhancing effect on fetal NT. In the same study, it was stated that the increase in NT did not change the results of the first-trimester fetal aneuploidy screening test and was independent of progesterone use. Nevertheless, a subsequent correspondence via the editor of the journal noted that the increase in NT was only in week 11 and did not include weeks 12 and 13 (14). On the other hand, it was stated that different preparations and uses might render the evaluation unhealthy. In another criticism, it was argued that bleeding in the progesterone group with the risk of miscarriage might change fetal circulation and have an impact on NT. Based on these criticisms, we planned to conduct our study with patients who used the same preparation for the same duration and had not had bleeding in pregnancy. Progesterone gel is administered intravaginally to all women

Table 1. Comparison of age, body weight, gravidity and parity, number of previous births and abortions, gestational week, crown-rump lengths, pregnancy-associated plasma protein-A, free β-human chorionic gonadotropin and nuchal translucency values in the progesterone and non-progesterone groups

Parameters	Use of progesterone						
	Non-progesterone group			Progesterone group			
	n	Mean ± SD	Median (minimum-maximum)	n	Mean ± SD	Median (minimum-maximum)	p
Maternal age (year)	164	31.1±4.4	31.0 (21.0-41.0)	121	31.6±4.9	31.0 (20.0-42.0)	0.406[a]
Maternal weight (kg)	164	65.0±11.4	64.0 (38.5-103.0)	121	68.0±10.7	67.0 (46.0-102.5)	**0.025[a]**
Gravidity	164	1.6±1.0	1.0 (1.0-5.0)	121	1.6±1.0	1.0 (1.0-4.0)	0.419[b]
Parity	164	0.4±0.7	0.0 (0.0-3.0)	121	0.3±0.6	0.0 (0.0-3.0)	0.136[b]
Number of previous abortions	164	0.2±0.5	0.0 (0.0-2.0)	121	0.4±0.7	0.0 (0.0-4.0)	**0.019[b]**
Gestational week	164	12.3±0.6	12.3 (11.0-13.6)	121	12.1±0.7	12.1 (11.0-13.6)	**0.006[b]**
CRL (mm)	164	61.1±8.5	60.0 (41.0-84.0)	121	58.6±9.3	58.3 (41.0-84.0)	**0.026[b]**
PAPP-A (mIU/mL)	164	3.5±2.7	2.7 (0.4-15.6)	121	3.7±3.6	3.0 (2.2-15.9)	0.493[b]
Free β-hCG (ng/mL)	164	54.1±43.1	39.2 (6.2-262.0)	121	59.5±45.3	48.0 (12.7-307.0)	0.114[b]
NT (mm)	164	1.2±0.3	1.1 (0.0-2.0)	121	1.3±0.4	1.2 (0.2-2.3)	**0.019[a]**

[a]Student's t-test; [b]Mann-Whitney U test; CRL: Crown-rump lengths; PAPP-A: Pregnancy-associated plasma protein-A; β-hCG: β-human chorionic gonadotropin; NT: Nuchal translucency; SD: Standard deviation

Table 2. Relationship between fetal nuchal translucency measurement and maternal and fetal parameters

Parameters	NT		
	n	r	p
Age	285	0.098	0.097
Weight	285	0.104	0.079
Number of pregnancies	285	-0.013	0.825
Number of previous birth	285	-0.040	0.497
Number of previous abortions	285	0.025	0.670
Gestational week	285	0.020	0.740
CRL	285	-0.009	0.886
PAPP-A	285	-0.044	0.455
Free β-hCG	285	-0.026	0.662

NT: Nuchal translucency; CRL: Crown-rump lengths; PAPP-A: Pregnancy-associated plasma protein-A; β-hCG: β-human chorionic gonadotropin

with post-ART pregnancies until week 12 in our collaborating IVF clinic; our study group was selected from among those women. No data regarding whether NT values were affected in ART pregnancies were observed in our literature review (15). Although it was reported in the literature that PAPP-A values were lower in ART pregnancies, PAPP-A values did not differ between the groups in our study (16). No relationship between NT values and gestational week was found; due to that fact and the limited number of patients, the progesterone-NT relationship was not evaluated by gestational weeks.

Keçecioğlu et al. (17) (2016) studied the subject with a group with low risk of miscarriage and reported that progesterone usage increased NT values and this change was positively related to the duration of progesterone usage. In our study, no such comparison was made as the progesterone usage durations and dosage remained fixed.

The relationship between NT values and maternal and fetal parameters was investigated in our study but no correlation was found. As maternal age advances, chromosomal anomaly incidence and consequently NT values increase; however, no study was observed in the literature to reveal the relationship between maternal age and NT value in fetuses with no chromosomal anomalies (18-20). It is normal that no relationship was established because no pregnancies with anomalies were included in our study. Ferreira et al. (21) (2015) investigated whether maternal age, which is known to affect NT measurement values by physicians who perform USG NT evaluations, and it was reported that measurements by expert ultrasonographers had no impact on the values, contrary to those of inexperienced operators. In our study, the physicians who evaluated NT with USG knew the maternal ages.

The most important limitation of this study is that some parameters differed statistically and significantly in the progesterone and non-progesterone groups. As for the differing parameters, the number of abortus being higher in the progesterone group is an expected result because that group included only ART pregnancies and this would not affect the NT value. Again, the fewer gestational weeks and lower CRL values in this group would not cause an increase in NT values. It was investigated whether there was a relationship between higher maternal weight and higher NT values in the progesterone group, and it was concluded that the maternal weight parameter did not affect the NT parameter. As we currently do not know how ART affects NT values in the first trimester, the study design would be more appropriate if all patients were similar in terms of the ways they became pregnant; this is also a limitation of our study.

In conclusion, it was seen in our study that fetal NT was increased in the first-trimester progesterone group compared with the untreated group. These data need to be confirmed by future studies with larger groups of patients such that it can be reflected in prenatal screening tests.

Ethics Committee Approval: *Ethics committee approval was received for this study from the Ethics Committee of Liv Hospital/Ankara.*

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

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Concept - M.N.K., Z.K., A.E.; Design - M.N.K., Z.K.; Supervision - A.E., T.G.; Materials - M.N.K., A.E., Z.K., B.B.; Writer - M.N.K., A.E.*

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

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

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Hysterectomy by vaginal-assisted natural orifice transluminal endoscopic surgery: Initial experience with twelve cases

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Abstract

Objective: To declare our initial experience on hysterectomy cases performed using vaginally-assisted natural orifice transluminal endoscopic surgery.

Material and Methods: The study was conducted with data from 12 patients in our department who were recommended for hysterectomies for various indications between January 2017 and May 2017. The following data were collected retrospectively: age, body mass index (BMI), parity, previous abdominal or pelvic surgery, total operating time, preoperative hemoglobin (Hb), postoperative Hb, peri-operative complications and Visual Analogue Scale scores for evaluating postoperative pain. All patients were laid in the dorsal lithotomy position under general anesthesia. A cervical circumcision, as well as anterior and posterior colpotomy were performed. A self-constructed glove port was then inserted through the anterior and posterior colpotomy openings into the abdominal cavity. After pneumoperitoneum was achieved, a 10-mm rigid zero-degree telescope, disposable conventional laparoscopic grasping forceps, and a tissue sealer were used as standard equipment. After exploration of the abdominal cavity, all uterine vessels and ligaments were sealed and transected using the tissue sealer. After removing the uterus, the vaginal opening was closed using a Vicryl 1-0 suture.

Results: The following are the mean values for each variable: patients' age: 55.75±9.8 years (range, 43-72 years), BMI: 29.4±5.4 kg/m^2 (range, 21-42 kg/m^2), operation duration: 66.8±25.3 min (range, 42-120 min), decrease in Hb: 1.5±1 (0-4) gr/dL, and hospital stay: 2.1±0.3 (2-3) days. There were no vaginal wound infections or dehiscence, and no patients reported pain during postoperative pelvic examinations.

Conclusion: Although these findings are from our initial experience, we can affirm the feasibility of this technique. (J Turk Ger Gynecol Assoc 2018; 19: 34-8)

Keywords: Hysterectomy, laparoscopic surgery, natural orifice transluminal endoscopic surgery

Received: 1 June, 2017 **Accepted:** 31 December, 2017

Introduction

Minimally invasive laparoscopic techniques have been widely used for gynecologic diseases. Advances in technological equipment such as flexible optics, reticulated graspers, and sealers have allowed umbilical single-port surgeries to become more common in daily practice. Single-site surgeries have benefits over conventional multiple-port laparoscopic surgeries, which include decreased anxiety from unintended surgical skin incisions and port site pain (1). In recent years, natural orifice transluminal endoscopic surgery (NOTES) has been used by general surgeons, urologists, and gastroenterologists for performing cholecystectomy, appendectomy or nephrectomy through natural body orifices such as the stomach, esophagus, bladder, rectum and vagina. It has also been reported that oophorectomies and even hysterectomies can be performed using NOTES by gynecologic surgeons (2). These procedures have the advantage of magnifying pelvic structures with optical systems, and thus increase the comfort of surgery through better visualization than open abdominal or vaginal surgeries. NOTES procedures also have the potential benefit of reduced umbilical or port site

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Journal of the Turkish-German Gynecological Association published by Galenos Publishing House.
DOI: 10.4274/jtgga.2017.0075

hernia rates and shorter recovery periods than conventional laparoscopic procedures (3).

Historically, the transvaginal route has been routinely used for diagnostic evaluation of infertility (4). Recently, a randomized controlled study was published by Baekelandt et al. (1) that compared transabdominal laparoscopy and NOTES. However, there is still a lack of knowledge on NOTES procedures in gynecologic practice. In this report, we aimed to declare our initial experience on hysterectomy cases that were performed with vaginal-assisted (Va) NOTES.

Material and Methods

The study was conducted with data from 12 patients in our department who were recommended for hysterectomies for various indications between January 2017 and May 2017. All procedures were performed by the same experienced surgeon in endoscopy (C.K.); ethical approval was obtained from our institutional ethics committee (Approval number: 2017/04/24). Data analyses of all patients who gave informed consent were included.

The inclusion criteria for patients undergoing VaNOTES hysterectomy were as follows: no contraindication for pneumoperitoneum or the Trendelenburg position, no fixed uterus or nodularity in the pouch of Douglas on bimanual pelvic examination, and no history of pelvic inflammatory disease, pelvic abscess or endometriosis. The following data were collected retrospectively: age, body mass index (BMI), parity, type of delivery, previous abdominal or pelvic surgeries, duration of anterior and posterior colpotomy, total operating time, preoperative hemoglobin (Hb), postoperative Hb, peri-operative complications, and decrease in Hb levels.

The duration of anterior and posterior colpotomy was defined as from the beginning of cervical circumcision to the point of accessing the abdominal cavity via both anterior and posterior openings. The total duration of surgery was defined as the time from the placement of the Foley catheter to the end of vaginal closure. Peri-operative complications included bowel, bladder, ureteral or vascular injuries and blood loss >300 mL. Postoperative complications included lower urinary tract infections, ileus, bleeding from the vaginal cuff or infections, and pelvic hematoma. Postoperative pain scores were assessed using a Visual Analogue Scale (VAS) (scoring from 0=no pain to 10=worst pain ever) after 6 and 24 hours postoperatively. Prophylactic antibiotic therapy was induced with 2 g of cefazolin during surgery. Each patient was called for postoperative follow-up after one and four weeks after surgery.

Anterior and posterior colpotomy

The patients were laid in the dorsal lithotomy position under general anesthesia. After appropriate sterilization and draping of the surgical field, a Foley catheter was inserted into the urethra. The cervix was identified using vaginal retractors and grasped at the upper and lower lips with two tenaculum forceps. A cervical hydrodissection was performed with a 20 cc saline injection, after which cervical circumcision was performed with a 20-mm scalpel. For the anterior colpotomy, the vaginal mucosa and bladder were pushed up along the uterine cervical fascia using a small surgical gauze. Once the anterior peritoneum was identified, it was opened with scissors, and the same dissection procedure was performed to access the posterior peritoneum until the pouch of Douglas was reached.

Insertion of self-constructed glove port

A size 8 surgical glove without powder was used. The middle finger of the surgical glove was incised with a 1-cm opening, through which a 10-mm disposable trocar was inserted for CO_2 insufflation and an endoscopic camera. The index and the 4th finger of the glove were incised with a 0.5-cm opening, through which two 5-mm disposable trocars were inserted for the laparoscopic instruments. All trocars were fixed to the glove using a number 0 silk suture. A small-sized Alexis Wound Protector/Retractor (Applied Medical, Rancho Santa Margarita, CA, USA), which was attached to the glove, as well as a self-constructed port, were inserted through the anterior and posterior colpotomy openings into the abdominal cavity.

Laparoscopic procedure

After achieving pneumoperitoneum with 10 mm Hg CO_2 insufflation, a 10-mm rigid zero-degree telescope was then inserted for optical imaging (Karl Storz visualization system; Karl Storz Tuttlingen, Germany). Disposable conventional laparoscopic grasping forceps and a tissue sealer (Enseal G2 articulating tissue sealer, Ethicon Endo Surgery, Cincinnati, OH, USA) were used as standard equipment. A suction-irrigation cannula was used when and where needed. After exploration of the abdominal cavity and pelvic organs, both sacro-uterine ligaments were sealed and transected using a tissue sealer. This procedure was repeated for the parametrium, both uterine arteries, ovarian ligaments and the Fallopian tube (Figure 1, 2). Both infundibulopelvic ligaments were sealed and transected if a bilateral salpingo-oopherectomy was planned. After complete resection of the uterus from its ligaments and vessels, it was placed into the glove (size permitting) or pulled into the abdominal cavity to obtain better visualization for hemostasis and rinsing of the peritoneal cavity. Afterwards, the pneumoperitoneum was deflated and the glove port was removed. The uterus and/or adnexa was extracted using tenaculum forceps. The vaginal opening was closed with a Vicryl 1-0 suture (Ethicon, Piscataway, NJ, USA).

Results

Twelve VaNOTES hysterectomy procedures were performed and no conversion to standard multi-incision laparoscopy was needed. The following are the mean values for each variable: patients' age: 55.75±9.8 years (range, 43-72 years), gravidity: 2.5±05 (2-3), parity: 2.5±05 (2-3), BMI: 29.4±5.4 kg/m^2 (range, 21-42 kg/m^2), uterus weight: 188.5±76.6 gr (100-300), size of uterus: 7.83±1.58 weeks (range, gestational week), operation duration: 66.8±25.3 (42-120) minutes, preoperative Hb: 12.1±1.3 (10-14) gr/dL, postoperative Hb: ±10.5 (9-13) gr/dL, decrease in Hb: 1.5±1 (0-4) gr/dL, blood loss: 170.83±68.95 cc (range, 100-300 cc), hospital stay: 2.1±0.3 (2-3) days, postoperative 6th hour VAS score: 6.2±0.5 (5-7), postoperative 24th hour VAS score: 2.7±0.6 (2-4). Five patients had bilateral salpingo-oophorectomy in addition to hysterectomy, and the remaining 7 patients had only bilateral salpingectomy in addition to hysterectomy. Table 1 presents an overview of the patient and perioperative data. Each patient was examined during the first and fourth weeks after surgery. There were no vaginal wound infections or dehiscence, and no patients reported pain during the postoperative pelvic examination.

