

TURKISH-GERMAN GYNECOLOGICAL EDUCATION and RESEARCH FOUNDATION

# Journal of the Turkish-German Gynecological Association



Cover Picture: 3D power Doppler angiography

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Violence during pregnancy Hüseyin Cengiz et al.; Istanbul, Turkey

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Official Journal of the Turkish-German Gynecological Education and Research Foundation www.tajev.org Official Journal of the Turkish-German Gynecological Association www.dtgg.de Volume 15 Issue 2 June

is now indexed in PubNied Central.

2014

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

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

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

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

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

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

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Present the detailed findings supported with statistical methods. Figures and tables should supplement, not duplicate the text; presentation of data in either one or the other will suffice. Emphasize only your important observations; do not compare your observations with those of others. Such comparisons and comments are reserved for the discussion section.

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State the importance and significance of your findings but do not repeat the details given in the Results section. Limit your opinions to those strictly indicated by the facts in your report. Compare your finding with those of others. Provide information on the limitations of the study. No new data are to be presented in this section.

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

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### Editorial



Dear Colleagues,

I would like to welcome you to another **attractive issue**, in which you will find an opportunity to read many important articles from Turkey and other countries. One of them is an interesting research article determining baseline laparoscopic and robotic skills of high school and college students and comparing them to those of current obstetrics and gynecology residents. You will also read an attractive paper determining the prevalence of domestic violence during pregnancy in a metropolitan city, İstanbul. Herein is presented another highly scientific article investigating the role of melatonin on uterine myoelectrical activity of non-pregnant rats. You will also find a review discussing cervical premalignant lesions and their management.

As the **Turkish - German Gynecological Education and Research Foundation (TAJEV)**, we are proud that our **Tenth Congress** was conducted in **Antalya** on **April 30th - May 4th, 2014** with a high scientific quality. The congress was organized at the brand new Titanic Deluxe Hotel - Belek with a great interest from the gynecology and obstetrics

society by more than **1.200 participants** and faculty members from **29 different countries**.

The congress started with six pre-congress courses and hands-on training sessions in different interests. The courses were followed by the impressive opening conference, presented by the **Lifetime Honorary Member** of our foundation, **Prof. Camran Nezhat** about "The Future Surgeon, the Future Surgeries and How it has Evolved". The program was presented in **43** sessions in 4 parallel halls by **125** Turkish and **42** international faculty members. The experts have shared their knowledge and experiences widely with the participants from the basic terms to the latest innovations in all topics of interest in the field of obstetrics & gynecology, and related disciplines.

We have enriched our scientific program this year with **two live surgeries**, one **from USA** and the other one **from Italy** performed by the worldwide experts. As well as our previous congresses, we continued our tradition of collaborated sessions this year with the **AAGL** - **TAJEV Joint Session on Endoscopy** and **NOGGO** - **TAJEV Joint Session on Oncology**.

Many prestigious pharmaceutical, instrumental and appliances companies found a great opportunity to introduce their current and brand new products and services to the participants in our congress. On the other hand, the participants had a chance to be informed of the latest innovations and technology in scientific medicine and devices industry at the exhibition area. **Five satellite symposiums** were also organized with the support of industry on up-to-date topics.

A total of **612 abstracts** have been collected by the online abstract submission system from many different countries. From these submitted abstracts; 534 poster, 35 oral, and 14 video abstracts have been accepted and presented. **Best three abstracts**, evaluated and selected by the scientific secretariat of the congress, were financially awarded with a total of 6.000 TL. The best abstract in endoscopic surgery category was also rewarded with **Dr. Aysun - Cihat Ünlü Special Prize** in the value of 4.000 TL.

The **gala** of the congress with the great performance of Turkish Pop Singer **Yalm** was a great relaxation and a cream of the crop for the participants before going back to their home after four days of scientific program.

The feedbacks of almost each participant were very positive about the organization, social activities, hotel selection and the high level of scientific program. These feedbacks and positive comments are encouraging and stimulating us to make even better and better activities and meetings in the future.

I wish you a beautiful summer holiday with a plenty of sun.

Best regards,

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

### Assessment of minimally invasive surgical skills of pre-medical students: What can we learn from future learners?

Mostafa A. Borahay<sup>1</sup>, Mary Jackson<sup>1</sup>, Ömer L. Tapısız<sup>1,2</sup>, Elizabeth Lyons<sup>3</sup>, Pooja R. Patel<sup>1</sup>, Ramsey Nassar<sup>4</sup>, Gökhan Sami Kılıç<sup>1</sup>

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of Medicine, Texas, USA <sup>4</sup>Taylor High School, Houston, Texas, USA

#### Abstract

**Objective:** Knowledge of baseline laparoscopic and robotic surgical skills of future learners is essential to develop teaching strategies that best fit them. The objectives of this study are to determine baseline laparoscopic and robotic skills of high school and college students and compare them to those of current obstetrics and gynecology residents.

**Material and Methods:** A cross-sectional (Class II-2) pilot study. Laparoscopic and robotic surgical skills of college and high (secondary) school students were evaluated using simulators and compared to those of obstetrics and gynecology residents. In addition, questionnaire data were collected regarding video game playing and computer use.

**Results:** A total of 17 students, both high school (n=9) and college (n=8), in addition to 11 residents, completed the study. Overall, students performed comparably to the residents in simple exercises (p>.05). However, students took significantly longer time to complete complex exercises (p=.001). Finally, students played video games significantly more than residents (p<.001).

**Conclusion:** Future learners may have a different background skill set. This difference may be related to improved hand-eye coordination, possibly due to playing video games. The results of this pilot study should spur more research into surgical teaching strategies. (J Turk Ger Gynecol Assoc 2014; 15: 69-73)

Key words: Simulation, education, robotic surgery, laparoscopy, video games

**Received:** 06 May, 2014

Accepted: 08 May, 2014

#### Introduction

Laparoscopic and robotic-assisted procedures, collectively known as minimally invasive surgery (MIS), are rapidly gaining popularity in the field of gynecology. Compared to laparotomy, they offer several advantages, including improved postoperative recovery, decreased postoperative pain and hospital stay, better visualization, better cosmetic outcome, and decreased blood loss (1, 2). Surgeons who adopt MIS must undergo a learning curve, the steepness of which varies among individuals. This learning curve involves mastering the hand-eye coordination associated with a 2-D screen, a skill similar those developed during video game and computer use. In fact, reports have demonstrated that video game playing is associated with improved reaction time and spatial visualization (3-7). It is therefore conceivable that individuals who have more video game exposure may have a different set of baseline skills for performing MIS, which has been the subject of multiple reports (8, 9).

New surgical technologies are usually introduced to practicing physicians before reaching residency training programs. Only after initial studies demonstrate safety and effectiveness do educational institutions begin incorporating the technology into residency training. For example, the first laparoscopic hysterectomy was described in 1989 (10); however, it did not become part of residency training objectives until the 2000s. Robotic surgery training seems to be following a similar trend. Although it was Food and Drug Administration (FDA)-approved in 2005, it is not yet part of the Accreditation Council for Graduate Medical Education (ACGME) training objectives. As a consequence of this delay, current teaching strategies are targeted towards practicing physicians, an older generation, rather than the younger generation of current and future gynecologic trainees. These strategies may not be appropriate, however, as the younger generation may possess

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a distinct set of background skills, given their early exposure to technology, that may render the current teaching strategies obsolete.

With the current widespread use of video games, it is reasonable to assume that future MIS learners may have a different set of "background" skills, impacting the design of more effective MIS teaching strategies. In this cross-sectional study, we evaluated the MIS simulation performance of college and high school students, as a proxy for future learners, in correlation to their exposure to screen time, defined as personal computer or video game use. To the best of our knowledge, this is the first study that assesses MIS simulation skills in high school and college students.

#### **Material and Methods**

The study was approved by the Institutional Review Board (IRB) at the University of Texas Medical Branch. This work was carried out in accordance with the Declaration of Helsinki, including, but not limited to, there being no potential harm to participants, guarantee of anonymity of participants, and informed consent from participants. This cross-sectional study included 3 groups of participants: high school students, college students, and obstetrics and gynecology residents. Questionnaires were used to obtain background information about the study subjects. The survey included questions about the subjects' education or PGY levels, years of computer use, and video game playing. Game use was further detailed by determining the number of gaming hours each day of the week, as well as average daily and weekly use. Furthermore, the types of consoles used e.g. Xbox (Microsoft Corporation, Redmond, WA, USA), PlayStation (Sony Corporation, Minato, Tokyo, Japan), personal computer, or Wii (Nintendo, Kyoto, Japan) and types of games played (war games, car racing, sports games, strategy games, or other) were delineated. Subjects were also asked how many minutes per day they used their computers, both for personal use and homework.

MIS simulation exercises were then performed, and data were recorded and analyzed. We used the Mimic Technologies dV-Trainer platform (Mimic Technologies Inc, Seattle, WA, USA) for robotic simulation and the 3-Dmed Trainer platform (3-DMEd, Franklin, OH, USA) for laparoscopic simulation testing. Four

robotic exercises mimicking EndoWrist manipulation were performed: "Pick and Place," "Ring and Rail," "Match Board-1," and "Peg Board-1." For laparoscopic simulation testing, we used the "Loops and Wire" exercise. Time to complete each exercise was recorded.

#### **Statistical Analysis**

All variables were examined for unusual and missing values using dot plots for each variable. Medians, interquartile ranges, and minimum and maximum values were examined for each of the background variables by group (High School, College, or Resident). A one-way analysis of variance procedure for nonparametric data (Kruskal-Wallis U test) was used to assess whether the variation among groups was significant for each of the variables. Separate pairwise comparisons for each group of subjects were performed using a Bonferroni adjustment. Data were analyzed using SAS Software, v. 9.2 (SAS Institute Inc., Cary, NC, USA).

#### Results

A total of 17 students, both high school (n=9) and college (n=8), were enrolled in this study. In addition, a group of obstetrics and gynecology residents (n=11) was included for comparison. As expected, the age range was significantly different among groups (p<.001).

Results of the laparoscopic and robotic simulation assessment are presented in Table 1. Pairwise comparisons with Bonferroni adjustment show that students took significantly longer time than residents to complete laparoscopic exercises (p=.027). However, the time needed by students to complete robotic simulation exercises was not statistically significant from residents (p-values ranged from .319 to .967). Only the Peg Board exercise showed a longer time to complete for high school students compared to residents (p=.006).