Discussion

Anatomically, the vaginal route is a well-known approach for surgical procedures by gynecologists. However, only a handful of institutions have started performing a transvaginal NOTES for benign gynecologic conditions (3,5,6). Current evidence is based on case series reported from these centers.

Baekelandt recently reported results of ten hysterectomies performed using transvaginal NOTES. In his study, there was no conversion to standard laparoscopy or laparotomy in any of the ten patients, with a mean operation time of 97 minutes (range, 60-120 min). He concluded that the NOTES approach may be useful for avoiding abdominal wall wounds and trocar-related complications (3). In another study by Lee et al. (5), the largest hysterectomy series yet with 137 patients undergoing the transvaginal NOTES technique, 94.9% (n=130) of patients were successfully treated, with the mean operative time and hospital stay being 88.2±4.1 minutes and 2.8 days, respectively. The mean uterine weight was 450.0±24.1 grams. Two patients had complications including intraoperative hemorrhage and cystotomy, and five had transvaginal colpotomy failure, cul-de-sac obliteration by bowel adhesions or mass obstruction. Another five patients had postoperative urinary retention or febrile morbidity (5). Yang et al. (6) reported 16 patients who underwent VaNOTES hysterectomies in comparison with 32 who underwent paired- and single-port laparoscopy-assisted vaginal hysterectomies (SP-LAVH). The authors reported no difference in intraoperative and postoperative outcomes such as estimated blood loss decrease in Hb on postoperative day 1, VAS scores, and febrile complications. However, the mean operative time was 70.6 minutes for VaNOTES hysterectomies and 93.2 minutes for SP-LAVH. The median postoperative hospital stay was 3.5 days for VaNOTES hysterectomies and 4 days for SP-LAVH (6). Jallad et al. (7) also recently reported eight salpingo-oophorectomy cases (six of them were unembalmed cadavers and two were live patients) in which the transvaginal NOTES technique was performed in the patients. First, they performed vaginal hysterectomy

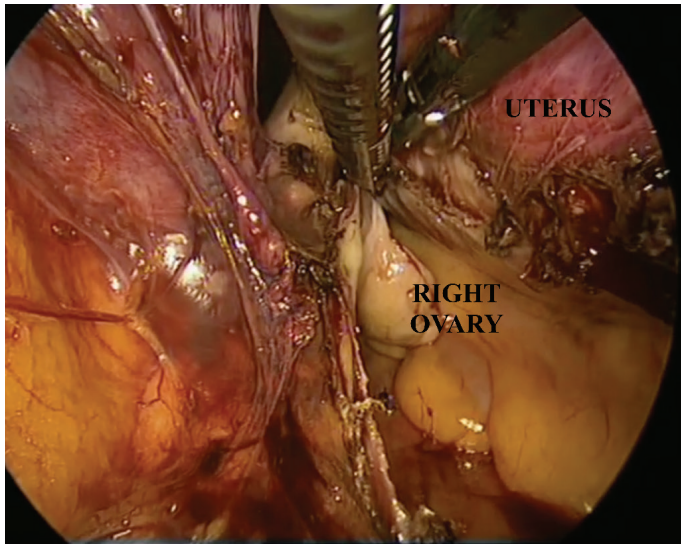

Figure 1. Endoscopic vaginal view of uterine cervix and corpus

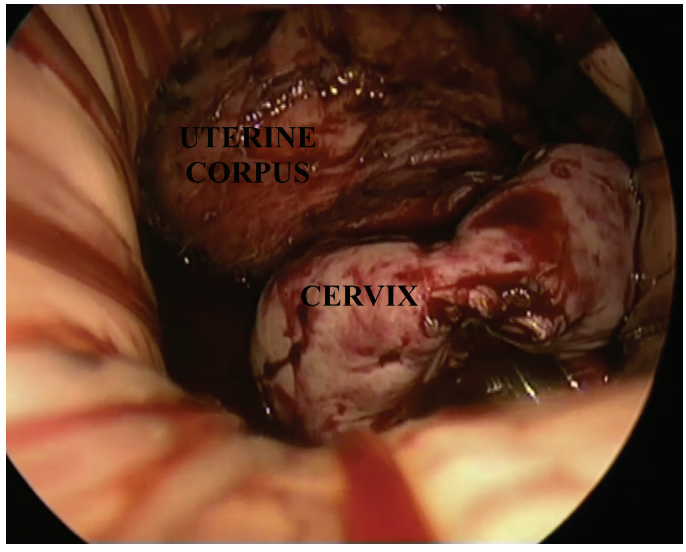

Figure 2. Endoscopic vaginal view of right salpingectomy procedure by using tissue sealer

Table 1. Overview of VaNOTES hysterectomy results and perioperative data

Case no	BMI kg/m^2	Previous Op	Size of uterus (week)	TVUS	Indication	Operation	Duration (min)	Blood loss (mL)	Pathology
1	25.7	None	6	-	HSIL	Hyst+BSO	60	100	HSIL
2	32.8	Append	8	-	HMB	Hyst+BSO	60	150	Adenomyosis
3	27.3	None	6	-	Endometrium Ca	Hyst+BSO	42	100	Endometrium Ca grade 1
4	23.4	None	8	40 mm myoma	HMB	Hyst+BSO	45	150	Myoma
5	21	None	10	70 mm myoma	Pain	Hyst+BS	80	200	Myoma
6	27.7	2 C/S	8	60 mm myoma	HMB	Hyst+BS	110	300	Myoma
7	31.2	None	8	60 mm myoma	HMB	Hyst+BS	70	150	Myoma
8	42.2	1 C/S, Umbl hernia	8	-	HMB	Hyst+BSO	120	300	Simple hyperplasia
9	31.3	1 C/S	6	-	HMB	Hyst+BS	60	150	Adenomyosis
10	33.3	None	10	70 mm myoma	Pain	Hyst+BS	45	100	Myoma
11	29.1	None	10	60 mm myoma	HMB	Hyst+BS	65	200	Myoma
12	28.2	1 C/S	6	-	HMB	Hyst+BS	45	150	Adenomyosis

*BMI: Body mass index; TVUS: Transvaginal ultrasound; HMB: Heavy menstrual bleeding; Hyst: Hysterectomy; BS: Bilateral salpingectomy; BSO: Bilateral salpingo-oophorectomy; HSIL: High grade squamous intraepitelial lesion

with a conventional technique and subsequently performed a salpingo-oophorectomy through a transvaginal NOTES technique. In this study, the ovaries appeared normal, and the main objective was to prove that transvaginal NOTES may facilitate salpingo-oophorectomy procedures after conventional vaginal hysterectomies.

In our study, we succeeded in finalizing the operations with a planned route using VaNOTES, with a mean operation duration that was lower than previously reported (3,5). This result may depend on the sealing device that we used for transection of the uterine attachments. In the studies mentioned above, the mean hospital stay varied between 2.8 to 3.5 days (3,5,6). On the other hand, the mean postoperative hospital in our study was merely 2.1 days, even after keeping patients as inpatients to observe any postoperative early complications. The mean Hb decrease and VAS scores in our study was similar to previous investigations.

Although these findings are from our initial experience, we firmly believe in the feasibility of this technique. By advancing the clinical experience of these cases, we may postulate that NOTES hysterectomies may take the place of total laparoscopic hysterectomies or single-port laparoscopic hysterectomies in the near future.

Ethics Committee Approval: *Ethics approval was obtained from institutional local ethical committee (Approval number: 2017/04/24).*

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

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Concept - C.K.; Design - C.K., İ.A.; Supervision - L.Y., M.E.; Materials - C.K.; Data Collection and/or Processing - C.K., İ.A.; Analysis and/or Interpretation - C.K., İ.A., Literature Review - C.K., İ.A.; Writer - C.K., Critical Review - C.K., L.Y., M.E.*

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

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

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Zika virus and pregnancy in Brazil: What happened?

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Abstract

The recent epidemic of Zika virus (ZIKV) infection in Central and South America is one of the most serious global public health emergencies since the Ebola outbreak in West Africa. In Brazil, especially in the north, northeast, and southeast parts of the country, the ZIKV outbreak is a cause of concern for pregnant women because ZIKV intrauterine infection has been found to be associated with multiple brain malformations and microcephaly. In Brazil, the number of newborns with confirmed microcephaly per year recorded during the ZIKV outbreak, has been approximately 15 times greater than previously reported. Considering that the infection is self-limiting and symptomatic, it is usually diagnosed at the time of routine prenatal scan, especially in the third trimester. In other cases, the disease is detected after childbirth through neuroimaging. This study provides an insight into the history and evolution of ZIKV in Brazil, including current knowledge concerning the transmission, diagnosis, and pathogenesis of the infection. In addition, this review describes the pre- and postnatal neuroimaging findings obtained using ultrasound, magnetic resonance imaging, and computed tomography. (J Turk Ger Gynecol Assoc 2018; 19: 39-47)

Keywords: Zika virus, intrauterine infection, microcephaly, ultrasound, magnetic resonance imaging

Received: 29 May, 2017 **Accepted:** 13 October, 2017

Introduction

A significant epidemiologic surge of registered cases of newborns affected by microcephaly has occurred in the past two years in Brazil. Further evidence has highlighted a possible association of microcephaly with fever, cutaneous rash, and Guillan-Barré syndrome (GBS) in pregnant women living in areas where Zika virus (ZIKV) is endemic (1-4). ZIKV was known to cause such symptoms since its discovery in Africa after the second world war raising health attention and concerns following viral spread and outbreak from French Polynesia and Yap island (5) to countries of Central and Latin America, where the virus has shown to be particularly harmful. Until the ZIKV genome was isolated and extracted from the brain cells of a third trimester aborted fetus affected by severe microcephaly in a mother who had a presumed ZIKV intrauterine infection in the first trimester of pregnancy (6), the relationship between viral infection and microcephaly remained a conundrum for healthcare providers. This discovery represents a milestone in the understanding of the infectious disease while confirming a direct pathogenetic and teratogenetic role of ZIKV in cases of congenital infection. However, does ZIKV act as other components of the *Flaviviridae* family or are its teratogenic and neurotropic effects exerted by a different pathogenetic mechanism? Secondly, is there a potential close relationship between ZIKV and specific environmental factors in many areas of Brazil that might have contributed to the degree and severity of congenital ZIKV infection? Some of these crucial questions have been answered by phylogenetic studies, which demonstrated how the Brazilian strain of the ZIKV ($ZIKV^{BR}$) was genotypically similar in 99% of cases to that isolated in French Polynesia (7). Nonetheless, although a large and rapidly growing number of microcephaly cases

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Journal of the Turkish-German Gynecological Association published by Galenos Publishing House.
DOI: 10.4274/jtgga.2017.0072

were recorded in women with suspected congenital ZIKV infection, serologic demonstration of the virus was only reported in a small subset of mothers (8) due to lack of laboratory tests at the time of the first Brazilian outbreak and availability of such tests in a low-resource clinical setting.

The present review aims to sum up historical and recent insights into the understanding of this severe congenital infection, from epidemiology to diagnosis, prognosis and prevention. Particular results are reported about antenatal and postnatal neuroimaging findings occurring in pregnancy after intrauterine ZIKV infection.

Etiology

The ZIKV is wrapped by a hicosaedryc capsid where the genome is formed by a single positive RNA helix of 10.794 nucleotides in length. It belongs to the *Flaviviridae* family, *Flavivirus* genus, which includes the yellow fever virus, dengue virus (DENV), and West Nile virus (WNV).

ZIKV virions are approximately 40-60 nm in size (9) and the viral genome encodes for a single polyprotein of approximately 3400 amino acids, which is further processed into ten different proteins consisting of three structural and seven non-structural proteins (10). It was first identified in a rhesus monkey in 1947 in the Zika forest in Uganda (11). Phylogenetic studies indicate the existence of two strains of the virus: Asian and African. The virus was first detected in humans in Nigeria in 1952 (12). In the following 60 years, benign and sporadic isolated cases of infection in humans were reported in Africa and Southeast Asia. The first outbreak of the disease occurred in the Yap Islands in 2007 where 49 confirmed and 59 suspected cases of ZIKV infections were detected. However, it was estimated that approximately 73% of the population was infected during this outbreak (13). Between 2013 and 2014, more than 28.000 suspected cases of infection were reported in French Polynesia and other Pacific islands. The ZIKV then spread into Brazil, Suriname, and Colombia *via* the Pacific Ocean. Genetic analysis revealed that the virus identified in Brazil belongs to an Asian lineage and originated from the strains from French Polynesia and the nearby islands (14,15). It has been demonstrated that the ZIKV^{BR} in humans may be able to infect the neural progenitor cells (NPCs) causing cell death by inducing apoptosis and autophagy and disrupting the cortical layers leading to microcephaly (7). The history of the ZIKV is summarized in Table 1.

Table 1. Place of discovery and epidemic outbreak of Zika virus

1947	Discovery of the Zika virus	Uganda	
1952	First human infection	Nigeria	
2007	First epidemic outbreak	Yap Islands	49 cases
2013	Second epidemic outbreak	French Polynesia	>400 cases
2015	Third epidemic outbreak	South America	>1.5 million cases in Brazil (according to the Brazilian Ministry of Health)

Transmission

As with other *Flaviviruses*, the transmission cycle of ZIKV between primates and mosquitoes is complex, and man is the occasional unintentional host. The intrinsic period of incubation of ZIKV in human hosts is 4-5 days. During this period, the virus infects other vectors that feed on infected blood, and after an extrinsic incubation period of 8-12 days, the virus is transmitted *via* vector saliva to other hosts (16). The virus is transmitted by mosquitoes of the *Aedes aegypti* species, which are also responsible for transmitting yellow fever, DENV, and chikungunya (CHIKV) fever. ZIKV has also been identified in *Aedes albopictus* mosquitoes (12).

It is noteworthy that *Arboviruses* (mosquito-borne viruses) usually replicate in the cytoplasm of dendritic cells (17), although a different replication mechanism has been hypothesized for ZIKV because its antigens have been observed within cell nuclei (18). It is interesting that the association between intrauterine ZIKV infection and microcephaly/GBS may be the result of the replication of ZIKV in a population with a high *Flavivirus* background- like pre-exposure to DENV infection (19).

The first case to suggest potential sexual transmission of the disease was reported in 2011. In 2015, the second case was reported, and in February 2016, the Centers for Disease Control in the United States recognized sexual transmission as a cause of ZIKV infection. Interestingly, ZIKV RNA can be identified in semen for up to 62 days, although the disease usually develops within 19 days following sexual intercourse (20).

Maternal–fetal transmission is one of the major concerns because ZIKV can cross the placenta at any stage of gestation, causing teratogenic effects (21). It has been confirmed that intrauterine infection occurring during the first trimester of pregnancy is associated with multiple congenital anomalies, mainly affecting the developing brain. The microcephaly risk due to ZIKV infection in the first trimester of pregnancy was estimated as 0.88 to 13.2% in cases of viral infection in the first trimester of pregnancy (22). ZIKV has been detected in the amniotic fluid, placenta, fetal tissues, and abortuses (23). The risk of transmission *via* blood transfusion from infected

individuals was confirmed when the ZIKV outbreak occurred in French Polynesia (24).