Given that the students' performance was close to that of residents in robotic simulation, we also analyzed screen time exposure. Background characteristics are shown in Table 2, where variables are presented as medians and interquartile ranges and stratified by group. Kruskal-Wallis tests showed that number of years of computer use, minutes of daily personal computer use, and minutes of weekly video game playing differed

						p value	28
	High School (n=9) median (IQ range)	College (n=8) median (IQ range)	Resident (n=11) median (IQ range)	Kruskal-Wallis	HS vs College <sup>‡</sup>	HS vs Resident <sup>‡</sup>	College vs Resident <sup>‡</sup>
Age (years)	16 (16-16)	19.5 (18.5-20)	29 (28-32)	<.001*	.001*	.001*	.001*
PC use (years of use)	10 (10-11)	15 (11-17.5)	15 (15-20)	.011*	.022	.002*	.429
PC use-work (min/day)	60 (45-120)	120 (75-150)	180 (90-240)	.148	.187	.071	.426
PC use-personal (min/day)	60 (60-90)	135 (82.5-240)	45 (30-60)	.003	.020	.075	.002
Video games (min/week)	675 (420-960)	1260 (1250-1650)	180 (30-200)	<.001*	.011*	.001*	.001*
IQ: interquartile range; PC: personal computer; HS: high school; n: number of subjects; min: minutes; vs: versus         *Statistically significant         *For pairwise comparisons, p-value is significant if less than .017.							

						p values	
	High School (n=9) median (IQ range)	College (n=8) median (IQ range)	Resident (n=11) median (IQ range)	Kruskal-Wallis	HS vs College <sup>‡</sup>	HS vs Resident <sup>‡</sup>	College vs Resident <sup>‡</sup>
Xbox	80 (50-85)	45 (5-55)	0 (0-5)	.005*	.144	.005*	.009*
PlayStation	15 (0-50)	5 (0-20)	0 (0-15)	.472	.541	.246	.474
Other	10 (0-15)	55 (27.5-82.5)	70 (0-100)	.016*	.004*	.029	.454
War	50 (40-85)	35 (10-55)	0 (0-10)	.012*	.111	.005*	.112
Car race	1 (0-20)	3.5 (0-12.5)	0 (0-50)	.977	.882	.842	.930
Sports	20 (5-34)	0 (0-10)	0 (0-0)	.001*	.016*	.001*	.298
Strategy	10 (0-10)	25 (12.5-30)	0 (0-85)	.319	.062	.597	.671
Other	0 (0-0)	135 (82.5-240)	0 (0-10)	.016*	.007*	.050	.117
IQ: interquartile ran *Statistically significa	ge; HS: high school; n: nui ant	mber of subjects; vs: ver	sus				

#### Table 2. Percentage of gaming time by game console and game type

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Percentages reflect division of playing time among console and game types at age of highest game use for each subject.

\*For pairwise comparisons, p-value is significant if less than .017.

#### Table 3. Minimally invasive surgery (MIS) simulation exercises data- time to completion in seconds

						p values			
	High School (n=9) median (IQ range)	College (n=8) median (IQ range)	Resident (n=11) median (IQ range)	Kruskal-Wallis	HS vs College <sup>‡</sup>	HS vs Resident <sup>‡</sup>	College vs Resident <sup>‡</sup>		
Pick and Place	86 (81-90)	86 (79.5-88.5)	78 (59-85)	.319	1.000	.148	.282		
Ring and Rail	58 (58-64)	62 (25.5-76)	62 (51-70)	.967	.772	1.000	.869		
Match Board-1	35 (31-45)	38.5 (29.5-43.5)	45 (26-48)	.693	.962	.543	.408		
Peg Board-1	62 (57-77)	51.5 (42-63)	45 (39-50)	.006*	.034*	.003*	.264		
Loops and Wire	159 (90-208)	74.5 (67.5-91)	55 (40-75)	.001*	.027*	.001*	.035*		
IQ: interquartile ran	IQ: interquartile range; HS: high school; n: number of subjects; vs: versus								

\*Statistically significant

\*For pairwise comparisons, p-value is significant if less than .017

among groups (p-values .0105, .0026, and <.001, respectively). Subsequently, we analyzed study groups according to types of consoles used and video games played (Table 2, 3). Our results show that high school and college students played the Xbox game console more than residents. The most common games played by high school students were war games, followed by sports and then strategy games, while college students mostly played other game types, followed by war and then strategy. High school students played statistically significantly more war games than residents. Of note, Xbox game use, other game console use, war games played, sports games played, and other types of games played were all significantly different among the 3 groups.

#### Discussion

The results of this study show that high school and college students' performance in basic MIS skills is comparable to residents. Initially, these results may seem unexpected; however, our finding that current students have more exposure to video games than residents have had in the past may provide

an explanation. These findings are important, as they may influence the way training programs are developed. We should emphasize that this is an initial pilot study that should spur larger-scale research in the field.

While our study is the first to assess MIS skills in nonmedical students, it is not the first to address the possible relationship of video games to MIS skills. In fact, several reports have been published in this area in recent years. For example, Shane et al. (11) showed that in a group of 26 medical students and first-year residents, individuals with previous video game experience took significantly less time to reach proficiency in laparoscopic skills than individuals without previous video game use. In 2 separate studies, Rosenberg et al. (8) and Glaser et al. (12) showed that in groups of 11 and 26 medical students, respectively, video game use correlated with both hand-eye coordination and reduced completion time for simple laparoscopic tasks. A potential bias with these studies, however, is the fact that prior exposure to laparoscopic surgery was not assessed. As medical students are exposed to MIS (both robotic and laparoscopic surgery) during their training, a study in younger students would be more pertinent. High school and college students, for example,

are less likely to be exposed to MIS. Clearly, a study comparing MIS skills in these students to those in residents who have already had some training would better assess the influence of video games on MIS skills.

Different types of video games likely produce different effects. Most studies of the cognitive effects of games have used action or "fast-action" games, typically first-person shooters, like the war games played by the students in this study (13, 14). In addition to shooter/war games, other games, such as Super Monkey Ball, which require complex navigation through virtual worlds, have successfully improved performance during surgical simulations (15-17).

These findings contribute to a growing body of literature investigating the potential of video games to influence visuospatial skills and psychomotor performance. Visual attention skills were shown to be higher in video game players than nonplayers in studies in both adults and children (18), with differences found in samples as young as 7 years old (19). The range of beneficial effects in studies that have tested video game training in nongamers suggests that these training effects may accrue in as little as 10 hours (15, 16) or may require longer periods of habitual use (17). Whether game-based training during childhood or adolescence may confer additional benefits over training in adulthood is an intriguing empirical question.

As previously mentioned, this study is a preliminary investigation; thus, its results must be interpreted with caution. The small sample size may have lacked sufficient power to detect differences among groups in their skills tests. There may also be unmeasured variables that contributed to these findings. It is possible that pre-existing differences in skills between those who choose to play games versus nongamers may confound the relationship between gaming and skill. Gender also may have played a contributing factor in producing these results. The students were male, while our sample of residents was mostly female (8/11). Though gender differences in visuospatial skills have been found in many studies, rigorous randomized controlled trials have shown that training women with action video games for as little as 10 hours can nearly eliminate gender differences (15). In fact, video-game-based spatial training may be as effective (19) or more effective (20) in women than men. Because action and shooter video games are typically marketed to and played by males, gender disparities are likely perpetuated by greater opportunities for skills training for young men (21). Balanced designs with larger samples are necessary to investigate how gender and gaming experience may interact in predicting surgical simulator performance.

As Spence and Feng (14) have pointed out, a major value of observational studies is in exploring fruitful new areas for subsequent research. Future studies are necessary to investigate potential cohort effects related to habitual video game use during childhood, the potential of video game-based interventions to increase surgical skills prior to residency, and the effectiveness of different educational strategies on gamers and nongamers.

Do we need to adapt our teaching strategies to fit the skills future learners already have? Clearly, we need to further investigate trends in hand-eye coordination in younger generations, which may lead to more successful teaching programs that use and build on skills already acquired. In addition, we can guide video game developers to create games that intentionally improve hand-eye coordination skills. It is intuitive to expect that such skills will not only improve MIS skills but also be advantageous to other professions requiring similar skills (eg, pilots, graphic designers, etc). Finally, we believe there should be close collaboration with developmental psychologists in building more effective MIS teaching strategies.

*Ethics Committee Approval: Ethics committee approval was received for this study from the Institutional Review Board (IRB) at the University of Texas Medical Branch.* 

*Informed Consent:* Written informed consent was obtained from participants for this study.

**Peer-review:** Externally peer-reviewed.

Author contributions: Concept - M.B., M.J., O.T., G.K.; Design - M.J., O.T., R.N., G.K.; Supervision - M.J., O.T., R.N., G.K.; Resource - M.B., E.L., G.K.; Materials - M.J., O.T., R.N., G.K.; Data Collection&/or Processing - M.J., O.T., R.N., G.K.; Analysis&/or Interpretation - M.B., M.J., O.T., G.K.; Literature Search - M.B., M.J., O.T., E.L., P.P.; Writing - M.B., O.T., M.J., P.P., G.K.; Critical Reviews - E.L., P.P., GK.

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

**Financial Disclosure:** This work was partially funded by an educational technology grant for Dr. Mostafa Borahay from the Academy of Master Teachers at the University of Texas System. Dr. Elizabeth Lyons is supported by NIH career development award (K12HD052023) and the Institute for Translational Sciences at the University of Texas Medical Branch.

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### Domestic violence against pregnant women: A prospective study in a metropolitan city, İstanbul

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#### Abstract

**Objective:** Violence against women, particularly against pregnant women, is increasingly being recognized as a significant problem around the world. Limited studies were found about domestic violence among pregnant women. The aim of this study was to determine the prevalence of domestic violence during pregnancy and the factors affecting it.

**Material and Methods:** This prospective study was conducted at the Department of Obstetrics and Gynecology, between January 2012 and April 2013. A total of 1349 pregnant women, irrespective of age and socioeconomic status, were interviewed using a questionnaire in the local language.

**Results:** The incidence of domestic violence in this study was 2.37%. The mean age of women who reported violence was  $29.06\pm5.53$  years. Age, marriage duration, gravidity, parity, educational level, partner's educational level, and monthly income exerted no significant influences on women who experienced domestic violence during their pregnancies (p>0.05). Women who resided in the same houses with large extended families were at significantly higher risk of domestic violence during pregnancy in comparison with the pregnant women who lived within a core family (p=0.018).

**Conclusion:** Domestic violence during pregnancy is a potential public health problem. Education, improvements in economic autonomy, and society's attitudes may reduce domestic violence. Health-care providers should increase their awareness of risk factors to protect women from domestic violence. (J Turk Ger Gynecol Assoc 2014; 15: 74-7)

Key words: Abuse, domestic violence, pregnant women, Turkey

Received: 30 July, 2013 Accepted: 15 October, 2013

#### Introduction

Violence against women, particularly against pregnant women, is increasingly being recognized as a significant problem around the world (1). Studies conducted in various countries have indicated that domestic violence against pregnant women varies between 4-40% (2, 3). Domestic violence exerts serious adverse effects. It has been reported that domestic violence has been associated with miscarriages, premature births, low birth weights, defective antenatal care, early placental separations, membrane ruptures, and fetal injuries (4, 5). In Turkey, pregnancy is the major reason why women enter health-care facilities. However, health-care providers generally remain unaware of domestic violence and do not take an interest in this problem.

There is a lack of robust data in Turkey on the prevalence of domestic violence during pregnancy. Only a limited number of studies that are focused on domestic violence during pregnancy have been conducted in Turkey. Therefore, our goal was to determine the prevalence of domestic violence during pregnancy, the factors affecting it, and the relationship between women's social status and domestic violence.

#### **Material and Methods**

This cross-sectional survey of pregnant women's experience with domestic violence was conducted in the antenatal ward of our clinic, between January 2012 and April 2013. Ethical approval was obtained from the institutional review board. Istanbul is the largest city in Turkey. It has a population of 13.9 million people. The city is one of the largest urban areas in Europe. It is the second largest city in the world with respect to urban populations. High numbers of immigrants travel from eastern and southeastern Turkey to Istanbul. Immigrants have cultural characteristics similar to general Eastern cultural characteristics. All pregnant women who attended the antenatal clinic at the participating health facility were eligible for the study. Participants were selected by simple random sampling performed on clinic days. Women who were too ill or had obstetric emergencies were excluded. The admitted pregnant women were interviewed by a clinic nurse in complete privacy. Informed consents were obtained, and interviews were conducted using a specific questionnaire. The questionnaire included questions that referred to physical violence when women were slapped, pushed, hit with a fist, choked, or threatened and sexual violence when women

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reported that they were forced to have some form of sexual intercourse. To assess intimate partner violence, women were asked: Has your husband/partner threatened you verbally, slapped you, pulled your hair, kicked you, or thrown you to the ground. A "yes" to any of these options was coded 1 versus 0 when none of these was reported. Types of abuse were defined as verbal and physical. Verbal violence included the use of degrading sentences, blaming, and swearing. Slapping, hitting, pushing, throwing women against walls, pulling hair, kicking, brandishing knives, and causing other injuries were classified as physical violence. Interview durations ranged between 30 minutes and 1 hour. Research assistants were trained on the importance of maintaining confidentiality. All women who were identified as at risk for violence were referred for counseling and further support.

SPSS 21.0 for Windows® software (Chicago, IL, USA) was used to conduct a statistical analysis of the data. Means, standard deviations, and percentages were used to evaluate descriptive statistics. The distribution of variables was controlled by the Kolmogorov-Smirnov test. The Mann-Whitney U-test was used to conduct a quantitative data analysis. Associations were tested using  $\chi^2$  or Fisher's exact test, as appropriate. The level of statistical significance was set at p<0.05.

#### Results

In total, 1349 pregnant patients were included in the study. Out of the total study population, 2.37% (n=32) reported they were victims of violence during their pregnancies. The mean age of women who reported violence was  $29.06\pm5.53$  years. The mean gravidity was  $2.78\pm1.54$ . The mean parity was  $1.34\pm1.18$ . Almost all participants (99%) were married, 49.9% had achieved educational levels of primary school or lower, 1104 (81.8%) were housewives, 10.4% smoked cigarettes, and none used alcohol. The results revealed that 47% of pregnant women's partners had graduated from preliminary school, 38.1% had graduated from high school, and 9.2% had earned bachelor's degrees.

Age, marriage duration, gravidity, parity, educational level, partner's educational level, and monthly income exerted no significant influences on women who experienced domestic violence during their pregnancies (p>0.05). Women who resided in the same houses with large extended families were at significantly higher risk of domestic violence during pregnancy in comparison with the pregnant women who lived within a core family (p=0.018). Table 1 shows the comparisons of the profiles of women who experienced violence during their pregnancies before they became pregnant with women who had not experienced violence. Remarkably, almost 30.5% of the women had unplanned pregnancies. However, this factor did not exert any influence on whether these women experienced violence during their pregnancies (p>0.05).

#### Discussion

Domestic violence (intimate partner or family violence) against women is a significant public health problem because of its prevalence, as well as because of its short- and long-term physical and mental health consequences (6). Violence during pregnancy demands special attention, because it affects women in a moment of great physical and emotional vulnerability. It has also been associated with adverse obstetric or neonatal outcomes, such as low birth weight and preterm delivery. Over both the short term and long term, physical injuries affect family life, which has a significant effect on children and loss of faith and trust in the institution of the family. These results not only affect the quality of life of individuals and society but also have long-term effects on social order. In a study focused on 290 pregnant women, Helton and Snodgrass (7) discovered a 15% prevalence of physical abuse prior to pregnancy and an additional 8% prevalence during current pregnancies. In the largest series conducted by Amaro et al. (8), they discovered a 7% prevalence of violence during current pregnancies. Hillard (9), Stewart (10), and Campbell (11) discovered a similar prevalence of violence during pregnancy (4%, 6.6%, and 8.2%, respectively). In the present study, we discovered a 2.37% prevalence of violence during pregnancy. In a study conducted in eastern Turkey, Arslantaş et al. (12) discovered an 18.2% prevalence of physical violence, and Taspinar et al. (13) also discovered a 24.8% prevalence of violence. A report published by the National Research on Domestic Violence Against Women in Turkey stated that the number of women who suffered from violence during pregnancy varied based on geographic regions (14). Generally, women's social status was worse in the eastern region. Despite high immigration rates, we were surprised to discover a low prevalence of violence (2.37%) in comparison with Western countries. We believe that pregnant women exposed to violence were afraid to disclose their experiences. They were afraid of their husbands, even if their husbands remained outside the clinic. In all likelihood, pregnant victims would hesitate to speak about violence, even in a tertiary center, and it is possible they might more easily disclose their experiences in first-line health-care units. It can be difficult to compare the differing results for the prevalence of violence, because women's understanding of the definition of violence may differ. These differences might be related to social norms. Some reports have noted that women who experience physical violence during pregnancy tend to be younger (10, 15). We were unable to discover any differences related to age. It has been well documented that women's exposure to violence tends to be lower among women who possess higher education levels (16-18). We achieved inconclusive results for educational levels in comparison with previous reports. However, we discovered a significant difference in the relationship between the number of people who resided together in the same house and exposure to violence. As the number of people who resided in the same house increased, the likelihood of exposure to violence also increased. In Turkey, some girls and boys continue to marry because they are pressured by their families and/or relatives. These couples continue to reside with their relatives after getting married. Turkish legal regulations state that any verbal or physical act of violence will be punished. However, women must lodge complaints against perpetrators. Unfortunately, women fail to make complaints because they

		l I	 No	Y	íes			
		Mean	±SD/n %	Mean	n±SD/n%	p value		
Frequency of violence (monthly)	)			5.0	±7.7			
Gravidity		2.38	±1.50	2.78	±1.54	0.086		
Parity		0.98	±1.07	1.34	±1.18	0.053		
Number of children		0.92	±1.03	1.28	±1.22	0.078		
Marriage duration (years)		6.46	±5.21	7.08	±5.61	0.514		
Age (years)		28.28	3±5.51	29.00	$29.06 \pm 5.53$			
Marital status	No	4	0.3%	0	0.0%	1 000		
	Yes	1.313	99.7%	32	100.0%	1.000		
	None	100	7.6%	4	12.5%			
	Primary school	555	42.1%	15	46.9%			
Women's educational status	Secondary school	217	16.5%	2	6.3%	0.189		
	High School	351	26.7%	11	34.4%			
	University	94	7.1%	0	0.0%			
Women's work status	Unemployed	1.078	81.9%	26	81.3%	0.020		
	Employed	239	18.1%	6	18.8%	0.000		
Partners' educational status	Primary school and below	616	46.8%	19	59.4%			
	High School	617	46.9%	12	37.5%	0.342		
	University	83	6.3%	1	3.1%			
Income/month	500 Dollars and below	679	51.6%	17	53.1%			
	500-1000 Dollars	474	36.0%	10	31.3%	0.422		
	1000-1500 Dollars	84	6.4%	1	3.1%			
	1500 Dollars and above	80	6.1%	4	12.5%			
Householder	Rental	704	53.5%	18	56.3%	0.754		
	Own	613	46.5%	14	43.8%	0.754		
Number of people residing together in house		3.68	±2.00	4.66	±2.73	0.018		
Systemic illness	No	1.166	88.5%	31	96.9%	0.140		
	Yes	151	11.5%	1	3.1%			
Smoking	No	1.180	89.6%	29	90.6%	0.851		
	Yes	137	10.4%	3	9.4%	0.001		
Planned pregnancy	No	398	30.2%	14	43.8%	0 101		
	Yes	919	69.8%	18	56.3%	0.101		
Gestational week		25.61	±8.75	24.75	5±8.82	0.569		
Coital frequency per week		2.16	±1.17	2.05	±1.15	0.469		
Contraception	No	440	33.4%	10	31.3%	0 709		
	Yes	877	66.6%	22	68.8%	0.190		

54

811

6.2%

93.8%

4

22

15.4%

84.6%

0.082

### Table 1. Comparison of characteristics of women who experienced violence during their pregnancies with women who did not experience violence

SD: standard deviation

History of unplanned pregnancy

No

Yes

fear discrimination or further exposure to violence perpetrated primarily by men. Turkish social customs contribute to females' poor self-esteem. In some areas, society excuses violence against women. In our study, with the exception of one case, all perpetrators were husbands. In the exceptional case, the perpetrator was the woman's father-in-law. Khosla et al. (19) reported that 51.8% of women in their study experienced violence perpetrated by their husbands' families.

The current study was limited, because it employed a small number of subjects. In addition, the current study's results solely reflect a small area of Turkey. Additional larger studies are required to evaluate the risk factors for violence during pregnancy. Researchers should interview men to determine risk factors and reasons for the perpetration of violence against women.

In conclusion, domestic violence during pregnancy is a potential public health problem. Education and improvements in economic autonomy and society's attitudes may reduce domestic violence. Health-care providers should increase their awareness of risk factors to protect women from domestic violence.

*Ethics Committee Approval: Ethics committee approval was received for this study from Bakırköy Dr. Sadi Konuk Research Hospital.* 

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

#### Peer-review: Externally peer-reviewed.

Author contributions: Concept - H.C.; Design - H.C., A.K.; Supervision - H.C.; Resource - H.C., Ş.Y.; Materials - H.C., Ş.Y., S.S.; Data Collection&/or Processing - A.K., S.S.; Analysis&/or Interpretation - H.C., Ş.Y.; Literature Search - H.C., Ş.Y.; Writing -H.C., Ş.Y.; Critical Reviews - Ş.Y.

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

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

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### Value of three dimensional power Doppler ultrasound in prediction of endometrial carcinoma in patients with postmenopausal bleeding

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#### Abstract

**Objective:** To determine whether endometrial volume or power Doppler indices measured by 3-dimensional (3D) ultrasound imaging can discriminate between benign and malignant endometrium in women with postmenopausal bleeding and endometrial thickness  $\geq$ 5 mm.

**Material and Methods:** The current diagnostic accuracy study was conducted at Ain Shams University Maternity Hospital. Eighty-four patients with postmenopausal bleeding and endometrial thickness  $\geq$ 5 mm underwent 3D power Doppler ultrasound examination of the corpus uteri. The endometrial volume was calculated, along with the vascularization index (VI), flow index (FI), and vascularization flow index (VFI) in the endometrium. The gold standard was the histopathological diagnosis of the endometrium.