Clinical presentations

ZIKV infection is asymptomatic in most cases. The rate of symptomatic individuals among those infected during the Yap Island outbreak in 2007 was 18% (13). There is no difference in the clinical presentation of pregnant and non-pregnant women infected by the virus, and individuals of all age groups are susceptible to the infection. Clinically, the symptoms appear a few days after being bitten by the mosquitoes and are characterized by generalized maculopapular rash, low intermittent fever, conjunctivitis, arthralgia, headache, myalgia, and asthenia. The symptoms have a short duration (2-7 days) and are usually self-limiting; the need for hospitalization is rare (25). In countries where ZIKV has active transmission, an increased incidence of neurologic syndromes, including encephalitis, meningoencephalitis, myelitis, acute flaccid paralysis, and GBS have been recorded (26).

Although ZIKV infection in the mother is accompanied by mild and less severe symptoms, it may cause multiple congenital malformations in the form of prematurity, placental insufficiency, and fetal growth restriction, which may progress to intrauterine fetal demise. Moreover, ZIKV intrauterine infection is associated with an increased number of spontaneous abortions (27).

Laboratory diagnosis

Laboratory diagnosis is based on the detection of ZIKV RNA using real-time reverse-transcriptase polymerase chain reaction (rRT-PCR) of serum within 5 days from the onset of symptoms. rRT-PCR of serum should be performed after the onset of symptoms because viremia is limited and the decrease in the viral load in maternal blood is very rapid after the onset of rash. The short-term positivity in serum and limited access to tests were the primary contributors to the difficulty in diagnosing the disease during the ZIKV outbreak in Brazil. ZIKV RNA can also be detected in urine with higher titers and for a longer period than in blood (usually within 20 days from the onset of symptoms). ZIKV has also been detected in saliva, semen, and cervical and uterine secretions. However, the suppression period has not yet been determined, and the virus may remain in the urine and semen for several months (11). rRT-PCR can also identify viral RNA in amniotic and cerebrospinal fluids (28). Serum immunoglobulin (Ig) M antibodies produced by ZIKV infection may be detected from the fifth day from infection using ELISA or immunofluorescence; however, these tests are not specific for ZIKV. Cross-reactivity with other *Flaviviruses* is common and precludes diagnosis in individuals with previous infections including DENV and CHIKV, and those vaccinated against yellow fever (16). Positive IgM antibody tests for ZIKV should be confirmed using the plaque-reduction neutralization test (PRNT), which is specific for ZIKV infection. Currently, this test is not available in Brazil. Although the conventional PRNT test provides a diagnosis of ZIKV infection within 7 days, a recent study reported the development of a rapid PRNT that may provide a diagnosis within 48 hours, without reducing the specificity of the PRNT test (29).

The disease in Brazil

ZIKV was first diagnosed in Brazil in the State of Bahia in 2015 based on the test results of serum from patients with DENV-like symptoms, including rash, fever, myalgia, arthralgia, and conjunctivitis (30). Later in September, a study reported a significant increase in the number of cases of microcephaly in newborns in northeastern Brazil and later in southeastern Brazil (31). ZIKV RNA was isolated from the amniotic fluid of pregnant women carrying fetuses with confirmed microcephaly and from the brains of fetuses with central nervous system (CNS) malformations (21).

In 2014, 147 cases of microcephaly were reported in Brazil, and in November 2015, the Brazilian Ministry of Health declared a public health emergency of national importance [Emergência em Saúde Pública de Importância Nacional (ESPIN)] because of changes in the pattern of occurrence of microcephaly in Brazil. Between 2015 and 2016, 10.232 cases of changes in the growth and development of newborns possibly due to ZIKV infection and/or overlapping infections were reported. However, the World Health Organization (WHO) did not recognize ESPIN until February 2016. Since March 2016, the number of reported cases of ZIKV intrauterine infection has significantly decreased in Brazil, partly due to the application of a "Rapid Action Strategy" [Estratégia de Ação Rápida (EAR)]. This program strengthens both healthcare and social protection for children with microcephaly. Additional federal funds were allocated to Brazilian States and municipalities to ensure access to diagnostic tests and monitoring the growth and development of these children. The cases of microcephaly registered by EAR were mainly concentrated in the northeast region of Brazil (67.0%), followed by the southeast (20.2%), midwest (5.1%), north (5.0%), and south (2.0%) regions. The States with the highest number of postnatally confirmed cases of microcephaly were Pernambuco (392 cases), Bahia (335 cases), and Rio de Janeiro (234 cases) State (32). In 2016, 215.319 probable cases of fever caused by ZIKV infection were reported in Brazil. Eight neonatal deaths due to ZIKV infection were confirmed through laboratory examination—four in Rio de Janeiro, two in Espírito Santo, one in Maranhão, and one in Paraiba. Although Rio de Janeiro and Piauí State did not report any suspected cases (3), 2347 newborns with microcephaly have been recorded in Brazil as of January 2017.

In 2017, 1653 probable cases of intrauterine ZIKV infection were reported, confirmed postnatally in 275 newborns. The incidence in the northern region of Brazil was higher than that in the other areas. As of February 2017, no cases of neonatal death due to ZIKV infection were confirmed in laboratory examinations (32).

Fetal infection

The mechanism by which ZIKV crosses the placenta is still unclear, but its neurotropism (6) and ability to destroy neural cells have been clearly studied (33). ZIKV infection induces abnormal mitotic and apoptotic cell death of human NPCs, causing disruptive lesions in the fetal CNS (34). NPCs are the primary target of the ZIKV, and this may partly explain the high number of abnormalities seen in the CNS and detected by neuroimaging examinations (35). The abnormalities affecting the developing brain are often diagnosed during the third trimester of pregnancy (36-38) or after birth. The teratogenic effectcs to the embryo, fetus, and newborn caused by ZIKV constitute what has been referred to as congenital ZIKV syndrome. These abnormalities include CNS disorders, impaired development of the eyes and ears, and arthrogryposis (39,40). The most common findings affecting the CNS are microcephaly, ventriculomegaly, brain calcifications, midline echo, and cerebellar defects. When microcephaly is considered, the most severe forms are those following congenital ZIKV infection occurring in the first trimester of pregnancy (41).

Microcephaly is a congenital malformation caused by impaired growth of the fetal brain, which leads to a decrease in the head circumference (HC). The Brazil Ministry of Health recommends an occipito-frontal circumference (OFC) of 32 cm or 2 standard deviation (SD) below the Fenton reference (42,43) for the diagnosis of microcephaly, wheres "severe microcephaly" is defined by an OFC of <3 SD (43-46). The reference ranges for HC most commonly used in Brazil are those reported in the InterGrowth chart (45,46), although the use of InterGrowth standards reduced the detection of microcephaly compared with Fenton growth chart (43). On November 18th, 2015, the Brazilian Ministry of Health defined newborns with gestational age (GA) of at least 37 weeks with HC smaller than 33 cm at birth as having microcephaly, for which registration is mandatory. However, due to the high numbers of false-positive cases, this cut-off value was reduced to 32 cm on December 9th, 2015 (32).

The role of ultrasound antenatal management

The International Society of Ultrasound in Obstetrics and Gynecology recommends the following ultrasound (US) monitoring protocols (47):

a) Confirmation of GA preferably before 14 weeks of pregnancy by measuring the crown–rump length;
b) Basic US: basic biometry with evaluation of fetal anatomy and growth rate;
c) Subsequent US: pregnant women with a clinical history of rashes, with or without serologic confirmation, should be scanned every 4-6 weeks;
d) In cases where US shows either fetal HC with 2 SD below the mean expected value for GA and sex or CNS malformations, the mother should be referred to a tertiary care center. A thorough neuroimaging examination should address morphology, integrity, and degree of skull ossification; presence of the cerebral midline echo throughout the length of the skull; and symmetry of the intracranial structures, such as lateral ventricles, cavum septum pellucidum, thalami, cerebellum, and cisterna magna. If available, magnetic resonance imaging (MRI) should be planned to search for other abnormalities affecting the corpus callosum, neural migration, and reduced gyration and sulcation. Other causes of overlapping infections should be discarded. Pregnant woman should be informed of the benefits and risks of amniocentesis to detect ZIKV RNA by rRT-PCR in amniotic fluid.

Prenatal radiological findings

Detailed knowledge of possible neuroimaging findings in newborns following congenital ZIKV infection is crucial for correct diagnosis, and enhancing parent counseling about the prognosis of the affected child (35).

Ultrasound: US is the method of choice for monitoring pregnant women living in areas at increased risk of congenital ZIKV infection. HC is easy to measure, and microcephaly is the most common finding in cases of congenital ZIKV infection. Studies conducted in Brazil to monitor fetal microcephaly using US indicated that microcephaly was severe in 73.7% of patients with HC <5 SD below the mean expected value for GA. Only 10.5% of fetuses had microcephaly alone, whereas 89.5% of fetuses had additional CNS malformations, including periventricular or parenchymal calcifications (63.2%), symmetrical or asymmetrical ventriculomegaly (47.4%), cerebellar abnormalities (42.1%), and cortical atrophy (15.8%). Doppler findings of the umbilical artery of these fetuses were unremarkable (48). Other abnormalities seen were craniofacial disproportion, agenesis or hypoplasia of the corpus callosum, congenital clubfoot, oligohydramnios, cardiac calcification, hepatomegaly, hyperflexion of the second and third fingers, and arthrogryposis. It is important to note that in almost all cases, the US findings were unremarkable until weeks 24, *i.e.*, pathologic findings are usually diagnosed only after GA of 24 weeks. Therefore, a normal morphologic examination *per se* does not rule out the possibility of congenital infection (48). For this reason, pregnant women with proven or suspected

congenital ZIKV infection should be followed up with US until delivery (Figure 1).

Magnetic resonance imaging: The MRI findings in fetuses with congenital ZIKV infections are similar to those reported with US. However, brain abnormalities such as polymicrogyria, lissencephaly, pachygyria, abnormal myelination, and changes to white matter and the cerebral cortex, are best evaluated using MRI. However, the sensitivity of MRI in the detection of intraparenchymal brain calcifications is lower than that reported using US (Figure 2) (35).

Postnatal radiologic findings

Ultrasound: Transfontanellar US is the examination of choice for the assessment of newborns because of its safety, ease of use, and low cost. It provides adequate visualization of the brain parenchyma and of the ventricular system. US is accurate in the evaluation of the location of cerebral calcifications, and is diagnostic in detecting ventriculomegaly, dysgenesis of the corpus callosum, subependymal cysts, malformations of cortical development, cerebellar and hypoplasia and brain stem (35). Small fontanels or premature closure of cranial sutures as well as bony structures of the skull are common findings in infected children and these factors may hinder US accuracy (Figure 3).

Computed tomography: Computed tomography (CT) scans provide excellent sensitivity in the assessment of parenchymal calcifications and skull bone deformities, particularly in cases where a three-dimensional reconstruction is used. Intracranial calcifications are clearly identified in CT scans; calcifications may be punctate or coarse and usually located in the corticomedullary junction or immediately below this junction in the frontal and parietal lobe or in the periventricular lobe. Calcifications may rarely occur in the basal ganglia, thalami and

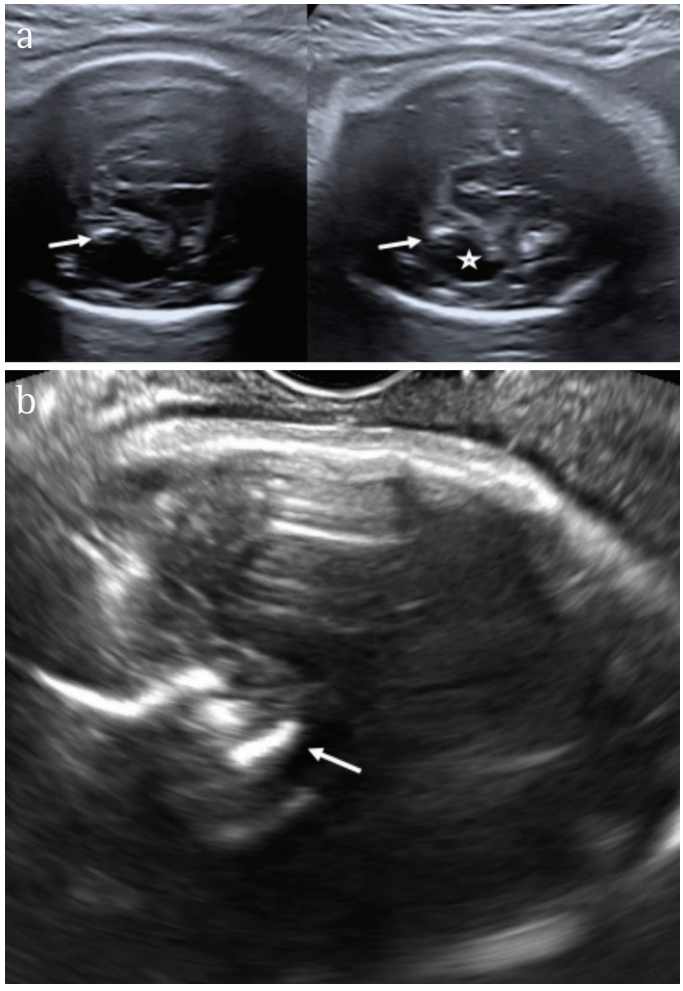

Figure 1. Prenatal ultrasound showing calcifications (arrows), ventricular dilatation (*) and microcephaly. Transabdominal axial plane (34 weeks) (a), ultrasound imaging of the fetal brain during third trimester of pregnancy is hinder by of the ossified skull base. Axial plane obtained by means of transvaginal probe (b) brain calcifications are more visible (arrow)

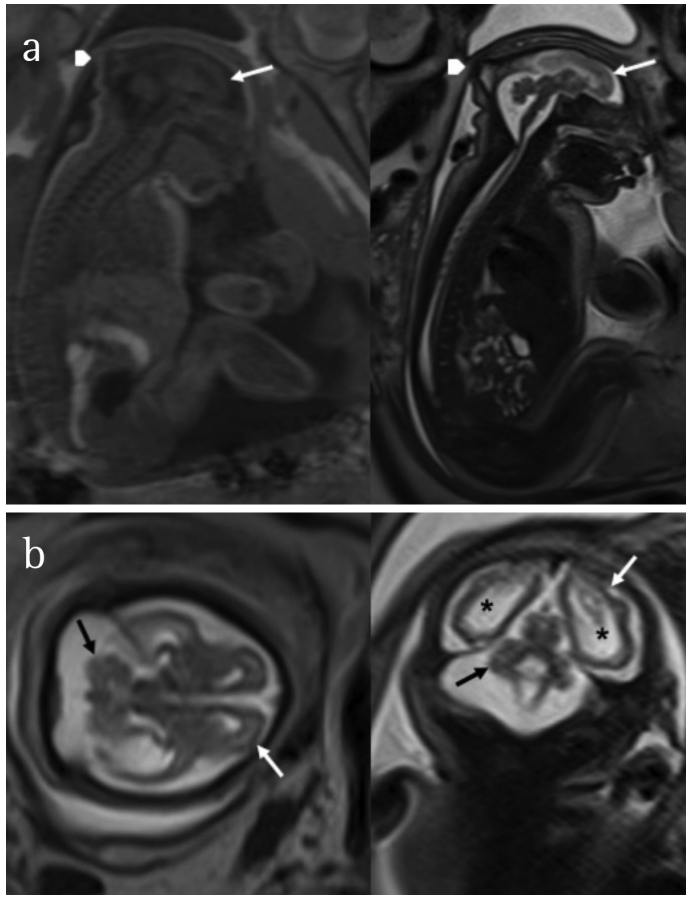

Figure 2. T1- and T2-wieghted magnetic resonance imaging in sagittal plane (37 weeks). Note microcephaly and smoothness of the brain surface (arrow) and redundant skin fold (arrow head) (a), axial and coronal planes showed cortical atrophy (white arrow), ventricular dilatation (*) and cerebellar hypoplasia (black arrow) (b)

cerebellum. Other phenotypic anomalies include craniofacial disproportion, depression of the frontal and parietal bones, overriding of sutures, small fontanels, demyelination, and abnormal density of white matter. Deformities of the cranial bones are secondary to acute cerebral atrophy and reduced intracranial pressure; these findings are commonly seen in infected children (49). The disadvantage of CT, compared with US and MRI, is the increased exposure to radiation (Figure 4).