**Results:** Of the 84 women included in the study, 56 (66.7%) had benign endometrial lesions, and 28 (33.3%) had malignant endometrial lesions. Endometrial thickness, endometrial volume, and flow indices (VI, FI, and VFI) were higher in patients with malignant endometrium than those with benign endometrium. The area under the receiver operator characteristic curve (AUC) of endometrial thickness was 0.83, that of endometrial volume was 0.73, and that of the best power Doppler variable, FI, was 0.93. The best logistic regression model for predicting malignancy contained the variables endometrial thickness and FI; its AUC was 0.93.

**Conclusion:** The diagnostic performance of endometrial volume measured by 3D imaging with regard to discriminating between benign and malignant endometrium was not superior to that of endometrial thickness measured by 2D ultrasound examination, but 3D power Doppler flow indices are good diagnostic tools in predicting endometrial carcinoma. (J Turk Ger Gynecol Assoc 2014; 15: 78-81)

Key words: Endometrial carcinoma, postmenopausal bleeding, power Doppler, 3-dimensional ultrasound

Received: 26 September, 2013 Accepted: 28 November, 2013

#### Introduction

Bleeding after menopause is a widespread problem, with 10%-15% of cases later suffering from cancer of the endometrium. It differs from other malignancies, in that early symptomatization is common, allowing early cure. Survival drops with late stages and so studies should strive to increase the precision of various diagnostic practices (1). The ideal diagnostic strategy is still debatable. A thin endometrium measuring less than 5 mm by vaginal scanning in PMB excludes about 99% of endometrial cancers. Sampling of the endometrium is considered necessary in those patients with an endometrium  $\geq 5$  mm. However, many "normal" women with PMB and thickened endometria will undergo unnecessary diagnostic procedures. (2). Three-dimensional (3D) ultrasonography and power Doppler angiography (PDA) is a novel sonographic diagnostic modality. This technology permits acquisition of the volume of the endometrium and assessment of its vasculature using 3D power Doppler mapping. Using Virtual Organ Computeraided AnaLysis (VOCAL<sup>™</sup>) software, three vascularity indices can be obtained automatically: the vascularization index (VI), the flow index (FI), and the vascularization flow index (VFI). This method has been proven to be highly reproducible for analyzing the volume of the endometrium and 3-dimensional power Doppler indices of patients with malignancy of the endometrium (3). This research aimed to establish if volume and power Doppler indices of the endometrium could differentiate cancerous and non-cancerous endometrium in patients who bleed after menopause and have endometrial thickening exceeding 5 mm.

#### **Material and Methods**

The current diagnostic accuracy study was performed at Ain Shams University Maternity Hospital between September 2010 and December 2012. The ethics committee of Ain Shams University confirmed the study methodology. Informed consent was taken from all cases after full counseling. Women with PMB and had endometrial thickening over 5 mm by vaginal B-mode scanning conformed with our inclusion criteria. Post-menopause was defined as at least 1 entire year of menstrual cessation after the age of 40. All cases had their histories taken; complete general and local examinations were done. For all included women, 3D transvaginal ultrasound

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imaging using (Philips<sup>™</sup> Amsterdam, The Netherlands, HD9, ultrasound system equipped with a 6-9 megaHertz transvaginal probe). Transvaginal color Doppler flow mapping was performed using the ascending branch of the uterine artery, which was located in the parametrial area at the level of the internal os. The body of the uterus only was centralized in the 3D sector on the monitor, so as to fill it, and the corporeal volume was taken; then, power Doppler mode was obtained, and the flow indices (VI, FI, VFI) in the endometrium were measured. Power Doppler settings were set to obtain maximum sensitivity to perceive low-velocity flow without noise (frequency=5 MHz; gain=7.4; dynamic range=20-40 dB; edge=1; persistence=2; color map=5; gate=2; wall motion filter=L1; pulse repetition frequency=0.6 kHz). Patients were requested to remain stationary, and volume was obtained in 15-20 seconds. VI reflects the number of color voxels, which represents the vessels in the tissue, and is written as a percentage. FI is the mean color value in the color voxels, which manifests the average intensity of blood flow, and is represented as a number from 0-100. VFI is the mean color value in all voxels in the volume, which represents both vascularization and flow, and is also written as a number from 0 to 100 (4). Endometrial volume and the power Doppler indices were measured using VOCAL.

A total of 84 cases with PMB were included in our study. Endometrial sampling was done for all of them. They were divided into two groups, according to the histological diagnosis obtained at the Early Cancer Detection Unit in Ain Shams University Maternity Hospital. Group 1 was 28 patients with histological diagnosis of endometrial carcinoma, and Group 11 was 56 patients with histological diagnosis of benign disorders.

#### Statistical Analysis

Statistical assessment was done on a computer using MedCalc© version 12.2.1.0 (MedCalc© Software, Mariakerke, Belgium). The D'Agostino Pearson test was done to assess the normality of the numerical data distribution. A statistically significant test denotes non-normally distributed data. Normally distributed numerical data are shown as mean and standard deviation (SD). Skewed numerical data are shown as median and interquartile range. Qualitative data are presented as number and percentage. Intergroup differences were compared using the independent samples t-test (for normally distributed quantitative data) or the Mann-Whitney U-test (for skewed quantitative data). Qualitative data were compared using the Pearson chi-square test or the chi-square test for trends (for ordinal qualitative data). Exact significance was calculated whenever the expected frequency was <5 in >20% of cells in any contingency table. To examine the value of various ultrasound indices for the prediction of the type of endometrial lesion (endometrial carcinoma versus benign lesions), a series of receiver-operating characteristic (ROC) curves were constructed, and the area under the curve was estimated. The best cut-off criterion of the ROC curve was identified as that associated with the highest Youden's index (J statistic), where J=(sensitivity + specificity) - 1. The DeLong method was used for calculation of the standard error (SE) for the area under the curve (AUC) and of the SE for the difference between any pair of AUCs (5). The 95% confidence interval (CI) for the AUC was calculated based on binomial exact probability, which was used to estimate the statistical significance for the difference between the AUCs of any

pair of ROC curves. The validity of study parameters was evaluated in terms of sensitivity, specificity, likelihood ratio (LR+), and negative likelihood ratio (LR-). All P values are two-tailed. p<0.05 was considered as denoting statistical significance.

#### Results

Eighty-four patients with postmenopausal bleeding were assessed with both B-mode transvaginal scanning and 3D power Doppler. Division according to endometrial pathology into 2 groups was done. Group 1 included 28 patients with malignant endometrium. Group 2 was 56 patients with benign endometrium. The histopathological diagnosis of the included women is shown in Table 1. Those with malignant endometrium tended to be older (mean age was 61 years versus 55 vears; p=0.001) and had a greater body mass index (BMI) (median,  $34 \text{ kg/m}^2$  versus  $28 \text{ kg/m}^2$ ; p=0.008) than those with benign endometrium, but there was overlap between the two groups regarding parity. On the contrary, medical disorders (e.g., diabetes mellitus and hypertension) were more prevalent among patients with benign endometrium. In Group 2, 24 women (43%) had diabetes mellitus, and 25 women (44.7%) were hypertensive, while in Group 1, 10 women (35.7%) were diabetic and 7 women (25%) had hypertension. Endometrioid adenocarcinoma was diagnosed in 12 cases (42.8%), adenocarcinoma was diagnosed in 10 cases (35.6%), clear cell adenocarcinoma and squamous cell carcinoma were diagnosed in 2 cases (7.2%), and serous papillary and sarcoma was diagnosed in 1 case (3.6%) (Table 1). Endometrial thickness, endometrial volume, and flow indices were greater in cases with endometrial malignancy than benign cases, as shown in Table 2. The ultimate discriminator between non-cancerous and cancerous endometrium was FI, with an AUC of 0.937. Endometrial thickness, VI, and VFI had a comparable diagnostic accuracy, all having an AUC of 0.83 (Table 3). As the model with endometrial thickness + FI showed greater specificity (96%), we considered it the best logistic regression model for differentiating between non-cancerous and cancerous endometrium. The mathematically optimal risk cut-off value of this model (0.19) had a sensitivity of 85%, specificity of 96%, an LR+ of 14, and an LR- of 0.15 (Table 4) (Figure 1).

Table 1. Flistopathological diagnosis of the included wom	iagnosis of the included women
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Histological diagnosis	n (%)
Polyps and myomas	30 (53.6)
Atrophic endometrium	4 (7.2)
Hormone-influenced endometrium	4 (7.2)
Hyperplasia without atypia	17 (30.3)
Hyperplasia with atypia	1 (1.7)
Endometrioid adenocarcinoma	12 (42.8)
Clear cell adenocarcinoma	2 (7.2)
Serous papillary	1 (3.6)
Adenocarcinoma	10 (35.6)
Squamous cell carcinoma	2 (7.2)
Sarcoma	1 (3.6)

Variable	Benign endometrial lesions (number=56)	Endometrial carcinoma (number=28)	p value
Endometrial thickness, mm	8 (7-9)	12 (9.5-16.5)	< 0.001
Endometrial volume, mm <sup>3</sup>	1.55 (0.88-3.16)	3.86 (2.94-6.57)	< 0.001
VI	0.063 (0.039-0.288)	0.687 (0.657-0.687)	< 0.001
FI	21.019 (19.231-22.228)	25.59 (24.039-28.403)	<0.001
VFI	0.013 (0.007-0.063)	0.193 (0.073-0.522)	< 0.001
Analysis using independent samples VI: vascularization index; FI: flow inc	s t-test dex; VFI: vascularization flow index		

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Ultrasound parameter	AUC	SE of AUC	95% CI of AUC
Endometrial thickness	0.834	0.045	0.737 to 0.906
Endometrial volume	0.737	0.059	0.692 to 0.827
VI	0.823	0.050	0.725 to 0.898
FI	0.937	0.029	0.863 to 0.979
VFI	0.838	0.047	0.741 to 0.909

AUC: area under the curve; CI: confidence interval; FI: flow index; ROC: receiver operator characteristic; SE: standard Error; VFI: vascularization flow index; VI: vascularization index



Figure 1. Comparison of areas under the three receiver operator characteristic (ROC) curves. Area under the ROC curve for flow index (FI) is significantly larger than the area under the ROC curve for either the vascularization index (VI) (p=0.031) or the vascularization flow index (VFI) (p=0.041) ROC curves

#### Discussion

The current study authenticated that even though the endometrial volumes were significantly greater in cancerous than in non-cancerous endometrium (mean volume of the endometrium was 1.55 cc and 3.86 cc in the two groups, respectively (p<0.001)), volume measurements by 3D imaging were not better than simple endometrial thickness measurements by B-mode examination (AUC 0.737 vs. AUC 0.834). They also showed that logistic regression models with thickness and flow indices (VI, FI, VFI) did better than models containing thickness alone (AUC 0.894, 0.931, and 0.904 vs. AUC 0.834). Four different studies have attempted to identify the diagnostic accuracy of 3D ultrasound scanning in differentiation between non-cancerous and cancerous endometria (6-9). A fifth study assessed the potential of 3D ultrasound to differentiate endometrial carcinoma and hyperplasia (3), and a sixth paper assessed the potential to differentiate malignancy and hyperplastic tissue from other non-cancerous conditions (10).

The six studies discussed the diagnostic accuracy of endometrial volume measurement, and four also reported on that of 3D power Doppler flow indices (3, 7, 9, 10).

In the study by Gruboeck et al. (6), ROC curves highlighted that endometrial volume was better than endometrial thickness in diagnosing endometrial malignancy. The best cut-off value of endometrial thickening in diagnosing malignancy was 15 mm, with a sensitivity of 83.3% and positive predictive value of 54.5%. A cut-off level of 13 ml for endometrial volume had a sensitivity of 100% and a positive predictive value of 91.7%. Both thickness and volume were greater in cases with late and less-differentiated malignancies. The measurements of endometrial volume were better than endometrial thickness as a diagnostic test for detecting endometrial malignancy in symptomatizing postmenopausal women (6). Yamen et al. (8) authenticated that both endometrial volume and thickness measurements by 3D and 2D scanning, respectively, were adequately reproducible but that the reproducibility of 3D was superior. Merce et al. (3) stated that endometrial volume and 3D power Doppler indices (VI, FI, and VFI) were significantly greater in carcinoma than hyperplasia and that a VFI of 2.07 was the ideal cutoff for prediction of carcinoma, with a sensitivity of 76.5% and specificity of 80.8%. No important differences were seen for endometrial thickness. As regard to the study by Odeh et al. (10), mean thickness was 11 mm and 15.5 mm in the normal and pathologic groups, respectively (p < 0.005). The mean volume was 6.87 cc and 15.5 cc in both groups, respectively (p<0.001). The VI was 2.27% and 2.95% in both groups, respectively (p=0.022). The FI was 18.6 and 23.6 in both groups, respectively (p=0.014). The VFI was 0.68 and 0.89 in both groups, respectively (p=0.018). Endometrial volume and 3D-PDA are valuable diagnostic modalities in the prediction of endometrial malignancy and hyperplasia in women with postand peri-menopausal bleeding (10).

Opolskiene et al. (9) showed that the diagnostic accuracy for differentiating between benign and malignant endometria by 3D ultrasonography was not better than endometrial thickness assessed by B-mode ultrasonography, and 3D power Doppler scanning yielded little more than thickness or volume. Models

	AUC	Cut -off value	Sensitivity	Specificity	LR+	LR-			
ET	0.834	>9 mm	75	80.36	3.82	0.31			
EV	0.737	>1.94 cm	78.57	62.5	2.1	0.34			
VI	0.823	>4.0 %	89.29	75	3.57	0.14			
FI	0.937	>23.3	85.7	98.2	14.6	0.15			
VFI	0.838	>1.4	89.29	75	3.57	0.14			
ET+EV	0.829	0.16	94	53	2.15	0.2			
ET+VI	0.894	0.19	92	80	4.73	0.089			
ET+FI	0.931	0.19	85	96	14	0.15			
ET+VFI	0.904	0.17	89	80	4.55	0.13			
AUC: area un likelihood rati	AUC: area under the curve; ET: endometrial thickness; EV: endometrial volume; FI: flow index; LR-: negative likelihood ratio; LR+: positive likelihood ratio; SE: standard error; VFI: vascularization flow index; VI: vascularization index								

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with volume and flow indices were inferior to thickness alone (AUC 0.79 vs. 0.82). Both thickness and volume were significantly greater in cancerous than in normal endometria, and flow indices in the endometrium and endometrial shell were significantly greater. The AUC of endometrial thickness was 0.82, while that od endometrial volume was 0.78, and those of the two best power Doppler variables (VI and VFI in the endometrium) were 0.82 and 0.82. The best logistic regression model for predicting malignancy contained endometrial thickness (odds ratio 1.2; 95% CI, 1.04-1.30; p=0.004) and VI in the endometrial 'shell' (odds ratio 1.1; 95% CI, 1.02-1.23; p=0.01) (9).

The variable findings in the six published studies, together with the differences in findings between our study and the other six, can confidently be explained by marked variations in study populations and design. Although all publications cited included only women with abnormal uterine bleeding, there were variations in menopausal state, rate of endometrial cancer, mix of benign histological findings, use of hormone replacement treatment, and endometrial thickening. Also, there were differences in group comparisons between the studies [cancer versus hyperplasia in the study by Merc´e et al. (3) and cancer or hyperplasia versus other benign conditions in the study by Odeh et al. (10)]. There was benign versus malignant in the studies by Gruboeck et al. (6), Yamen et al. (8), Alcazar et al. (7), and Opolskiene et al. (9), and there were also variations in the methods used to determine diagnostic performance. There were 2 limitations to the analysis reported in the current study. The first was the relatively small sample size, and the second concerned the fact that ultrasound examination was done by a single observer; so, there was no comment on the reproducibility of the 3D ultrasound imaging. The diagnostic performance of endometrial volume measured by 3D imaging regarding discrimination between benign and malignant endometria was not better than that of endometrial thickness measured by B-mode scanning, but 3D power Doppler flow indices are good diagnostic tools in predicting endometrial carcinoma.

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

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

**Peer-review:** Externally peer-reviewed.

Author contributions: Concept - S.A.; Design - S.H.; Supervision - S.H.; Resource - A.A.G.; Materials - H.A.B.; Data Collection&/or Processing -H.A.B.; Literature Search - H.A.B.; Writing - S.H., H.A.; Critical Reviews - S.A.

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

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

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# The effect of intrauterine insemination time on semen parameters

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#### Abstract

**Objective:** The purpose of this observational study was to determine whether semen parameters (concentration, motility) were affected by the interval between the onset of postwash sperm incubation and intrauterine insemination (IUI) time.

**Material and Methods:** Semen specimens of 100 normozoospermic men collected at the clinic were allowed 20 minutes for liquefaction at room temperature. Semen samples were subjected to both macroscopic and microscopic examinations. After centrifugation in a density gradient column and sperm-washing medium, the samples were kept in an incubator. After 30 minutes, 60 minutes, and 120 minutes, the concentration and motility were recorded.

**Results:** According the results of the Bonferroni post hoc test, there were significant differences in values of mean sperm count, percent progressive sperm motility, and total motile sperm count between 30 minutes and 120 minutes (p=0.000, p=0.000, and p=0.000) and between 60 minutes and 120 minutes (p=0.000, p=0.000, and p=0.000), but there was no significant difference between 30 minutes and 60 minutes (p=1, p=0.173, and p=1).

**Conclusion:** This study demonstrated that sperm parameters are negatively affected from prolonged incubation time. A maximum 60-minute limit of the interval between the onset of postwash sperm incubation and IUI time may increase pregnancy rates. (J Turk Ger Gynecol Assoc 2014; 15: 82-5)

Key words: IUI time, sperm wash, semen parameters

Received: 23 October, 2013 Accepted: 16 December, 2013

#### Introduction

Intrauterine insemination (IUI) is a method that has been used for infertile couples in fertility treatment for many years. IUI is the first referenced assisted reproductive technique (ART) for mild to moderate male infertility. IUI is noninvasive and very simple, and it is less expensive than classical in vitro fertilization (IVF) and intracytoplasmic injection (ICSI). IUI success depends on many factors such as drugs, the timing and number of cycles, and total motile sperm count after washing. Another important factor affecting the success of IUI is the amount of motile sperm that is inserted into the uterus (1). Most studies have suggested that the success of IUI therefore decreases in pregnancy rates if there is not a sufficient number of motile sperm after washing (2-5).

A semen analysis is the first step to accurately diagnosing male infertility. Sperm count, percentage of moving sperm (sperm motility), and the percentage of sperm with normal morphology are the main criteria for the quality of semen. Determining sperm quality often uses values established by the World Health Organization (WHO) 2010 (6). However, the cut-off values for total progressive motile sperm count are still controversial and vary between 0.3x10<sup>6</sup> and 20x10<sup>6</sup> (7).

The intervals between semen collection and IUI time could affect the sperm parameters and clinical pregnancy rates negatively. However, there are few studies on this issue (8).

According to WHO criteria (2010), the interval between semen collection and giving it to the laboratory should not exceed 60 minutes (6). But, a few studies suggest that this interval should be shorter (8).

In this study, we aimed to determine the ideal interval between the onset of postwash sperm incubation and IUI time to increase IUI success.

#### **Material and Methods**

#### Subjects

In this study, 100 normozoospermic men underwent semen analysis at the Dokuz Eylül University IVF Unit. The mean age of the men was  $32.7\pm5.2$  years (range 23-47).

Semen samples were obtained by masturbation after sexual abstinence periods (from 2 to 6 days). All semen specimens collected at the clinic were allowed 20 minutes for liquefaction at room temperature.

After liquefaction, the semen variables that were considered were ejaculate volume, sperm count, total motility, and total progressive motility (TPM). Standard semen parameters were analyzed according to the 2010 WHO guidelines (6).

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Over 15 million sperm per milliliter of semen was considered normal. The lower reference limit for total sperm number was 39x10<sup>6</sup> spermatozoa per ejaculate. Total sperm count was calculated by multiplying semen volume by sperm concentration. Spermatozoa were categorized by the following different motility grades: 1) rapid progressive motility, 2) slow progressive motility, 3) nonprogressive motility, and 4) immotile sperm.

Total motility (normal  $\geq$ 40%) includes rapid progressive motility, slow progressive, and nonprogressive motility. Progressive motility (normal  $\geq$ 32%) includes rapid and slow progressive motility. Absolute values for total progressive motile spermatozoa count per ejaculate were also calculated by multiplying the percentage of total progressive sperm motility (a+b) by sperm concentration per milliliter and by the volume (in milliliters) of the single ejaculate.

Morphology was evaluated by the Spermac staining procedure (FertiPro, Beernem, Belgium). The smears were stained with Spermac, and 200 spermatozoa were evaluated for morphology. These were abnormal acrosome, head, mid-piece, and tail forms. The smears were evaluated under a light microscope at  $\times 1000$  magnification according to the method described by Kruger. The percentage of normal morphology in a sample was 4%. If it was below this percentage, this condition was diagnosed as teratozoospermia (6).The ejaculation time, the beginning of sperm wash time, and the onset of incubation were recorded.

Semen samples were processed using density gradient medium (PureSperm®, Nidacon, International AB, Sweden) and sperm wash medium (Sperm Wash, Nidacon, International AB, Sweden). For the experiment, we used PureSperm gradients of 90% and 50%. All procedures were conducted under sterile conditions. Media that was used for the processes was brought to 37°C. For the lower layer, PureSperm gradient 90% was transferred into a conical centrifuge tube using a sterile 1 mL

Table 1. The baseline characteristics of prewash semen samples

Semen parameter	Median value (IQR)*
Semen volume (mL)	3 (3)
Sperm count (10 <sup>6</sup> /mL)	60 (41.5)
Total progressive motility (%)	50 (10)
Total motility (%)	60 (10)
Morphologic normal form (%)	4 (1)
*Data are presented as median IQR: interquartile range	

pipette. For the upper layer, PureSperm gradient 50% was gently dispensed on top of the lower layer using a new sterile 1 mL pipette. Liquefied semen sample were then placed on top of the upper layer, and the tube was centrifuged for 15 minutes at 1800 rpm. The upper and lower layers were carefully aspirated without disturbing the pellet. Sperm wash medium was added, and the pellet was re-suspended using a 4 mL transfer pipette; then, the tube was centrifuged for 5 minutes at 1500 rpm. The supernatant was then removed, and 0.3-0.5 mL of sperm wash medium was dispensed on the top of the pellet gently. The tube was then incubated, and after 30 minutes, 60 minutes, and 120 minutes, the concentration and motility were recorded.