Magnetic resonance imaging: The quality of MRI in neuroimaging makes it the first choice in the evaluation of children with congenital ZIKV infection. MRI offers high sensitivity and specificity to detecting brain pathology in fetuses and newborns. Defects in the development of the cerebral cortex are associated in almost all cases of microcephaly and are characterized by cortical atrophy and abnormalities in the pattern of the brain gyri (polymicrogyria) and sulci. Demyelination or delayed myelination causes changes in the brain mantle in 88-100% of cases. Moderate-to-severe ventriculomegaly occurs in 85-100% of cases and usually affects the entire ventricular system. Interventricular septa are found in 10-30% of cases, and changes in the corpus callosum (agenesis or atrophy) may affect up to 94% of newborns. Cerebellar hypoplasia may be unilateral or bilateral and affects 27-82% of infected newborns (35). Congenitally-infected children often have sleep disturbances and restlessness requiring sedation before undergoing MRI (Figure 5).

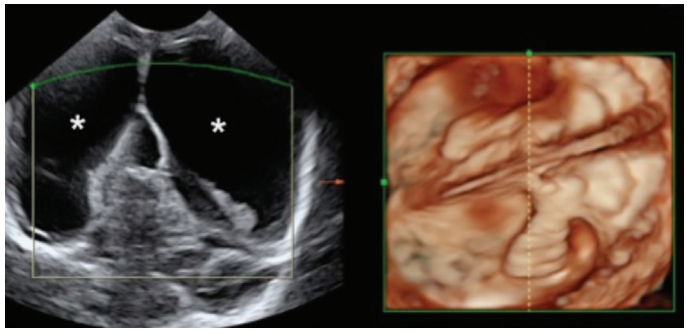

Figure 3. Postnatal transfontanellar ultrasound performed with three-dimensional volume reconstruction showing ventricular dilatation (*)

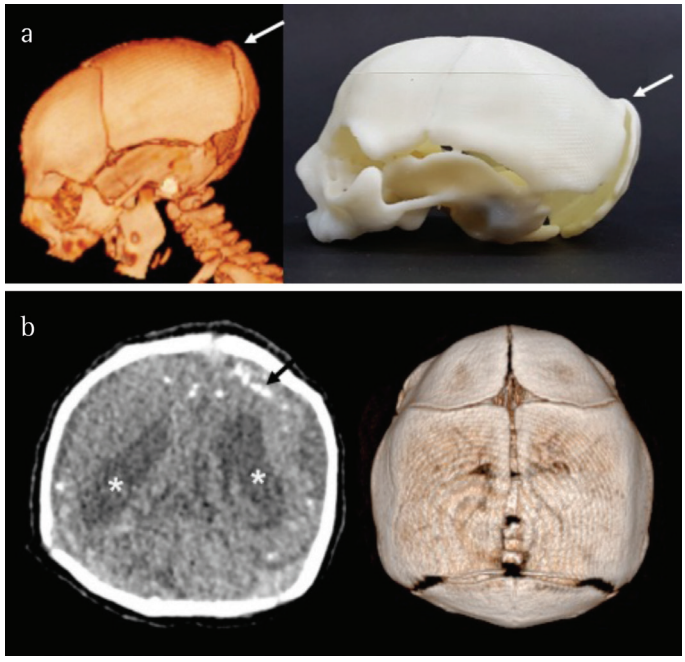

Figure 4. Three-dimensional sagittal reconstruction from computed tomography scan and corresponding three-dimensional printing. The skull has collapsed appearance (arrow) (a), axial plane shows frontal lobe calcifications (arrow), ventricular dilatation (*) with three-dimensional axial reconstruction (b)

Congenital infection without microcephaly

Congenital microcephaly may be a marker of intrauterine ZIKV infection but the disease may not always be detected at birth. A Brazilian study involving 13 newborns with congenital ZIKV infection but born without microcephaly documented the presence of other CNS abnormalities, including ventriculomegaly, cerebral atrophy, subcortical calcifications, and cortical malformations, underlining the need to use

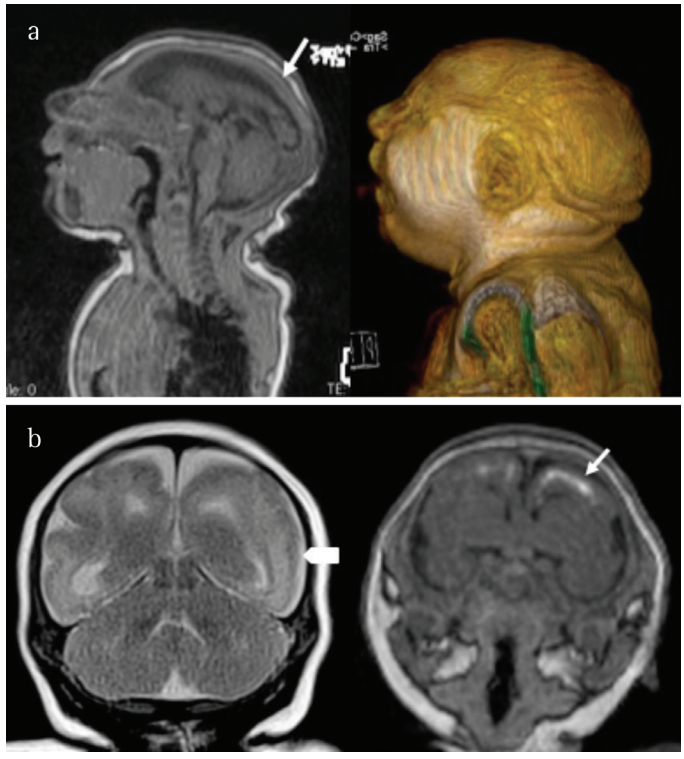

Figure 5. Postnatal T1-weighted magnetic resonance imaging in sagittal plane showing cortical atrophy (arrow) and three-dimensional reconstruction showing microcephaly (a), coronal T2- and T1-weight images showing smoothness of the brain (arrow head) and calcifications (arrow) (b)

neuroimaging in these assessments. In these clinical series, the development of microcephaly only presented after birth and was accompanied by severe neurologic disorders, including hypertension, hemiparesis, dystonia, dysphagia, epilepsy, and the persistence of primitive reflexes. These symptoms did not differ from those found in children where microcephaly was detected at birth. However, children born without microcephaly showed better social interaction and made and maintained eye contact and social smiles. However, in this group, 60% of the children had epilepsy and all presented with neuromotor disorders (50).

Prevention

The most effective prevention strategy is vector control, either by integrated insect management or personal prevention with repellents, long-sleeved clothes, and cooled air. The WHO has also proposed the possibility of using genetically modified mosquitoes to control the populations of *Aedes aegypti*. Since March 2016, academic and pharmaceutical institutions have been working on the development of various types of vaccines against ZIKV, which include the use of live and attenuated viruses (16).

Treatment

Treatment primarily consists of supportive measures and rest because ZIKV is usually a self-limiting infection, although long maternal viremia has been reported (33). To date, no drugs have been approved for the treatment of ZIKV or other *Flavivirus* infections. A recent *in vitro* study suggested that ZIKV infection could respond to treatment with interferon. However, more studies are needed to confirm this finding (16).

Prognosis

The biologic effects of ZIKV infection are variable in degree and severity, with different clinical manifestations. The more severe the case of microcephaly, the worse the prognosis is. Early diagnosis allows for early encouragement and improvement of neuromotor performance in children with ZIKV infection.

In conclusion, the outbreak of ZIKV as seen in Brazil in the last two years has contributed to increased numbers of notified cases of microcephaly in newborns; when microcephaly was diagnosed antenatally it was usually at the time of the third trimester scan, and was confirmed, in the vast majority of cases, postnatally by means of CT scans or MRI; antenatal and postnatal diagnosis of microcephaly should be performed according to specific constructed US reference curves; primary microcephaly should be excluded by genetic testing; ZIKV infection as well as overlapping viral infectious diseases (yellow fever, WNV, DENV and CHIKV) and TORCH should be investigated and excluded with diagnostic laboratory testing; amniocentesis to search for viral ZIKV RNA should be recommended after 14-16 weeks in all pregnant women with proven intrauterine ZIKV infection; a thorough US examination, especially a neuroscan, should be performed by expert sonographers in tertiary care centers and fetal MRI incorporated as a complementary neuroimaging investigation; ZIKV has high neurotropism and causes teratogenic effects in the fetus mainly affecting the developing brain; the most severe forms of ZIKV-related congenital infections are associated with prolonged viral shedding and direct placental action is also hypothesized; in targeted cases the ZIKV genome should be sought and isolated from the placenta, and brain tissue and phylogenetic studies should be conducted to investigate the different ZIKV strains; newborns with ZIKV-associated microcephaly should undergo neurodevelopmental outcomes. Although ongoing research is trying to develop specific vaccines against ZIKV, appropriate counselling is advised to prevent congenital infection in pregnant women, especially for those living in epidemic areas of the virus.

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Concept - A.M.P., D.L.M.M., H.W.; Design - A.M.P., P.D., H.W.; Supervision - H.W., E.A.J.; Materials - T.F., B.G.; Writer - A.M.P., G.T., A.B.P., E.A.J.*

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

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

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The implications of male human papilloma virus infection in couples seeking assisted reproduction technologies

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Abstract

Human papilloma virus (HPV) is one of the most common viral sexually-transmitted diseases worldwide. The prevalence of HPV is higher in infertile males when compared with fertile men and ranges between 10 and 35.7% in men affected by unexplained infertility. HPV can bind to spermatozoa and can potentially be transferred to fertilized oocytes. Viral detection in blastocysts and trophoblastic cells is associated with impaired embryo development and poor pregnancy outcomes. Nevertheless, attempts to eliminate HPV-DNA from sperm samples through routine washing techniques have failed. In assisted reproduction technologies (ART), intracytoplasmic sperm injection involves no natural selection of the sperm cell, which means that these procedures have a plausible risk of injecting sperm containing HPV. The possible detrimental effects of HPV on ART in couples with infected male partners are summarized in this review. (J Turk Ger Gynecol Assoc 2018; 19: 48-52)

Keywords: Human papilloma virus, assisted reproduction, male infection

Received: 21 April, 2017 **Accepted:** 31 July, 2017

Introduction

Human papilloma virus (HPV) is one of the most common viral sexually-transmitted diseases in the world. The prevalence of HPV is 40% in the general population (1). HPV infection in men is considered transient and usually clears spontaneously over time. The clinical finding in men is usually penile warts on the external genitalia. At present, there is neither an effective treatment nor a control strategy for HPV infection in men. Men are generally treated through removal of the infected tissue. The presence of HPV has also been documented in the reproductive system (testis, epididymis, and ductus deferens) (2-6) and in semen (4,7).

The consequences of HPV in the male reproductive system have been the focus of recent research. Meanwhile, many authors reported that HPV infection was associated with reduced sperm motility and idiopathic asthenozoospermia (8,9). In addition, in infected patients, there are significantly more anti-sperm antibodies positive sperm cells (10). Male fertility impairment in HPV-DNA infection was widely reviewed by Gizzo et al. (11). Whether HPV infection in males is a casual factor for infertility still needs to be elucidated. Another debate is the possible detrimental effects of HPV on assisted reproduction technologies (ART) in couples with infected male partners, which will be summarized in this review.

The prevalence of HPV in infertile patients

Among the sexually transmitted diseases, HPV is the most prevalent in semen (38.1%). The prevalence of HPV semen infection is 53.8% in patients with genital warts, 40.9% in males with infected partners, 10.2% in infertile patients, and 2.2% fertile controls (12). In a literature review of the last two decades, higher percentages of HPV infection were found in infertile men compared with fertile controls, and a possible correlation between HPV sperm infection and unexplained male infertility (9). Recently, the data from 1138 males showed that 6.7% of fertile males (n=523) and 17% of idiopathic infertile

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Journal of the Turkish-German Gynecological Association published by Galenos Publishing House.
DOI: 10.4274/jtgga.2017.0031

males were infected with HPV (n=615) (13). In a meta-analysis that evaluated HPV prevalence, 16% [95% confidence interval (CI): 10-23%] was reported from seven studies in the infertile group, whereas it was 10% (95% CI: 7-14%) in 11 reports from other populations (7). In 2015, a systematic literature review of the last two decades found that the prevalence of HPV in men ranged between 10 and 35.7% in unexplained infertility (9).

In a large study, seminal HPV was positive in 16% of fertility clinic attendees (14). In infertile patients undergoing *in vitro* fertilization (IVF)-intracytoplasmic sperm injection (ICSI) cycles, the reported prevalence of male HPV infection was 9.5% in a prospective study by Perino et al. (15), and 7.8% in the study of Schillaci et al. (8). In sperm donors, the prevalence of HPV was 16% in the study of Kaspersen et al. (16).

According to a recent epidemiologic classification, the HPV genotypes were considered low or high (oncogenic) risk (17). In most infected men, oncogenic type HPV is detected (7). Kaspersen et al. (16) reported oncogenic type HPV in 66.7% of cases, and Gimenes et al. (18) reported it in 82.7% of cases. HPV16 is the most prevalent type (23.4%), followed by HPV31, HPV51, and HPV56 (each type detected in 10.9%) and HPV42 (9.4%) (14). Infection with two or more genotypes simultaneously was observed in 5.3% of the cases in the study of Kaspersen et al. (16); 34% in the study of Foresta et al. (19), and up to 58.6% (17/29) in that of Gimenes et al. (18).

The prevalence of HPV in specific groups of patients needs to be confirmed in larger populations with an assay that is validated for use in sperm samples. Unfortunately, a 'gold standard' test for subclinical HPV infection for men is lacking. Unlike cervical liquid-based cytology tests, the problem of detecting HPV in semen samples is that sperm contains many inhibitors, and the currently available commercial HPV tests are not validated for use in sperm samples. Polymerase chain reaction is usually used to detect male infection in semen (12). However, HPV DNA present in semen plasma only represents part of the detectable HPV DNA. HPV has been detected in semen and in spermatozoa, particularly in the sperm head (20). HPV16 capsids can bind to live human sperm cells (21). In infected males, HPV was detected at the sperm head in 25% of the whole sperm population (22). HPV types 6, 16, 18, and 31 can bind to the sperm cell head at or near the equatorial segment (16). High-risk HPV type 16 can attach to negatively charged glycosaminoglycans on the sperm cell surface (21,23). Moreover, syndecan-1, which is localized in the equatorial segment of the sperm head, can interact with L1 capsid protein of HPV (3). The most effective method for evaluating whether the virus is located in sperm or exfoliated cells is fluorescence in situ hybridization analysis (12). In infertility, especially men undergoing ART, detection of HPV at the spermatozoa level seems to be a prerequisite to clarify outcomes.