Ethics committee approval was received for the study, and all patients who participated in this study gave written informed consent forms.

#### **Statistical Analysis**

Statistical analysis of the data was done by SPSS software (version 16, SPSS, Chicago, USA). The data were analyzed by general linear models. A *P value* <0.05 was considered statistically significant.

#### Results

The median age of males was 32 years (range 23-47). The median ejaculatory abstinence interval was 4 days (range 2-5). The baseline characteristics of prewash semen samples are shown in Table 1.

According to Table 2, values for mean sperm count and total motile sperm count after 60 minutes of incubation were the highest and the lowest after 120 minutes. The mean total percent progressive sperm motility after 30 minutes was the highest and the lowest after 120 minutes. It was found that when using the ANOVA test with repeated measures with a Greenhouse-Geisser correction, the mean scores for sperm concentration (mil/mL), percent progressive sperm motility (%), and total motile sperm count (mil) were significantly different between groups (F=13.065, p<0.0005, F=20.128, p<0.0005, F=11.454, p<0.0005).

According the results of the Bonferroni post hoc test, there were significant differences in values of mean sperm count, percent progressive sperm motility, and total motile sperm count between 30 minutes and 120 minutes (p=0.000, p=0.000, and p=0.000) and between 60 minutes and 120 minutes (p=0.000, p=0.000, p=0.000, and p=0.001), but there was no significant difference between 30 minutes and 60 minutes (p=1, p=0.173, p=1).

Table 2. Margina	l means (mean±SD	)) of postwas	h semen para	ameters in di	ifferent pe	eriods
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Semen parameters (mean±min SD)	Groups						
	30 min	60 min	120 min	F	p value		
Sperm count (x10 <sup>6</sup> /mL)	$30.2 \pm 25.5$	$30.8 \pm 29.0$	$21.5 \pm 19.1$	13.065	0.000		
Total progressive motility (%)	96.8±8.3	95.2±10.4	87.5±21.9	20.128	0.000		
Total motile sperm count (x10 <sup>6</sup> )	8.8±7.5	9.2±10.9	5.4±4.8	11.454	0.000		

#### Discussion

Among the ARTs, IUI is considered a first-line procedure. IUI is simple, easy, and cheap, and it has the absence of potentially serious complications. Prior to an IUI, the semen to be used during the procedure must be prepared; this procedure is commonly referred to as sperm washing. According to the 2010 WHO guidelines, semen samples should be collected in a private room near the andrology laboratory, because freshness of the samples could affect fertility (6). Within minutes after ejaculation, the number of living sperm cells and their activity begin to drop off. Semen is affected from temperature changes and prolonged time (9-12). A freshly ejaculated semen sample might resume an admissible sperm progressive motility for up to 12 hours, and sperm cells will survive up to 24-48 hours (11, 12). Analysis of sperm should be conducted in a laboratory within 1 hour of collection according to the 2010 WHO guidelines (6).

Consumption of energy sources in the sperm-washing medium by the motile spermatozoa during a prolonged sperm wash-IUI interval could affect fertilization negatively, because sperm motility requires energy sources, such as glucose and fructose, and washed motile spermatozoa that are deprived of energy sources during a prolonged sperm wash-IUI interval might not be able to reach the fertilization site in the fallopian tubes after IUI. Another such factor could be premature (*in vitro*) capacitation of washed motile spermatozoa in the sperm-washing medium during a prolonged sperm wash-IUI interval, because seminal decapacitation factors are removed along with seminal plasma during the sperm wash procedure (8, 13).

Shimuzu et al. (14) published a study in 2009 to assess the relationship between the time interval from semen collection to sperm wash and IUI outcome; 1054 IUI treatment cycles were analyzed. The time interval from semen collection to sperm wash was divided into three groups: a) less than 3 hours, b) 3-5 hours, and c) over 5 hours. Total pregnancy rate was 14%, and IUI pregnancy rate was not different between groups. Another study was done by Alexander et al. (15) to correlate pregnancy outcomes with the subject time intervals in 210 clomiphene citrate (CC)-IUI cycles. Mean intervals from collection to sperm washing, from sperm washing to IUI, and from collection time to IUI were similar in pregnant and non-pregnant CC-treated women. A similar study conducted in 2003 analyzed 132 IUI cycles to determine if IUI outcome was affected by place of semen collection (home or clinic) and intervals from semen collection to sperm wash, from sperm wash to IUI time, and from semen collection to IUI time. Yavas et al. (16) concluded that CC and human menopausal gonadotropin (hMG) result in similar pregnancy rates when semen is collected either at home or at the clinic. Pregnancy is not affected by intervals of semen collection-sperm wash, sperm wash-IUI, and semen collection-IUI in CC-treated women but is associated with shorter intervals in hMG-treated women. Yavaş and Selub (8) published another study in 2004. In this study, it was found that semen collection at the clinic resulted in a higher pregnancy rate than collection at home in hMG-treated but not in CC-treated women. Intervals

of semen collection-sperm wash, sperm wash-IUI, and semen collection-IUI were shorter in pregnant than in nonpregnant hMG-treated women. Semen washed within 30 minutes after collection resulted in a higher pregnancy rate than that processed 31-60 minutes after collection in hMG-treated but not in CC-treated women. IUI performed within 90 minutes after collection resulted in a higher pregnancy rate than IUI performed at 91-120 minutes or >120 minutes after collection in hMG-treated but not in CC-treated women (8).

In a recent study, it was found that if washed sperm can be incubated for a minimum of 30 minutes at 37°C, the pregnancy rate is optimum. The authors concluded that a longer period up to 180 minutes does not compromise the pregnancy rate (17). Different results in the literature could be based on the dif-

ferent techniques used to prepare the spermatozoa for IUI or technicians in the andrology laboratory. In our study, all semen specimens were collected at the clinic in private rooms near the andrology laboratory. All semen analyses and sperm wash procedures were performed by the same investigator (E.K.). There were no data related to IUI outcomes, because IUI was not performed on women. In this study, normozoospermic semen samples of 100 men who applied to our laboratory for sperm testing were analyzed.

In this study, independent from woman factor and thus from the hormone preparations used, we aimed to see whether semen parameters (concentration, motility) were affected from the interval between the onset of postwash sperm incubation and IUI time. We set a fixed duration for the liquefaction (20 minutes) and sperm wash procedure (25 minutes).

According to statistical data, sperm parameters are not affected adversely by the start of the washing process of semen samples followed by a fixed semen liquefaction time of 20 minutes and by the extension of the insemination period up to 60 minutes, on the condition of appropriate incubation of the semen sample. Nevertheless, as this period reached 120 minutes, we observed a significant decline in number of sperm, total progressive sperm percentage, and total motile sperm averages.

As a result, especially considering the human chorionic gonadotropin (hCG) injection time at busy centers, this period, with the condition of not exceeding 60 minutes from the beginning of the incubation up to the time of insemination, probably affects pregnancy rates positively. It is also aimed to review the pregnancy results by repeating this study on IUI patients with their recorded incubation start times and IUI times.

### *Ethics Committee Approval: Ethics committee approval was received for this study.*

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

Peer-review: Externally peer-reviewed.

Author contributions: Concept - E.K., R.E.O., O.E.D.; Design - E.K., M.K., S.D.; Supervision - E.K., B.G.; Resource - E.K., O.E.D., R.E.O., B.G.; Materials - E.K., O.E.D., R.E.O., B.G.; Data Collection&/or Processing -E.K.; Analysis&/or Interpretation - E.K.; Literature Search - E.K.; Writing -E.K.; Critical Reviews - E.K.

**Conflict of Interest:** No conflict of interest was declared by the authors. **Financial Disclosure:** The authors declared that this study has received no financial support.

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### Dual effects of melatonin on uterine myoelectrical activity of non-pregnant rats

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#### Abstract

**Objective:** In this experimental study, we aimed to investigate the role of melatonin on uterine myoelectrical activity of non-pregnant rats. **Material and Methods:** Forty-six female rats were assigned to six groups: (1) control; (0.2 mL 0.9% NaCl was injected intravenously (IV), n=6); (2) melatonin applied as 0.4 mg/kg/IV (n=8); (3) melatonin applied as 4 mg/kg/IV (n=8); (4) single dose of oxytocin (100 mU/kg) injected IV (n=8); (5) melatonin (0.4 mg/kg) plus oxytocin (100 mU/kg) (n=8); and (6) melatonin (4 mg/kg) plus oxytocin (100 mU/kg) injected IV (n=8). Each rat underwent a laparotomy, and uterine myoelectrical signals were recorded. The mean spectrum, averaged over the spectral content of signals in each group, was compared.

**Results:** Melatonin induced uterine myoelectrical activity in a dose-dependent manner. Treatment of melatonin after oxytocin suppressed the mean power of the signals. Serum melatonin concentrations were significantly higher in melatonin-treated rats.

**Conclusion:** Melatonin itself at two different dose levels was found to be equally effective in stimulating the uterine electrical signals, although oxytocin-induced uterine electrical activity was suppressed by melatonin. These findings merit further investigations on the possible beneficial role of melatonin in the treatment of conditions associated with abnormal uterine activity. (J Turk Ger Gynecol Assoc 2014; 15: 86-91) **Key words:** Uterine contraction, melatonin, oxytocin, electrical stimulation

Received: 15 December, 2013 Accepted: 29 December, 2013

#### Introduction

Plasma oxytocin throughout pregnancy is very low in the daytime and changes to the highest level at nighttime (1, 2). This change has been reported to parallel diurnal changes in uterine contractions (2). This is why the onset of labor, particularly in human primates, generally transpires during nighttime (3). It has been reported that uterine contraction frequency increases between 20:30 and 02:00 in humans, which interestingly coincides with maximal pineal melatonin secretion (4).

Melatonin is a peptide hormone secreted by the pineal gland in a circadian manner and is released into the vascular system. Its higher levels occur during midnight in all species (5). Several physiological processes, such as the sleep-wake cycle and endocrine function of various glands, have been regulated by melatonin secretion (6). It was suggested that serum melatonin levels differ throughout gestation. The first half of pregnancy is characterized by elevated levels of melatonin; however, between 20-36 weeks, melatonin levels decrease, and from that time to the end of pregnancy, the levels rise again (7).

In a few previous investigations, it has been shown that uterine myocytes express both melatonin-1 and melatonin-2 receptors (8). In recent years, several studies have been conducted for investigating a significant correlation between uterine contraction dynamics and melatonin level; nonetheless, inconsistent results have been reported (9-11). Despite these results being contradictory, in addition to melatonin's prime effects on reproductive mechanisms, all of these studies indicate the existence of an uncharacterized relation between melatonin level and uterine contractility.