Sperm washing techniques and HPV

Even if little is known about the consequences of HPV infection in couples with infertility, this infection might affect the outcome and safety of ART. Nevertheless, routine sperm washing techniques do not eliminate HPV-DNA from samples (19), and in ICSI, sperm selection techniques do not guarantee non-infected sperm injection. After sperm washing centrifugation, infected samples and infected cells do not change. A minimal effect was observed in Ficoll and swim-up protocols regarding the number of infected samples (30 and 26, respectively) (19). Much better results were obtained using the modified swim-up with heparinase-III (24,25). As mentioned before, HPV can bind to negatively charged cell-surface glycosaminoglycans. Heparin is a sulfated polysaccharide that resembles GAGs in chemical structure. Heparin molecules can directly bind to papillomavirus capsids and block their association with the sperm cell surface (21). Hence, this method for washing sperm with heparinase-III for removing HPV is promising (24) but the question remains as to whether real virions can be removed or eliminated in modified swim-up technique with heparinase-III.

HPV transmission to fertilized oocytes

HPV is able to bind to the sperm head at or near to the equatorial segment (3,10,21). At the sperm head, HPV DNA positivity is about 25% but integration into the sperm nucleus is unclear (20). E6 gene specific mRNA of HPV type 16/18 is expressed in infected sperm cells *in vitro* (26). Infected sperm are able to transfer HPV E6/E7 genes and L1 capsid protein into the oocytes (3). In *in vitro* studies, exogenous HPV-DNA, brought by sperm cells, has been observed in cumulus cells during fertilization (26,27). Considering the data above from experimental studies, the infected spermatozoa is a vector for HPV transmission into fertilized oocytes (3,28,29) and transmission of virus-destabilized genes to oocytes during fertilization is possible.

HPV infected male and embryo development

In addition, during recent years, investigations on HPV implications in couples seeking fertility indicated poor reproductive outcomes. Numerous authors indicated reduced fertility potential in couples with HPV-infected males because the negative impact of HPV infection was observed at various steps of human embryo development (3,21,28,29).

The negative effects of HPV infection on the early development of infected embryos depend on viral genome expression at blastocysts and trophoblasts (11,29,30). The oncogenic HPV types 16 and 18 are able to inhibit embryo development only at the 2 cells stage, and there is a reduction in blastocyst formation (31). After HPV16 exposure, embryo development inhibition can be up to 30% (31). After HPV18 exposure, hatching at the

blastocyst stage can be disturbed (31). The current evidence from *in vitro* studies in blastocyst stage embryonic cells also demonstrates impaired embryo development (32). When mouse blastocysts are incubated with HPV-DNA-16 fragments, DNA fragmentation and apoptosis increases (32). Studies of patients undergoing ART also support the negative effect of HPV on human embryo development. A significantly reduced blastocyst formation rate was found in infected couples when compared with non-infected couples (54% vs. 27%, $p<0.05$) (33). The causal effects of HPV infection start within the very early stages of embryo development. Therefore, the possible negative role of HPV infection needs to be clarified with further *in vitro* studies.

Implantation and pregnancy rates

Regarding implantation rates, HPV infection resulted in a 37% reduction in implantation in murine embryos (34). Supporting these data, in human ICSI cycles cumulative pregnancy rates were significantly lower in infected couples when compared with non-infected couples (38% vs. 14%, $p<0.05$) (33). In another prospective study that investigated infertile HPV-infected couples undergoing ART, the pregnancy rate was lower in infected couples compared with non-infected couples (15). The reported pregnancy rates in HPV negative and positive men were 33.3% and 31.6%, respectively (15). In the same study, the pregnancy rates in HPV negative and positive women were 31.1% and 42.9%, respectively (15). Others also reported that cervical HPV infection in women undergoing IVF resulted in significant reduction in pregnancies when compared with HPV-negative women (23% vs. 57%) (35). In intrauterine insemination cycles, conceiving was six times less likely to occur in women with HPV infection in cervical cytology specimens compared with women without HPV infection (1.87% vs. 11.4%) (36). Data about HPV positivity and pregnancy rates are limited; however, they imply a trend towards lower pregnancy rates. However, more scientific evidence is needed to clarify this issue.

Pregnancy outcomes

HPV is suggested as an etiologic agent of some miscarriages depending on the data, reporting that HPV is more prevalent in spontaneous abortions compared with elective pregnancy terminations (60% vs. 20%) (37). Moreover, in a recent study in infertile couples, a significantly higher abortion rate was observed in infected couples compared with non-infected couples (62.5% vs. 16.7%, $p<0.05$) (33). Concordantly, the rate of spontaneous abortion in women with HPV infection (n=66) was two-fold higher than that of HPV-negative women (n=900) (12% vs. 6%) (38).

The possible negative impact of HPV infection on early embryo development could result in abnormal placentation and early pregnancy loss (15,33). A few studies have shown apoptosis of embryonic cells through fragmentation in infected embryos as the probable cause of pregnancy loss (30,34,39). In 3A trophoblastic cells cultured with different types of HPV (11,16,18,31), active expression of early and late genes of HPV was observed (39). Moreover, trophoblastic cell number is reduced and trophoblastic-endometrial cell adhesion was abnormal (39,40). In trophoblastic cells transfected with HPV16, the apoptosis rate was 3- to 6-fold higher (30). In addition, HPV viral genome expression in early stages of embryo development reduces the invasiveness of trophoblastic cells (30). A progressive decrease in the invasion ability of trophoblastic cells (25-57%) from day 3 to 15 after transfection was reported (30). The down regulation of adhesion protein E-cadherin in trophoblastic cells with HPV16 viral genome expression was suggested as the possible explanation for the reduction of invasiveness of trophoblastic cells (41).

In ART cycles, the consequences of HPV infection in men might be more serious due to the plausible risk of injecting sperm containing HPV. When HPV infection is diagnosed in sperm cells of the male, the risk of pregnancy loss significantly increases (15). An abortion rate of 66% was reported in couples with HPV-infected males (15% in non-infected males) (15). According to these data, cervical HPV infection increases pregnancy loss (40% vs. 13.7%), but all pregnancies resulted in miscarriages when both partners had HPV infection (15).

On the other hand, authors investigating cervical HPV in women undergoing IVF showed no significant difference in miscarriage rates (35). The analysis of HPV-DNA in placenta from term deliveries was positive in 24%, and 17% in the placenta from spontaneous miscarriages (total n=129) (42). These rates were 15% and 24%, respectively, in the study by Conde-Ferráez et al. (43), which had 127 women in each group. Other authors (44) also confirmed that there was no causal association between HPV and pregnancy loss in a retrospective analysis of cervical HPV infection prevalence in unexplained recurrent miscarriages (n=49) and healthy controls (n=475) (26% vs. 61%, respectively).

To date, the negative impact of HPV infection demonstrated by *in vitro* studies has not been confirmed by *in vivo* studies. The adverse outcomes depending on the very early stages of embryo development cannot be demonstrated by clinical studies. Speculation will continue until a better understanding about HPV's possible role in adverse pregnancy outcomes is obtained.

In conclusion, in couples seeking ART, despite the risk of viral transmission, HPV is not part of viral screening protocols (45).

The viral screening of couples undergoing ART includes HIV, hepatitis B and hepatitis C, but not HPV (45) in the United States and also in Europe.

It remains unclear if sperm infection may have a negative impact on assisted fertilization and pregnancy outcomes. HPV is highly prevalent in the general population. Nearly 16% of the infertile population is infected (7). HPV can bind to spermatozoa and can potentially be transferred to fertilized oocytes. Viral detection in blastocyst and trophoblastic cells is associated with impaired embryo development and poor pregnancy outcomes. All the above-mentioned points raise concerns about the consequences of HPV infection in males undergoing ART. Nevertheless, the paucity of clinical data enables to draw strong conclusions.

As a first step, semen samples of risky subjects (those with genital warts and infected partners) can be screened for HPV. Secondly, caution should be recommended to eliminate HPV infection from sperm cells or reduce the infectiousness of samples before use in ART or in sperm banking. Another option for HPV-positive men might be to wait for viral clearance, which is approximately seven months for men (45,46). Moreover, detection of HPV at the spermatozoa level might be explanatory for pregnancy losses as indicated by previous studies (15,35). However, screening for HPV infection at the spermatozoa level of all couples before IVF needs an effective validated method before use in clinical practice.

Finally, large population-based studies will help to develop clinical guidelines to improve ART outcomes and ART safety in HPV-infected males. Vaccination of males might have secondary benefits because HPV is one of the most common viral sexually-transmitted diseases in the world and also a possible casual factor for infertility. However, cost-effectiveness of such a protocol needs to be confirmed before application.

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Concept - G.S.C.; Design - G.S.C.; Supervision - N.G.; Materials - G.S.C.; Data Collection and/or Processing - G.S.C.; Literature Review - G.S.C.; Writer - G.S.C., N.G.; Critical Review- N.G.*

Conflict of Interest: *The authors declare no conflict of interest.*

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

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What is your diagnosis?

A 25-year-old, second gravida woman was referred to the fetal medicine clinic at 19 weeks' gestation for a fetal skin biopsy. Her first baby was a home delivery and had abnormally thick, rough skin and expired on day 2 of life. Two previous ultrasound scans in this pregnancy at 16 weeks and 18 weeks were reported to be normal. On repeating the ultrasound, we saw clenched fists, contractures of toes, and minimal fetal movement with stiff limbs in a semi-flexed position. A 3-dimensional (3D) scan showed thick pouting lips, flat nasal bridge, distal contractures of the toes with incurving, clenching of fingers, and flattened ears (Figure 1). Taking into account the history of consanguinity, a previous baby with a skin disorder, and these ultrasound findings, the parents were counseled and after deliberation, they opted for termination of the pregnancy. A postnatal examination confirmed the features seen on 3D ultrasound (Figure 1). The fetus showed facial dysmorphism, clenched fists, and contractures of the toes of both limbs. A histopathologic examination of the skin biopsies (from the fetal forearm, trunk, lower limbs, and buttocks) revealed marked orthokeratotic hyperkeratosis with a granular layer.

Four years later, the woman conceived again and consulted us at 18 weeks' gestation. Unfortunately, even in this pregnancy, the fetus on 2D and 3D ultrasound was found to have similar features (Figure 2) as observed in her last pregnancy; therefore, after counseling, the pregnancy was terminated. Post-natal examination of the fetus corroborated the findings seen on the 3D scan (Figure 2).

Received: 5 October, 2017 **Accepted:** 31 December, 2017

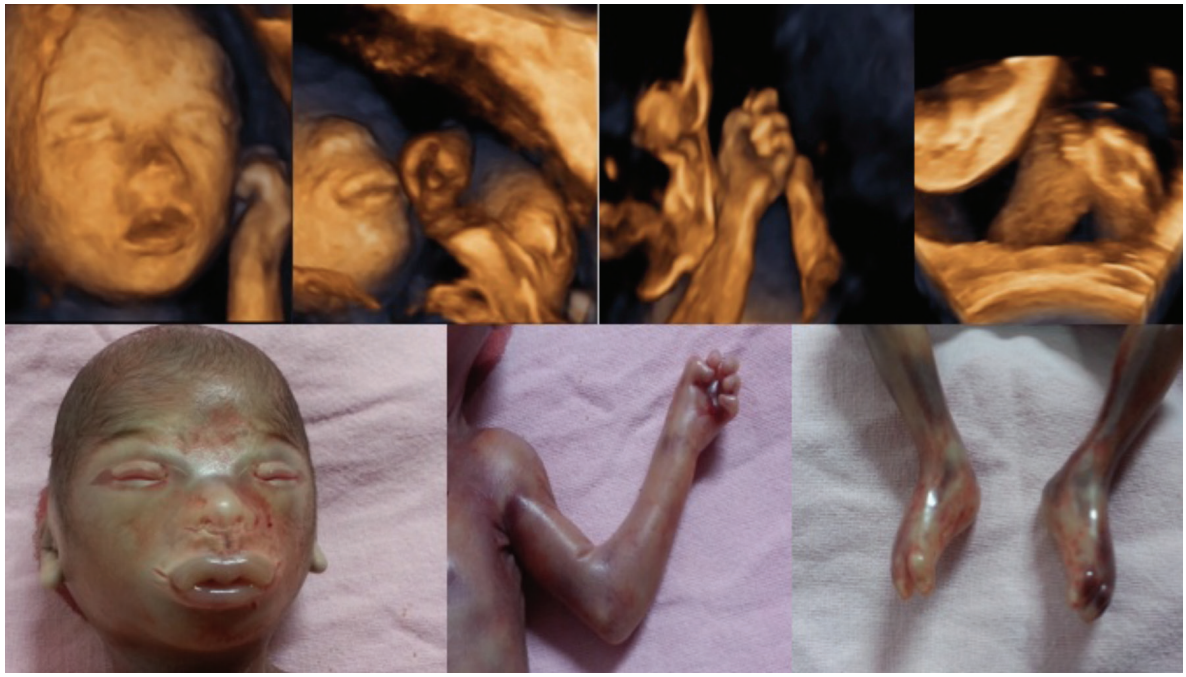

Figure 1. Antenatal 3-dimensional-ultrasound images and post-natal images of the fetus in the second pregnancy showing the typical phenotypic features of Harlequin ichthyosis (eclabium, ectropion, small nose, clenched fists and incurved toes)

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Journal of the Turkish-German Gynecological Association published by Galenos Publishing House.
DOI: 10.4274/jtgga.2017.0117