While the beneficial effects of melatonin in different tissues have been studied by researchers, to our best knowledge, there is no any information regarding the effects of melatonin in different concentrations on contraction patterns of the nonpregnant rat uterus (12-15). On the basis of this background, using an in vivo experimental model, we aimed to explore the physiological and pharmacological concentrations of melatonin on spontaneous and oxytocin-induced contraction patterns of a non-pregnant rat uterus.

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#### **Material and Methods**

#### Animal Care

Forty-six adult female Wistar albino rats, aged 11-13 weeks and weighing 200-350 g, were purchased from the Inonu University Laboratory Animals Research Center and placed in a temperature ( $21\pm2^{\circ}$ C)- and humidity-controlled ( $60\pm5\%$ ) room, in which a 12:12 h light: dark cycle was maintained. The rats had free access to standard dry pellets ad libitum and tap water to the end of the investigation. Each step of the study protocol was done in accordance with the animal research guidelines of the National Institutes of Health, and ethical approval was obtained from the local ethic committee of Inonu University Faculty of Medicine.

Before uterine exposition, all animals were synchronized hormonally in their 4-day estrus stages in order to eliminate the differences in steroid synthesis, and thus, uterine myoelectrical activity was recorded between the individual animals, and then the obtained signals were analyzed. Hormonal synchronization was done by subcutaneous injections of 55 mg/kg body weight estradiol with two doses 24 h apart, followed by one injection of 7.5 mg/kg body weight of progesterone 20 h after the last estradiol dose (16).

In order to determine the estrous cycle of each rat, vaginal smears of the rats were taken by a cotton swab daily; the swab was inserted into the vagina of rats and rotated 360° clockwise; then, the swab was smeared onto a glass slide. Smears were stained by Papanicolaou method and then evaluated through a Leica DFC280 light microscope by a pathologist who was blind to the groups. The estrous cycle was diagnosed as follows: proestrus period (epithelial cells with centrally nucleolated), estrus period (unnucleated and cornified epithelial cells), metestrus period (a few cornified cells with leukocytes and mucus), and diestrus period (a number of epithelial cells, mucus, and leukocytes).

#### **Drug Preparation and Treatment**

All rats were first weighed and then anesthetized with xylazine (8 mg/kg) and ketamine hydrochloride (75 mg/kg), which were intraperitoneally (i.p.) administered. The rats were randomly assigned (using random number tables) to six groups as follows: (1) control; (0.2 mL 0.9% NaCl was injected intravenously (IV) via the jugular vein, n=6; (2) melatonin applied as 0.4 mg/ kg/IV (physiological dose, n=8); (3) melatonin applied as 4 mg/ kg/IV (pharmacological dose, n=8); (4) single dose of oxytocin (100 mU/kg) injected IV (n=8); (5) melatonin (0.4 mg/kg) plus oxytocin (100 mU/kg) (n=8); and (6) melatonin (4 mg/kg) plus oxytocin (100 mU/kg) injected IV (n=8). The drug treatments were administered as a bolus injection via a right jugular venous catheter, which was performed under general anesthesia. Melatonin (Sigma Chemical Co., St Louis, MO, USA), used in this experiment, was dissolved in ethanol and diluted in saline to achieve a final concentration of 1% in ethanol.

#### **Myoelectrical Activity Measurement**

The recording of the electrical activity of uterine myofibrils was performed with a BIOPAC MP100 A-CE data acquisition system

(model MP100; version 3.7.2; Goleta, CA, USA) with a 1000 preamplifier gain (this preamplifier gain was compensated later through signal processing) and a sampling frequency of 500 Hz. Each rat underwent a laparotomy, and bipolar electrodes were subserously inserted into the uterine horn 1 cm apart from each other. The reference electrode was inserted on the left leg of animals, and the uterine myoelectrical activity was recorded for at least 5 min under general anesthesia. The signal recording was started 60 seconds after the injection of medication in each group for the stabilization period. The signals were examined by a specific program that was developed by the same research group using the Matlab (R6; The MathWorks, Natick, MA, USA) environment.

#### The Signal Analysis Method

The signals recorded from the uterine horns of each rat were analyzed for assessment of the effect of the given remedy. Since the strategy in this study was not based on exciting and recording the response, it was not thought that the time domain signals would provide a good marker for the given treatment common to all subjects within one group. The temporal average of signals over the subjects in each group may cancel the characteristic representations that can be linked to the medication. This cancellation could be due to unpredictable phase shifts between individual signal components. However, it was considered that a sort of correlation that goes between all the signals in each group should exist and that this correlation signal should give information regarding the format of the uterine contractions common to the individual group under consideration. Obviously, a permutative correlation process intervening between each pair of uterine signals would take too much time. So, instead of the correlation process in the time domain, the mean spectrum averaged over the spectral content of the signals of subjects in each group was simply taken into account for the assessment. Although these signals are, in fact, time-varying signals that may not be really processed through Fourier transform, we only focused on the existence of a common spectral content rather than the temporal evolution of each component. Otherwise, the process would be meaningless. Basically, the mean spectrum averaged over the signals in each group was calculated as:

$$S(f) = \frac{1}{N} \sum_{n=1}^{N} \int_{-\infty}^{+\infty} s_n(t) e^{-j2\pi ft} dt$$

(1) where s(t) stands for temporal uterine electrical activity and N is the number of subjects per group. The spectral signal, S(f), obtained from this process was considered as carrying characteristic features common to the group, which, therefore, should correlate to the medication given to the rats in the group. Note that the temporal signal is first converted into an analytical form through Hilbert transform, such that s(t)=u(t)+jH(u(t)). The Hilbert transform of a given uterine electrical signal u (t) can be defined as:

$$H(u(t)) = \frac{1}{\pi} P.v \sum_{n=1}^{N} \int_{-\infty}^{+\infty} \frac{u(\tau)}{t-\tau} d\tau$$

(2) where Pv. is the Cauchy principal value and  $\tau$  is the time delay (17). Then, the analytical signals were filtered with a second-order Butterworth band-pass filter with cutoff frequencies of 0.3 Hz-45 Hz. Finally, the power spectra of these complex analytical uterine signals were accomplished using equation (1).

#### **Extraction of Melatonin in Serum Samples**

To measure the melatonin concentration,  $100 \ \mu L 2$  M tricarboxylic acid (TCA) was added to 1.0 mL of serum samples. After 10 min in an ice bath, the mixture was centrifuged (5000 g, 10 min), and the pH of the supernatant was adjusted to 7.0 by the addition of  $10 \ \mu L 2$  M NaOH. The aqueous sample was added to the top of disposable Waters Oasis HLB (1 cc, 30 mg), conditioned using methyl alcohol (1 mL) and water (1 mL). Organic extraction was performed with 2 ml dicholoromethane. The extract was concentrated by evaporation in a nitrogen gas system. The residue was then reconstituted in 100  $\mu$ L of mobile phase, and 20  $\mu$ L of this solution was injected into the chromatograph.

#### Chromatography

Plasma melatonin concentration was determined by a sensitive high-performance liquid chromatographic (HPLC) method. The HPLC system was equipped with an LC-10 AD pump, fluorescence detector, and an SIL-AAAD Autosampler (Agilent, USA). Melatonin was separated using an ODS-2 (150x4.6 mm, 5  $\mu$ m) column. The HPLC mobile phase consisted of 75 mM sodium acetate-acetonitrile (72:28, v/v) (pH 5.0). The flow rate was 1.0 ml/min. The fluorimetric detector was set at an excitation wavelength of 275 nm and an emission wave length of 345 nm. The peak area was used as a measure of the detector response. The level of melatonin in serum samples was calculated by using an external melatonin standard.

#### **Statistical Analysis**

For detecting even minor effects, the required sample sizes used in this experiment were identified using statistical power analysis. The sample sizes necessary for a power of 0.80 were estimated using NCSS software. The normality of the distribution was controlled by Shapiro-Wilk test. According to the results obtained from the normality test, Kruskal-Wallis H test was used for the statistical analysis as appropriate. After a significant Kruskal-Wallis H test, Conover test was carried out. p<0.05 was accepted as statistically significant. The values were given as medians (Min-Max). Data were analyzed using the SPSS software program, version 18.0, for Windows (SPSS Inc., Chicago, IL, USA).

#### Results

#### Analysis of the Electrical Signal

The recorded uterine electrical signals were vague; so, many statistical means were tried for classifying them, but the success rate was very low. So, these trials were not taken into account here. Figure 1 shows the typical uterine electrical activity of each group. These temporal signals show variations, even within groups, so that we could not succeed in classifying them. The group-based mean spectral signals obtained in this study are shown in Figure 2. These signals contain some information regarding the uterine contractions that may indirectly specify the format of the uterine contractions common to the group. As the mean spectrum signals given in Figure 2 were analyzed, one can easily notice the fundamental narrow spectral bands that may be named spectral components (each band may be composed of two or more very closely time-limited oscillating harmonics). From this point of view, Group 1 comes up with two significant spectral components centered around 0.6 Hz and 1.1 Hz. Group 2 also comes up with two components: one centered around 0.65 Hz and the other around 1.2 Hz. The significant spectral components for Group 3 were somewhat different, as three narrow-band components centered at 0.55 Hz, 0.67 Hz, and 0.75 Hz, respectively. These components were apparently much more powerful than the components that appeared in other groups. In the case of Group 4, the components were centered at 0.48 Hz, 0.8 Hz, and 1.21 Hz, respectively, with a much narrower band (as seen from the figure, each band almost contained a single oscillating component). As these three components were artificially created, the signal showed the characteristics of the natural burst demonstrated by the oxytocin-induced rats (Figure 3). These results clearly show the precision of the experiment conducted here. Interestingly, Group 5 demonstrated only one component at 0.55 Hz, while Group 6 also showed one component, located at 1 Hz.

#### Serum Melatonin Results

The mean melatonin levels in each group are shown in Table 1. In brief, both the endogenous physiological dose of melatonin (0.4 mg/kg) and pharmacological dose of melatonin (4 mg/kg) caused an elevation in serum melatonin concentrations when compared to the control group. There was no difference between the control and oxytocin groups. Supplementations of both melatonin doses to the oxytocin group showed significant elevation, according to the oxytocin-treated alone group.

#### Discussion

In this experimental study, it is clearly presented that melatonin treatment alone can induce uterine myoelectrical activity in a dose-dependent manner, similar to the oxytocin in nonpregnant rats. Another remarkable finding of the present study is that the treatment of melatonin after oxytocin caused the mean power of the signal to reduce, which thereby means that it inhibits the physiological contractile effect of oxytocin on the uterus. To the best of our knowledge, this is the first time that these observations have been presented in an in vivo experimental study.