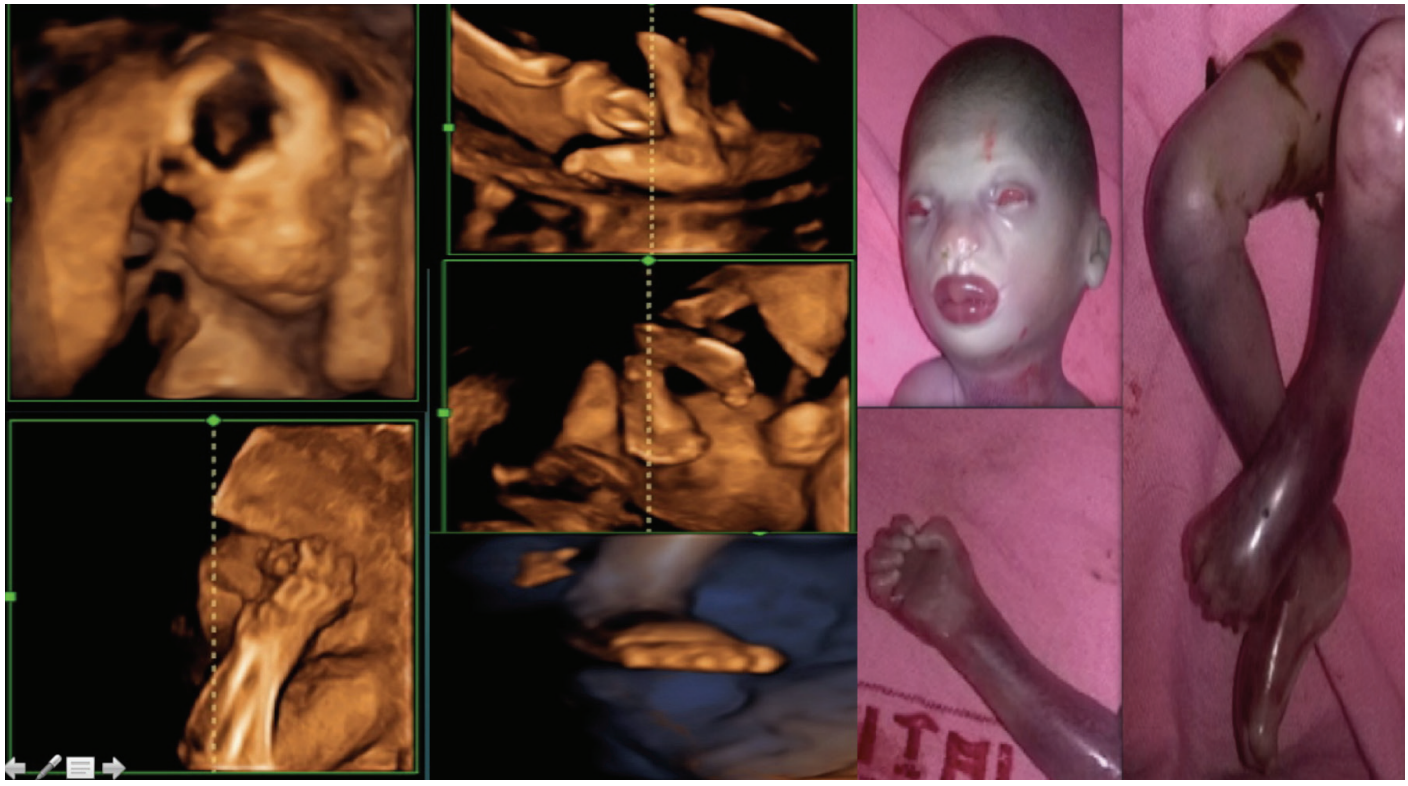

Figure 2. Antenatal 3-dimensional-ultrasound images and post-natal images of the fetus in the third pregnancy showing the classic phenotypic features of Harlequin ichthyosis (eclabium, ectropion, small nose, clenched fists, incurved toes and contracture at the bilateral knee joints)

Answer

On the basis of the characteristic phenotypic features of the first born who succumbed and ultrasound findings in second pregnancy, a suspicion of ichthyosis was entertained, the couple was counseled, and they agreed for the termination of pregnancy. Post abortion, fetal skin biopsy confirmed the diagnosis. Also in the third pregnancy, diagnosis was made on ultrasound because the couple could not afford a molecular prenatal diagnosis.

Ichthyosis is described as a group of skin disorders of keratinization, characterized by generalized scaling of the skin with varying severity. The great majority are inherited. The molecular basis and pathophysiology of most inherited ichthyosis is described with the identification of mutations in gene coding for various proteins or enzymes involved in a broad variety of cellular functions from DNA repair to skin barrier homeostasis. They vary from less severe to more severe forms. The mode of inheritance is autosomal semi-dominant, X-liked or recessive (1). Harlequin ichthyosis (HI), the most severe form, is an extremely rare autosomal recessive skin disorder that is almost always fatal in the early days of life (2). If both parents are carriers for genetic mutation, there is a 25% chance of each pregnancy being affected. It was unfortunate that 3 consecutive pregnancies were affected in our case. The skin is shiny, white, thick, with hyperkeratotic plaques and polygonal or diamond shaped fissures/cracks (3). The skin thickening results in clown-like faces with an eclabium (eversion of lip), ectropion (eversion of eyelids), a hypoplastic nose, small rudimentary/absent ears, and contractures in the upper and lower limbs with incurved toes and clenched fists (4). The abnormal keratinization of the skin leads to dehydration and infection as a result of the defective skin barrier, from which newborns succumb. Mutations on the Adenosine Triphosphate Binding Cassette Transporter Protein A12 (ABCA12) gene, a keratinocyte lipid transporter, lead to this condition (5).

Prenatal diagnosis of HI was first described in 1983 by Blanchet-Bardon et al. (6) in a couple with a previous harlequin birth, through fetal skin biopsy at 20-22 weeks. Fetal skin biopsy between 17-22 weeks remained the standard of care for prenatal diagnosis till recent times, with pathologic analysis consistent with accelerated hyperkeratosis (7). There have been reports of cytologic analysis of centrifuged amniotic fluid showing the same pathology (8). The isolation of the ABCA12 gene has made prenatal diagnosis possible early in pregnancy through chorionic villous sampling and amniocentesis with the option of terminating affected fetuses (9,10). Attempts have been made to make a diagnosis on ultrasound, mostly

late in the second and third trimester. Unfortunately, medical termination of pregnancy cannot be offered after 20 weeks' gestation in Turkey.

Many authors have used ultrasound to make a diagnosis by looking at the characteristic features as described above. Mihalko et al. (2) were probably the first to report suggestive findings on 2D-ultrasound at 28 weeks' gestation but did not make a prenatal diagnosis. They reported the presence of a thick discontinuous membrane floating in front of the fetus, restricted fetal movements, masses anterior to each orbit, and a thickened scalp.

Bongain et al. (11) were amongst the first to use 3D-ultrasound for the diagnosis of HI in a woman in two pregnancies. In the first pregnancy, diagnosis was made at 30 weeks when the woman was referred with an abnormal ultrasound and the pregnancy was terminated without a prenatal diagnosis because characteristic features were seen on 2D/3D scans. In the second pregnancy, thick lips and echogenic liquor were noted at 17 weeks. At 22 weeks, on 3D-ultrasound, an open mouth and thick lips were observed and a fetal skin biopsy confirmed the diagnosis of HI. Holden et al. (12) described another case with distal arthrogryposis at 24 weeks in a couple with a previously affected child. Flattening of the facial profile was noted only at 32 weeks.

Ultrasound diagnosis can be challenging in the index case. This has been highlighted in case reports with reports of typical findings on 2D/3D scans without a primary diagnosis of HI and confirmation of the diagnosis only after delivery (13,14).

This case report is one of the very few that shows accurate early detection (before 20 weeks' gestational age) using ultrasound without invasive testing. All the typical facial features, clenched hands, and toe contractures were appreciated on 2D and 3D-ultrasound. It highlights the fact that in centers where molecular diagnosis/fetal skin biopsy is unavailable, it is possible to use 3D-ultrasound in addition to 2D-ultrasound as a useful tool in clinching the diagnosis, especially in couples with a previously affected child. 3D images have a photograph-like realism, especially when looking at typical "fish-like facial morphology". This gives confidence in making a diagnosis in the absence of skin biopsy or molecular prenatal diagnosis. It also emphasizes the fact that diagnosis in a high-risk case requires serial ultrasound monitoring for suspicion of these subtle features because they may not develop at the routine anomaly screening at 18-20 weeks. There have been a few case reports of diagnoses of skin fissures/cracks in other types of ichthyosis on ultrasound imaging (15).

In conclusion, the availability of molecular testing without doubt simplifies the process of prenatal diagnosis of HI. However, with the non-availability of molecular testing/skin biopsy, careful and serial 2D/3D- ultrasound by experienced sonographers can achieve early diagnosis without resorting to invasive testing in couples with a previously affected child.

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Communication, orientation, coordination: A model for a laparoscopy team

To the Editor;

There are many residency programs, courses, congresses, and articles on training laparoscopists, but very few on developing teams. Communication, Orientation, Coordination (COC) is simple team training for the gradual and safe advancement in laparoscopy and natural orifice transluminal endoscopic surgery. From 1991 until 2003 at today's Mount Sinai of Queens, New York, we began special sessions for training teams of general surgeons, gynecologists, urologists, and assistants for video laser laparoscopy. We trained on simulators and in the animal laboratory. For lasers, we practiced in accordance with what later would become the United States of America (USA) Occupational Safety and Health Administration guidelines. The communication and coordination that we learned from laser safety complemented the COC. For video laparoscopy, we used our years of experience in laparoscopic gynecology to teach less experienced surgeons and assistants to follow the commands of the surgeon. Some beginners had a tendency to direct the laparoscope opposite to the surgeon's request and had difficulties with depth perception. We added concepts from football, also known as soccer, and the aviation cockpit or Crew Resource Management (CRM).

Football is the most popular sport in the world and most fans are familiar with its signs and verbal commands. Coaches establish communication among players as part of the collective strategy and tactics for the team. Players learn from repetitive actions, and short verbal communications are used when possible during a game. However, in laparoscopy there is time for verbal commands.

Since 1979, CRM has continued to evolve, a dedicated paper on this subject is available for free on ResearchGate (1). We focused on the communication in the cockpit between the captain and the first and second officer, which is similar to surgeons and assistants, using the analogy that the surgeon is the captain. Cockpit Voice Recorders (CVR) are reviewed by dedicated committees after unusual events or accidents; we used a video recorder instead of the CVR. It is important to review our recorded surgeries and compare them with expert videos. In 1997, we began training teams for more advanced procedures. The orientation problems with depth perception were overcome by experience. Next, we had to overcome large off-axis views. Orientation was more difficult when the angle increased from 0° to 180° in relation to the working area. Orientation was achieved by changing forms of communication in these situations. The commands are given as topographic directions rather than directional movements. Additionally, we limited the higher angles off-axis to a specific step or shorter parts of the operation. Disorientation is tiresome, it delays procedures and increases risk. COC team training promotes sharing knowledge, experience, expense, and equipment. Like in football, we need good players and a good team to excel. In my opinion, COC training was time well spent.

Daniel A. Tsin

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Reference

1. Guerlain SA, Turrentine FE, Bauer DT, Calland JF, Adams R. Crew resource management training for surgeons: feasibility and impact. Cognition, Technology and Work 2008; 10: 255-64.

Received: 5 October, 2017 **Accepted:** 31 December, 2017

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Journal of the Turkish-German Gynecological Association published by Galenos Publishing House.
DOI: 10.4274/jtgga.2017.0138

CONGRESS CALENDER

INTERNATIONAL MEETINGS

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

International Society of Gynecological Endocrinology 18th World Congress 2017 (ISGE 2017)

- **Venue** : Firenze Fiera
- **Location** : Florence
- **Start Date** : March 7, 2018
- **End Date** : March 10, 2018

26th European Congress of the European Board & College of Obstetrics and Gynaecology 2018 (EBCOG 2018)

- **Venue** : Palais des Congrès de Paris
- **Location** : Paris
- **Start Date** : March 8, 2018
- **End Date** : March 10, 2018

Society of Gynecologic Surgeons 44th Annual Meeting 2018 (SGS 2018)

- **Venue** : Hyatt Regency Grand Cypress
- **Location** : Orlando
- **Start Date** : March 11, 2018
- **End Date** : March 14, 2018

6th Congress on Maternal-Fetal-Nutrition in the First 1000 Days 2018

- **Venue** : Gloria Golf Resort Antalya
- **Location** : Antalya
- **Start Date** : March 14, 2018
- **End Date** : March 18, 2018

Society of Obstetricians & Gynaecologists of Canada West/Central CME 2018 (SOGC 2018)

- **Venue** : The Rimrock Resort
- **Location** : Banff
- **Start Date** : March 15, 2018
- **End Date** : March 17, 2018

6th European Pelvic Course 2018

- **Venue** : Dorint Hotel Hamburg-Eppendorf
- **Location** : Hamburg
- **Start Date** : March 22, 2018
- **End Date** : March 24, 2018

Society of Gynaecologic Oncologists 48th Annual Meeting on Womens Cancer 2018 (SGO 2018)

- **Venue** : Hyatt Regency New Orleans
- **Location** : New Orleans
- **Start Date** : March 24, 2018
- **End Date** : March 27, 2018

19th Annual National Conference on Fetal Monitoring 2018

- **Venue** : Bally's Las Vegas Hotel & Casino
- **Location** : Las Vegas
- **Start Date** : April 5, 2018
- **End Date** : April 7, 2018

CONGRESS CALENDER

INTERNATIONAL MEETINGS

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

American College of Osteopathic Obstetricians and Gynecologists 85th Annual Conference 2018 (ACOOG 2018)

- **Venue** : Waldorf Astoria Bonnet Creek
- **Location** : Bonnet Creek
- **Start Date** : April 8, 2018
- **End Date** : April 13, 2018

American College of Obstetricians and Gynecologists 66th Annual Clinical Meeting 2018 (ACOG 2018)

- **Venue** : Austin Convention Center
- **Location** : Austin
- **Start Date** : April 27, 2018
- **End Date** : April 30, 2018

12th Congress of Turkish German Gynecology Association (TGGF 2018)

- **Venue** : Elexus Hotel Resort & Spa - Convention Center
- **Location** : Cyprus
- **Start Date** : April 27, 2018
- **End Date** : May 1, 2018

British Society for Gynaecological Endoscopy 28th Meeting 2018 (BSGE 2018)

- **Venue** : Dynamic Earth
- **Location** : Edinburgh View Map
- **Start Date** : May 9, 2018
- **End Date** : May 11, 2018

Japan Society of Obstetrics and Gynaecology 70th Annual Congress 2018 (JSOG 2018)

- **Venue** : Sendai International Center
- **Location** : Sendai View Map
- **Start Date** : May 10, 2018
- **End Date** : May 13, 2018

NATIONAL MEETINGS

March 1-4, 2018	**Palandöken Kadın Doğum Kongresi, Erzurum** (http://palandokenkadindogum.com/)
March 8-11, 2018	**7. Jinekoloji Endoskopi Sempozyumu ve Çalıştayı, Bursa** (http://www.infertilitendoskopi.org/)
April 27-May 1, 2018	**12. Türk Alman Jinekoloji Kongresi, Kıbrıs** (http://www.tajev2018.org/)

TGGF

TURKISH - GERMAN GYNECOLOGICAL
EDUCATION and RESEARCH FOUNDATION

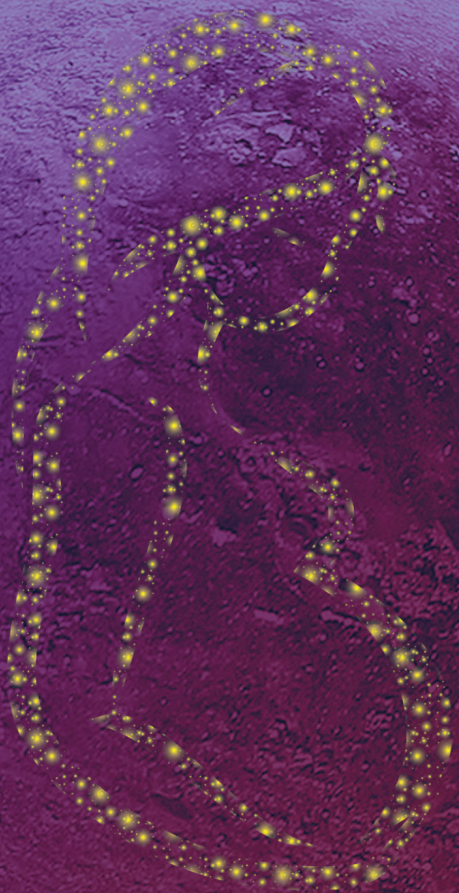
XII. TURKISH GERMAN GYNECOLOGIC CONGRESS

www.tajev2018.org

April 27 - May 01, 2018

Elexus Hotel, Kyrenia

Turkish Republic of Northern Cyprus



PROGRAM

Scientific Program
28th April 2018, Saturday

HALL 1	HALL 2	HALL 3
08:45 - 09:15 *KEYNOTE LECTURE 1* *Chair:* ***L. Cem Demirel*** *The importance of mitochondria on early embryonic development and possible therapeutic interventions for the future in ART* *HALL 1*  ***Thomas Ebner***		
09:15 - 10:35 *ISUOG Session I* *Chairs:* ***Emine Çetin, Özlem Pata***	**09:15 - 10:35** *General Concepts in Ob&Gyn* *Chairs:* ***Fatma Ferda Verit, Murat Ulukuş***	**09:15 - 10:35** *Oral Presentations*
09:15 - 09:35 *Anomaly Scan 11-14 Weeks* ***Sevgi Tercanlı***	09:15 - 09:35 *Reducing professional liability and improving patient safety: An achievable and necessary task* ***Frank Chervenak***	
09:35 - 09:55 *Early fetal echocardiography* ***Emine Çetin***	09:35 - 09:55 *Overdiagnosis and overtreatment in obstetrics and gynecology* ***Esat Orhon***	
09:55 - 10:15 *Increased nuchal transluceny and pregnancy outcome* ***İsmail Tekesin***	09:55 - 10:15 *Liquid Biopsy in Gynecological Cancers* ***Dilek Aktaş***	
10:15 - 10:35 *Anomaly Scan 20-23 Weeks* ***Sevgi Tercanlı***	10:15 - 10:35 *Complications and Prevention of Laparoscopic surgery: Safety First* ***Gazi Yıldırım***	
10:35 - 11:00	**COFFEE BREAK**	
11:00 - 12:00	*SATELLITE SYMPOSIUM 1* *HALL 1*	
12:00 - 13:20 *ISUOG Session II* *Chairs:* ***Sevgi Tercanlı, Bülent Tandoğan***	**12:00 - 13:20** *Oncology* *Chairs:* ***Ali Ayhan, U. Fırat Ortaç***	**12:00 - 13:20** *Cesarean Scar Problems - Nerve Sparing Surgery* *Chairs:* ***Talip Gül, Cengiz Alataş***
12:00 - 12:20 *NİPT clinical implementation - state of the art* ***Emine Çetin***	12:00 - 12:20 *Management of Postmenopausal adnexal masses* ***Çağatay Taşkıran***	12:00 - 12:20 *Effect of isthmocele on fertility* ***Hüseyin Görkemli***
12:20 - 12:40 *Implementation of NIPT in routine antenatal care in Switzerland* ***Sevgi Tercanlı***	12:20 - 12:40 *S3-Guideline 2018: Diagnosis and treatment of endometrial cancer* ***Peter Mallmann***	12:20 - 12:40 *Minimally invasive management of isthmocele* ***Ahmet Göçmen***
12:40 - 13:00 *Prenatal assessment of fetal cortical anomalies* ***Atıl Yüksel***	12:40 - 13:00 *Current management of endometrial hyperplasia* ***M. Faruk Köse***	12:40 - 13:00 *Cesarean scar pregnancies and their management* ***Mutlu Ercan***
13:00 - 13:20 *Fetal ventriculomegali* ***Rıza Madazlı***	13:00 - 13:20 *Organ sparing approach in gynecological oncology, place of minimally invasive surgery* ***U. Fırat Ortaç***	13:00 - 13:20 *Nerve sparing surgical approach in the treatment of deep infiltrating endometriosis* ***Ercan Baştu***

12:00 - 13:20 *ISUOG Session II*
Chairs: Sevgi Tercanlı, Bülent Tandoğan

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Emine Çetin

12:20 - 12:40 *Implementation of NIPT in routine antenatal care in Switzerland*
Sevgi Tercanlı

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Atıl Yüksel

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Rıza Madazlı

12:00 - 13:20 *Oncology*
Chairs: Ali Ayhan, U. Fırat Ortaç

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Peter Mallmann

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M. Faruk Köse

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Mutlu Ercan

13:00 - 13:20 *Nerve sparing surgical approach in the treatment of deep infiltrating endometriosis*
Ercan Baştu

13:30 - 14:30 **LUNCH**

14:30 - 15:50 *Antenatal-Postpartum Bleeding*
Chairs: Ateş Karateke, Yaprak Üstün

14:30 - 14:50 *Maternal Mortality*
Yaprak Üstün

14:50 - 15:10 *Medical management of uterine atony and postpartum bleeding*
Recep Has

15:10 - 15:30 *Postpartum bleeding, factors to determine success in surgery*
Ateş Karateke

15:30 - 15:50 *Placental anomalies, placenta previa and ablatio placenta*
İsmail Özdemir

14:30 - 15:50 *New Trends in Reproductive Medicine*
Chair: Sezai Şahmay

14:30 - 14:50 *Reproduction and microbiota of genital system: What is new?*
TBA

14:50 - 15:10 *Does the microflowable fluid technology improve pregnancy outcomes in IVF?*
Bülent Tıraş

15:10 - 15:30 Seminal plasma application to improve ART outcome
Barış Ata

15:30 - 15:50 *What are the new alternatives in the treatment of unresponsive thin endometrium?*
Bülent Gülekli

14:30 - 15:50 *General Gynecology*
Chairs: Engin Oral, Selçuk Söylemez

14:30 - 14:50 *Management of Patients with Premature Ovarian Failure*
Cavidan Gülerman

14:50 - 15:10 *ERAS (Enhanced Recovery After Surgery) protocol in Gynecologic surgery*
Fatih Durmuşoğlu

15:10 - 15:30 *Evidence-based management of of UI (Urinary incontinence)*
Orhan Ünal

15:30 - 15:50 *Vulvodynia and vestibulectomy: Treatment of an important problem for a gynecologist*
Tolga Taşcı

15:50 - 16:10 **COFFEE BREAK**

16:10 - 17:30 *Endoscopic Surgery*
Chairs: Mete Güngör, Gürkan Uncu

16:10 - 16:30 *UNCU Modification: The new way of using peritoneum and remnants in Mayer Rokitansky Küstner Hauser Syndrome*
Gürkan Uncu

16:30 - 16:50 *Techniques for challenging laparoscopic hysterectomy*
M. Murat Naki

16:50 - 17:10 *Laparoscopic sacrocalpopexy*
Yakup Kumtepe

17:10 - 17:30 *Complications of hysteroscopy*
Gülnur Özakşit

17:30 - 18:10 *Oral Presentations*

16:10 - 17:30 *AWARD WINNING PRESENTATIONS*
Chairs: L. Cem Demirel, Yaprak Üstün

17:30 - 18:10 *Oral Presentations*

16:10 - 17:50 *Perinatology*
Chairs: Recep Has, İbrahim Polat

16:10 - 16:30 *New ultrasonographic approaches in diagnosis of diaphragmatic hernia*
H. Fehmi Yazıcıoğlu

16:30 - 16:50 *Antepartum, intrapartum and postpartum management of hypertensive disorders of pregnancy*
Yusuf Üstün

16:50 - 17:10 *Current management of maternal sepsis*
Yaprak Üstün

17:10 - 17:30 *Diagnosis and management of gestational diabetes*
Dilek Şahin

17:30 - 17:50 *How to diagnose and manage the heart disease in pregnancy? The role and obligations of the obstetrician...*
Şevki Çelen

17:30 - 18:10 *Oral Presentations*

HALL 1	HALL 2	HALL 3
08:45 - 09:15	*KEYNOTE LECTURE 2* ***Chair: Peter Mallmann*** *Professionalism: The foundation of obstetrics and gynecology* *HALL 1*	 ***Frank Chervenak***
09:15 - 10:35 *Endometriosis Management* ***Chairs: Emine Yüksel, Barış Kaya***	**09:15 - 10:35** *Infertility - ART* ***Chairs: Human Fatemi, Bülent Urman***	**09:15 - 10:35** *Recurrent pregnancy losses* ***Chairs: Sedat Kadanalı, Yavuz Emre Şükür***
09:15 - 09:35 *Management of endometriosis refractory to current treatments* ***Serdar Bulun***	09:15 - 09:35 *BRCA function, mutations and DNA Repair in oocyte aging* ***Kutluk Oktay***	09:15 - 09:35 *Recurrent pregnancy loss: current ESHRE guidelines* ***Engin Oral***
09:35 - 09:55 *Endometriosis: Etiopathogenesis based treatments & nutrition* ***Erkut Attar***	09:35 - 09:55 *What to do to maximize oocyte count in older women?* ***Bülent Urman***	09:35 - 09:55 *Does low molecular weight heparin prevent recurrent pregnancy losses?* ***Serdar Ural***
09:55 - 10:15 *Emerging therapies for endometriosis and endometrioma* ***Vasilios Tanos***	09:55 - 10:15 *Aging oocyte: from an embryologist's perspective* ***Cengiz Karakaya***	09:55 - 10:15 *Decreased ovarian reserve as a cause of RPL and the value of PGS for the treatment of RPL* ***Yılmaz Güzel***
10:15 - 10:35 *ART in pelvic endometriosis-related infertility cases* ***Kutay Biberoğlu***	10:15 - 10:35 *Social egg freezing* ***L. Cem Demirel***	10:15 - 10:35 *Unexplained recurrent pregnancy losses: treat or not to treat* ***Sedat Kadanalı***
10:35 - 10:50	**COFFEE BREAK**	
10:50 - 11:50	*SATELLITE SYMPOSIUM 2* *Iron Deficiency/Anemia During Pregnancy and Postpartum, Treatment and Role of IV Iron* *HALL 1*	ABDIIBRAHIM
11:50 - 12:50	*LIVE SURGERY 1 FROM ISTANBUL* ***Chair: Mete Güngör*** ***Surgeons: M. Faruk Köse, M. Murat Naki*** *HALL 1*	
12:50 - 13:45	**LUNCH**	

13:45 - 15:05 *Miscellaneous*
Chairs: Kadir Güzin, Özgüç Takmaz

13:45 - 14:05 *New fetoscopic treatment modalities in lower urinary system obstructions*
Selahattin Kumru

14:05 - 14:25 *Prevetion of Respiratory Morbidity in Preterm Delivery: Antenatal Steroids*
Talat Umut Kutlu Dilek

14:25 - 14:45 *Practical clues for thrombosis profilaxis in obstetrics*
Yusuf Üstün

14:45 - 15:05 *Clinical use of Mifepriston (RU 486) in early pregnancy*
Cemil Yaman

13:45 - 15:05 *Oncology*
Chairs: Macit Arvas, Nejat Özgül

13:45 - 14:05 *Management of early stage ovarian cancer*
Macit Arvas

14:05 - 14:25 *Primary cytoreductive surgery vs NAC in epithelial overian cancer surgery*
Ali Ayhan

14:25 - 14:45 *Place of lymphadenectomy in advanced overian cancer*
Mete Güngör

14:45 - 15:05 *How to identify patients who benefit from surgery of recurrent ovarian cancer?*
Beyhan Ataseven

13:45 - 14:45 *Infertility - ART*
Chairs: Erol Tavmergen, Tevfik Yoldemir

13:45 - 14:05 *Non-conventional IVF stimulation protocols: For only cancer patients? When and for whom?*
Özgür Öktem

14:05 - 14:25 *Fresh or frozen ET in PCOD*
Ege Nazan Tavmergen Göker

14:25 - 14:45 *Patient tailored COH management*
Erol Tavmergen

15:05 - 15:30 **COFFEE BREAK**

15:30 - 16:50 *Oncology*
Chairs: Sinan Özalp, Demir Özbaşar

15:30 - 15:50 *Cervical screening programmes*
Nejat Özgül

15:50 - 16:10 *Management of low grade cervical preinvasive lesions*
Salih Taşkın

16:10 - 16:30 *Management of high grade cervical preinvasive lesions*
Kunter Yüce

16:30 - 16:50 *Early cervical cancer - Sentinel approach and organ conserving surgery*
Peter Hillemanns

16:50 - 18:00 *Oral Presentations*

15:30 - 16:50 *Urogynecology*
Chairs: Peter Hillemanns, M. Faruk Köse

15:30 - 15:50 *Functional anatomy of the urogynecological point of view*
İbrahim Alkatout

15:50 - 16:10 *Is there still a place for the vaginal approach in Urogynaecology?*
Eckhard Petri

16:10 - 16:30 *Minimally invasive apical defect repair*
Gökhan Kılıç

16:30 - 16:50 *Complications and their management in midurethral sling operations*
Funda Güngör Uğurlucan

16:50 - 18:00 *Oral Presentations*

15:30 - 16:50 *Contoversies in Gynecology*
Chairs: Kubilay Ertan, Yusuf Üstün

15:30 - 15:50 *Is there a future for robotic surgery in gynecology?*
Kubilay Ertan

15:50 - 16:10 *Tethered vagina syndrome: A new disease? How to manage?*
Akın Sivaslıoğlu

16:10 - 16:30 *When and how to treat T-shaped uteri?*
Recai Pabuçcu

16:30 - 16:50 *Overview Expression I-V: Patient´s Expectations and Preferences regarding Physician-Patient Relationship and Clinical Management*
Gülten Oskay-Özçelik

16:50 - 18:00 *Oral Presentations*

HALL 1	HALL 2	HALL 3
08:45 - 09:25 **08:45-09:05** *KEYNOTE LECTURE 3* ***Chair: Cihat Ünlü*** *Hereditary Cancer Risk A ssessment* *HALL 1*  ***Farr Nezhat***		**09:05-09:25** *KEYNOTE LECTURE 4* ***Chair: Cihat Ünlü*** *Management of GU Endometriosis to Prevent Silent loss of Kidney* *HALL 1*  ***Camran Nezhat***
09:25 - 10:25 *Perinatology* ***Chairs: Atıl Yüksel, Recep Has***	**09:25 - 10:25** *Fertility Preservation* ***Chairs: Botros Rizk, Ahmet Zeki Işık***	**09:25 - 10:25** *Endometroisis Surgery* ***Chairs: Jörg Keckstein, Ertan Sarıdoğan***
09:25 - 09:45 *The fruitful marriage of ultrasonography and prenatal genetics* ***Wolfgang Holzgreve***	09:25 - 09:45 *Current techniques, success and efficiency of ovarian cryopreservation and transplantation* ***Kutluk Oktay***	09:25 - 09:45 *ESGE/ESHRE/WES Recommendations on surgical techniques for treatment of endometriomas* ***Ertan Sarıdoğan***
09:45 - 10:05 *Ultrasound findings of intrauterine infections* ***Özlem Pata***	09:45 - 10:05 *Ovarian stimulation protocols in breast cancer patients* ***Cem Atabekoğlu***	09:45 - 10:05 *Advanced surgery in Deep Infiltrating Endometriosis: Strategies for key steps* ***Jörg Keckstein***
10:05 - 10:25 *"I want the best done for my baby" Why Omega-3 in pregnancy?* ***Serdar Ural***	10:05 - 10:25 *Where are we in oocyte cryopreservation?* ***Ahmet Zeki Işık***	10:05 - 10:25 *Cancer arising from endometriosis and its clinical implications* ***Farr Nezhat***
10:35 - 12:10	*LIVE SURGERY 2 FROM TUBINGEN* ***Chair: Cihat Ünlü*** ***Surgeon: Diethelm Wallwiener*** *HALL 1* 	
12:10 - 13:30 *Controversies in ART - Reproductive medicine* ***Chairs: Kutluk Oktay, L. Cem Demirel***	**12:10 - 13:30** *Endometriosis Research* ***Chairs: Serdar Bulun, Sun Wei Guo***	**12:10 - 13:30** *Oncology* ***Chairs: Michael Stark, M. Murat Naki***
12:10 - 12:30 *Blastocyst transfer and PGS: Do we have enough evidence?* ***Mohamed Aboulghar***	12:10 - 12:30 *Stem cells, epigenetics and steroids in endometriosis, uterine myomas and breast cancer* ***Serdar Bulun***	12:10 - 12:30 *Nerve sparing approach in radical surgery of cervical cancer* ***Hüsnü Çelik***
12:30 - 12:50 *Role of mitochondrial function and treatments in oocyte quality: Fact or fiction?* ***Kutluk Oktay***	12:30 - 12:50 *Why did all biomarker identification studies fail in endometriosis?* ***Sun Wei Guo***	12:30 - 12:50 *The NESA's VIEZION Oncological Project - Toward optimisation of post-surgical treatment* ***Michael Stark***
12:50 - 13:10 *The new concept of Luteal coasting* ***Barbara Lawrenz***	12:50 - 13:10 *Endometriosis surgery or medical treatment, if pregnancy is desired* ***Abolfazl Mehdizadehkashi***	12:50 - 13:10 *Striking vulva findings: How to detect, how to treat?* ***Peter Mallmann***
13:10 - 13:30 *Time-lapse imaging: results and current perspectives* ***Thomas Ebner***	13:10 - 13:30 *It's time to treat Endometriosis based on Pathophysiology* ***Shahla Chaichian***	13:10 - 13:30 *Fluorescence-guided Sentinel node dissection for vulvar cancer* ***Peter Hillemanns***

13:30 - 14:30 LUNCH

14:30 - 15:50 *Pitfalls in Gyn Surgery*
Chairs: ***Ceana Nezhat, Erol Tavmergen***

14:30 - 14:50 *Myomectomy and morcelation Dilemma*
Ceana Nezhat

14:50 - 15:10 *Impact of sarcoma thread on myomectomies and hysterectomies*
Liselotte Mettler

15:10 - 15:30 *Power morcellation complications in Europe: results of an ESGE Survey*
Vasilios Tanos

15:30 - 15:50 *Accidental diagnosis of endometrial and cervical cancer after hysterecomy: What to do?*
Peter Mallman

14:30 - 15:50 *MEFS Session*
Chairs: ***Mohamed Aboulghar, Michel Abou Abdallah***

14:30 - 14:50 *Do women in the middle eastern countries have specific causes of infertility?*
Barbara Lawrenz

14:50 - 15:10 *Infertility due to the absence of gametes*
Mohamed Aboulghar

15:10 - 15:30 *Ovarian Aging*
Michel Abou Abdallah

15:30 - 15:50 *Preservation of fertility*
Botros Rizk

TGGF

14:30 - 15:30 *Adenomyosis*
Chairs: ***Mustafa Bahçeci, Ercan Baştu***

14:30 - 14:50 *The use of transvaginal elastography in diagnosing and dating of adenomyosis and in guiding the choice of treatment modality*
Sun Wei Guo

14:50 - 15:10 *Severe Adenomyosis: Surgical techniques to preserve the uterus and improve fertility and quality of life*
Jörg Keckstein

15:10 - 15:30 *Management of adenomyosis prior to IVF*
Mustafa Bahçeci

15:50 - 16:10 COFFEE BREAK

16:10 - 17:30 *Surgical Gynecology*
Chair: ***Camran Nezhat, Liselotte Mettler***

16:10 - 16:30 *A New Method of Hysterectomy in Patients With Prior History of C-Section, Anterior Myomectony or Segmental Bladder Resection*
Camran Nezhat

16:30 - 16:50 *Subtotal and Total Laparoscopic Hysterectomies - their comparative advantages*
Liselotte Mettler

16:50 - 17:10 *Non obstetric Surgery in Pregnancy*
Ceana Nezhat

17:10 - 17:30 *Management of submucosal fibroids: Is hysteroscopic approach always the best?*
Ertan Sarıdoğan

16:10 - 17:10 *PCOS*
Chairs: ***Erhan Şimşek, Gazi Yıldırım***

16:10 - 16:30 *How to diagnose PCOS?*
Gürkan Bozdağ

16:30 - 16:50 *Ovarian stimulation for PCOS*
Botros Rizk

16:50 - 17:10 *How accurate is the "Freeze All" approach in IVF cycles*
Rıfat Gürsoy

17:10 - 17:30 *Rational Drug Use*
Özgüç Takmaz

16:10 - 17:30 *Prolapse Surgery*
Chairs: ***Eckhard Petri, Fuat Demirci***

16:10 - 16:30 *The dark side of alloplastic meshes in prolapse surgery*
Eckhard Petri

16:30 - 16:50 *Effective Mesh-free surgery for uterine/vaginal vault prolapse: The vaginal sacrocolpopexy*
Peter Hillemanns

16:50 - 17:10 *Alternative laparoscopic treatment modalities of apical prolapse: Pectopexy, lateral suspension, uterosacral plication*
Fuat Demirci

17:10 - 17:30 *Colpocleisis for advanced POP*
İsmail Mete İtil

20:30 *GALA CONCERT*

Course Programme
27th April 2018, Friday

Colposcopy Course
Course Presidents: **Kunter Yüce, M. Faruk Köse**

10:30 - 11:30 *Session 1*

Chairs: ***Kunter Yüce, M. Faruk Köse***

10:30 - 11:00	*HPV Infections and Epidemiology*	*Ali Ayhan*
11:00 - 11:20	*HPV Infections and Epidemiolog*	*Murat Gültekin*
11:20 - 11:30	*Discussion*	

11:30 - 12:00 *Coffee Break*

12:00 - 13:30 *Session 2*

Chairs: ***Nejat Özgül, H. Gökhan Tulunay***

12:00 - 12:20	*Colposcopy*	*Kunter Yüce*
12:20 - 12:40	*Normal cervical colposcopy*	*H. Gökhan Tulunay*
12:40 - 13:00	*Colposcopy of abnormal transformation zone*	*Nejat Özgül*
13:00 - 13:20	*Colposcopy of LSIL (+ ASC-US)*	*Müfit C. Yenen*
13:20 - 13:30	*Discussion*	

13:30 - 14:30 *Lunch*

14:30 - 16:10 *Session 3*

Chairs: ***Macit Arvas, U.Fırat Ortaç***

14:30 - 14:50	*Colposcopy of HSIL (ASC-H +)*	*M. Faruk Köse*
14:50 - 15:10	*Colposcopy of Invasive Cervical Cancer*	*Ali Haberal*
15:10 - 15:30	*Abnormal Cervical Cytology Management*	*Murat Dede*
15:30 - 15:50	*Histological Management of Premalignant cervical lesions*	*Macit Arvas*
15:50 - 16:00	*Cryotherapy, LEEP and Conization*	*U. Fırat Ortaç*
16:00 - 16:10	*Discussion*	

16:10 - 16:30 *Coffee Break*

16:30 - 17:30 *Session 4*

16:30 - 17:30	*Interactive Case studies*	*Kunter Yüce, Nejat Özgül*

Minimal Invasive Gynecological Surgery Course
Course Presidents: **U. Fırat Ortaç, Mete Güngör**

Opening ***U. Fırat Ortaç, Mete Güngör***

10:30 - 11:45 *Session 1*

Chairs: ***Gürkan Uncu, Vedat Atay***

10:30 - 10:45	*Diagnostic and Hysteroscopic Interventions with Office Hysteroscopy*	*Bülent Urman*
10:45 - 11:00	*Hysteroscopic Myomectomy, polypectomy and tissue removal / morcellation techniques*	*Onur Topçu*
11:00 - 11:15	*Hysteroscopic surgery in Intrauterine Synechiae*	*Barış Ata*
11:15 - 11:30	*Hysteroscopic Surgery in uterine abnormalities*	*Gürkan Bozdağ*
11:30 - 11:45	*Discussion*	

11:45 - 12:00 *Coffee Break*

12:00 - 13:15 *Session 2*

Chairs: ***Bülent Urman, Cem Atabekoğlu***

12:00 - 12:15	*Safe Laparoscopic Trocar Access Techniques*	*Kubilay Ertan*
12:15 - 12:30	*Laparoscopic Suturing Techniques (intracorporeal, extracorporeal, barbed sutures / vaginal cuff closure techniques)*	*Erhan Şimşek*
12:30 - 12:45	*Laparoscopic myomectomy - step by step*	*Gürkan Uncu*
12:45 - 13:00	*Morcellation in Laparoscopic Surgery / large uterus and tissue removal techniques*	*Hüsnü Çelik*
13:00 - 13:15	*Discussion*	

13:15 - 14:15 *Lunch*

14:15 - 15:30 *Session 3*

Chairs: ***Mete Güngör, Fatih Güçer***

14:15 - 14:30	*Laparoscopic simple hysterectomy - Step by step*	*Kemal Özerkan*
14:30 - 14:45	*Laparoscopic Surgical Approach in Frozen Pelvis*	*Çağatay Taşkıran*
14:45 - 15:00	*Case selection and laparoscopic approach in adnexal masses*	*Salih Taşkın*
15:00 - 15:15	*Preservation of Ovarian Reserve in Laparoscopic Surgery of Endometrioma*	*Ercan Baştu*
15:15 - 15:30	*Discussion*	

15:30 - 15:45 *Coffee Break*

15:45 - 17:00 *Session 4*

Chairs: ***Orhan Ünal, Yakup Kumtepe***

15:45 - 16:00	*New Laparoscopic Techniques in Pelvic Organ Prolapse*	*Fuat Demirci*
16:00 - 16:15	*Minilaparoscopy techniques*	*M. Murat Naki*
16:15 - 16:30	*Laparoscopic and hysteroscopic isthmocele treatment*	*Murat Api*
16:30 - 16:45	*Laparoscopic complications and their management*	*Fatih Güçer*
16:45 - 17:00	*Discussion and Closing*	

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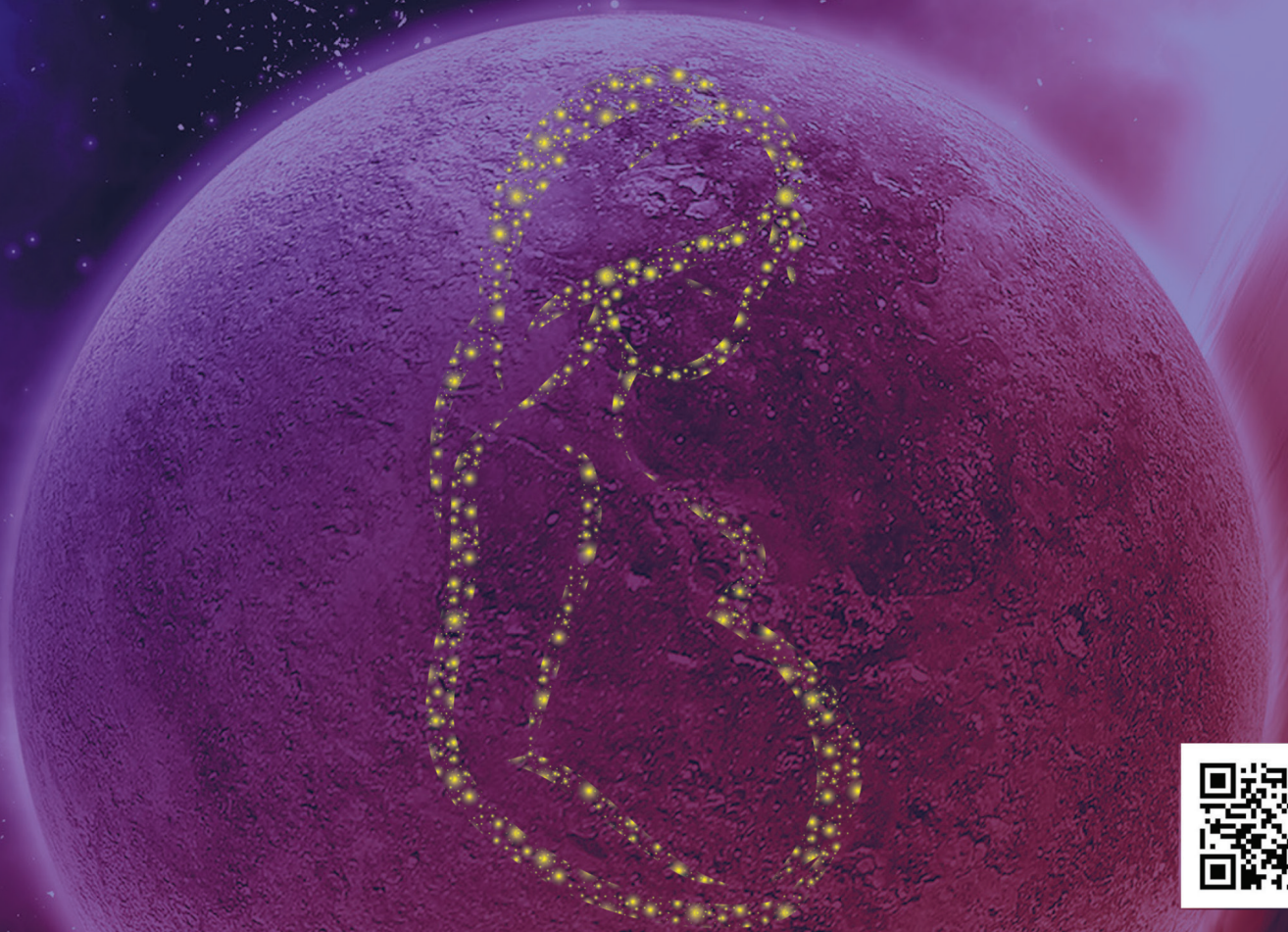

Planlı Gebelikte Kontrol Sizde!

BAYER

Tecrübesiyle Güven Verir[1,2]

- Akne tedavisi ve tüylenmede azalma[1]
- Antimineralokortikoid etkiyle kilo aldırmama[1]
- İyi siklus kontrolü[2]

Kesintisiz Konfor Sağlar[3,4]

- Düşük dozla daha iyi tolerabilite[3,4]
- 24+4 rejim ile kullanım kolaylığı ve yan etkilerde azalma[3,4]
- Akne tedavisi ve tüylenmede azalma[3,4]
- Antimineralokortikoid etkiyle kilo aldırmama[3,4]
- Adet öncesi gerginlik endikasyonu[4]

Doğal Ritmi Korur[5-8]

- E_2V - doğala özdeş östrojen ile yan etkilerde azalma[5]
- Kesintisiz ve dinamik doz rejimi ile doğal hormon seviyelerine uyum[5]
- Şiddetli adet kanaması endikasyonu[6,7]
- Yüksek kullanıcı memnuniyeti[8]

Referanslar: 1. Yasmin® Kısa Ürün Bilgisi. **2.** Kelly S, et al. Clin Drug Investig 2010;30 (5):325-36. **3.** Bachmann G, et al. Contraception 2004;70:191-8. **4.** Yazz® Kısa Ürün Bilgisi. **5.** Fruzetti F, et al. Gynecological Endocrinology 2012; 28(5): 400-8 **6.** Qlairista® Kısa Ürün Bilgisi. **7.** Fraser Iset al. Eur J Contracept Reprod Health Care. 2011;16(4):258-69 **8.** S. Palacios et al. European Journal of Obstetrics & Gynecology and Reproductive Biology 149 (2010) 57–62

L.TR.MKT.WH.01.2018.0659