Previously, Ayar et al. (18) reported that melatonin can prevent prostaglandin production and suppress native and oxytocinstimulated uterine contraction. On the contrary, however, Ma<sup>¬</sup>rtensson et al. (19) reported that melatonin exposure after

Groups	Melatonin level (pg/mL) Median (min-max)	Different from (p<0.05)
Group 1 (Control)	3.93 (1.96-28.52)	(2), (3), (5), (6)
Group 2 (0.4 mg/kg melatonin)	554.87 (289.36- 791.17)	(1), (4)
Group 3 (4 mg/kg melatonin)	617.23 (288.00-754.28)	(1), (4)
Group 4 (100 mU/kg oxytocin)	42.90 (3.73-693.50)	(2), (3), (5), (6)
Group 5 (0.4 mg/kg melatonin + 100 mU/kg oxytocin)	513.21 (267.54-615.04)	(1), (4)
Group 6 (4 mg/kg melatonin + 100 mU/kg oxytocin)	658.44 (458.05-1325.44)	(1), (4)

 Table 1. The mean melatonin levels in each group

noradrenalin administration to human myometrial strips can induce the contraction of myofibrils. More recently, Sharkey et al. (20) reported that melatonin acts synergistically via the melatonin receptor 2/phospholipase C/protein kinase C (MT2R/PLC/ PKC) signaling pathway to markedly increase the sensitivity of myofibrils to oxytocin and augment oxytocin-stimulated contractility. In this report, they suggested that treatment with physiological concentrations of melatonin stimulates both basal and oxytocin-triggered contractility of uterine myofibrils. Contrary to the results above, Taketani et al. (21) recently suggested that melatonin protects ovarian granulosa cells from reactive oxygen species-dependent damage and stimulates progesterone production, which suppresses uterine contractility in women undergoing in vitro fertilization and embryo transfer procedures. In the present study, after oxytocin induction, melatonin treatment caused suppression of the rat's uterine myoelectrical activity-that is, our finding is in concordance with those reporting the inhibitory role of melatonin on the uterine contractility induced by oxytocin.

The exact mechanisms for the contractile or relaxing actions of melatonin, however, remain to be elucidated. According to Schlabritz-Loutsevitch et al. (22), these differences are likely to relate to differences in the phase relation between nocturnal melatonin secretion and maximal myometrial contractile activity (high at night in primates; high during the day in rodents). Gimeno et al. (23), on the other hand, suggested that melatonin can have a suppressive effect on uterine contractility by inhibition of prostaglandin production in vitro. Furthermore, our data show for the first time that melatonin has a unique dual effect on the rat uterus in vivo, including the individual excitation of uterine myoelectrical activity and the suppression of oxytocininduced uterine contractile signals in a dose-dependent manner (Table 1) (Figure 1, 2).

Although the signal components for Groups 5 and 6 seem to be powerful enough from a signal point of view, since they are single oscillating components, they do not able to generate impulsive components to stimulate myometrial muscular cells. As also seen from Figure 1, treatment with higher-dose melatonin after oxytocin caused the mean power of signal to reduce, which means that it inhibits the physiological effect of oxytocin. From a power viewpoint in Group 3, the pharmacological dose of melatonin provokes powerful components, but this may not lead directly to powerful contractions, due to possessing lower



Figure 1. Typical uterine electrical signals recorded from nonpregnant rats

frequencies than those rats treated with oxytocin alone.

Similarly, Abd-Allah et al. (24) reported that melatonin pretreatment of rats for 15 consecutive days markedly reduced the number of uterine estrogen receptors by 59%, with a concomitant increase in progesterone receptors by 34.7%. Additionally, they showed prominent suppression in the uterine response to oxytocin in rats treated with melatonin. These observations may suggest that suppression of estrogenic activity on the myometrium is one of the possible mechanisms of a blocking effect of melatonin on oxytocin induced-uterine contractions.

In the current experiment, the effects of oxytocin and melatonin on the uterine myoelectrical activity of non-pregnant rats were investigated using signal processing techniques. Signal processing techniques provide useful information regarding uterine activity. The effectiveness of such methods has previously been discussed by our group (25). Therefore, the results obtained here have the potential to be translated into clinical therapy, as melatonin is a common product that is available as a dietary supplement that is suitable for use during pregnancy (26). In obstetrical practice, preterm delivery is one of the most common pregnancy complications and the leading factor of neonatal mortality and morbidity (27, 28). It has been concluded that the use of currently known tocolytic agents in cases of preterm



Figure 2. Group-based spectra of uterine electrical activity obtained from non-pregnant rats



Figure 3. a, b. A synthetic signal simulating the uterine signal treated with oxytocin (a) and its spectrum (b)

labor does not reduce neonatal morbidity and mortality (29). Concerning both the results of previous studies and the results obtained in this study, as an alternative option, it might be valuable to suggest that melatonin itself or its agonists for treatment of pregnants with premature uterine contractions. Since Parlakpinar et al. (30) reported that the pineal gland atrophies and its melatonin secretion significantly decreases in geriatric cases, who are not capable of pregnancy, a new experimental study can be planned in pinealectomized rats for evaluating the effects of the lack of chronic physiological melatonin on

#### uterine tissue.

In the current study, also, the mean melatonin levels among the groups were determined for reflecting the basal melatonin status (for Groups 1 and 4) and the response sensitivity of melatonin treatments in the melatonin alone and combined groups (for Groups 1, 2, 5, and 6) (Table 1). Therefore, the discussion section was designed and more detailed, according to the obtained serum melatonin results and uterine myoelectrical signals.

In fact, this experimental study has a number of limitations, such as the small size of groups and lack of intrauterine pressure measurement. The small size issue was due to our ethical concern; however, much larger numbers would be useful to make a brilliant conclusion on the topic. Evaluation of intrauterine pressure together with the results obtained through signal processing would certainly be more valuable to discuss directly the effects of melatonin on either spontaneous or oxytocininduced uterine contractions.

As a final conclusion, it is possible to report that melatonin alone stimulates, but melatonin after oxytocin treatment reduces the myoelectrical activity in non-pregnant rats, which demonstrates the dual effect of melatonin on the contractility of the uterus.

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

#### Informed Consent: N/A.

Peer-review: Externally peer-reviewed.

Author contributions: Concept - Y.Ş., H.P., M.E.T.; Design - H.P., Y.Ş. M.E.T, ; Supervision - Y.Ş.; Resource - Y.Ş., H.P.; Materials -Y.Ş., H.P., U.T., M.E.T, B.A. ; Data Collection&/or Processing - H.P., Y.Ş., U.T.; Analysis&/or Interpretation - M.E.T, Y.Ş.; Literature Search - Y.Ş., H.P., M.E.T.; Writing - Y.Ş., M.E.T., H.P.; Critical Reviews - M.E.T.

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

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

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### The role of TWIST, SERPINB5, and SERPIN1 genes in uterine leiomyomas

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#### Abstract

**Objective:** The aim of this study is investigate the role of the Twist homolog 1 (TWIST), serine peptidase inhibitor (SERPINB5), and plasminogen activator inhibitor 1 (SERPIN1) genes in uterine leiomyoma etiopathogenesis.

**Material and Methods:** Twelve patients, aged between 39 and 58, and had a hysterectomy, were included in the study. The size of the leiomyomas was between 20 and 130 mm based on gross pathology after hysterectomy. Tissue samples were obtained from normal myometrium and leiomyoma (1 cm<sup>3</sup>) tissue of the uterus of the patients and stored at -86°C. Samples were divided to two groups after histopathological evaluation of the uterus: normal myometrial tissues as control group (Group 1) and leiomyoma tissue as the study group (Group 2). The TWIST, SERPINB5, and SERPIN1 genes were studied for uterine leiomyoma etiopathogenesis.

**Results:** TWIST gene expression was significantly higher in the uterine leiomyoma tissue (p < 0.001). SERPINB5 and SERPIN1 gene expression was decreased in the uterine leiomyoma tissue, but the differences were not statistically significant.

**Conclusion:** TWIST gene activity is significantly increased in leiomyoma tissue when compared to normal myometrium. In spite of the fact that the development of uterine leiomyomas is estrogen- and progesterone-dependent, myometrial cells could be triggered by the TWIST gene for uterine leiomyoma development. (J Turk Ger Gynecol Assoc 2014; 15: 92-5)

Key words: Uterine leiomyomas, etiopathogenesis, TWIST, SERPINB5, SERPIN1

Received: 07 January, 2014

Accepted: 07 May, 2014

#### Introduction

Uterine leiomyomas (ULs) are typically defined as benign tumors of the myometrial smooth muscle tissue and found in up to 70% of women by the fifth decade of a woman's life (1, 2). UL is the most common type of solid tumor in women of reproductive age, with an incidence of 20-25% (3). UL is a monoclonal tumor of uterine smooth muscle cells and consists of large amounts of extracellular matrix that contains collagen, fibronectin, and proteoglycan (3). Although the majority of ULs is asymptomatic, up to 20% causes symptoms, like menorrhagia, chronic pelvic pain, pressure symptoms on the adjacent pelvic organ, and postpartum hemorrhage (3). Even though the pathogenesis of UL is not clearly known, there is considerable evidence that estrogens and progesterone play a role in growth (3, 4). Effective treatment strategies are limited by the narrow understanding of the pathogenesis of this disease. Cytogenetic analysis has demonstrated that ULs have multiple chromosomal abnormalities (5). Studies of UL specimens have shown that approximately 50% of these tumors have cytogenetic chromosomal alterations (5).

Twist homolog 1 (TWIST), also known as TWIST1, is a class II member of the basic helix-loop-helix transcription factor family (6). During embryonic development, TWIST plays an important role in specification of the mesoderm and differentiation of the mesoderm-derived tissues (6). Germline mutations in the coding sequence of the human TWIST gene have been observed in diverse types of cancers (7). TWIST also plays an important role in regulating smooth muscle cell differentiation and phenotypic modulation (8). The role of TWIST in the human uterus remains to be fully elucidated, but TWIST protein expression was observed in human myometrial smooth muscle cells (9). Serine peptidase inhibitor (SERPINB5), also known as maspin, is a member of the serpin family of serine protease inhibitors, and structurally, it is homologous to plasminogen activator inhibitor 1 (SERPIN1) (10). It is proposed that SERPINB5 is a tumor suppressor. However, the molecular role of SERPIN1 remains to be eluci-



dated (10). SERPINB5 has recently been shown to bind directly to collagen, an interaction that may contribute to cell adhesion (11). SERPINB5 is shown to be differentially regulated in the progression of many types of solid tumors, like prostate, breast, and lung (12).

The aim of this study is investigate the role of the TWIST, SERPINB5, and SERPIN1 genes in uterine leiomyomas etiopathogenesis.

#### **Material and Methods**

#### **Tissue Collection and Preparation**

Twelve patients, aged between 39 and 58, who had a hysterectomy, were included in the study. Total abdominal hysterectomy was performed in 11 patients, and only 1 patient was operated on by vaginal hysterectomy technique. Tumor size was based on gross pathology after hysterectomy. The sizes of the leiomyomas were between 20 and 130 mm. Tissue samples were obtained from normal myometrium and leiomyoma (1 cm<sup>3</sup>) tissues of the uterus of the patients and stored at -86°C. The present study complied with the ethical guidelines of the institutions involved, as it was approved by the Gazi University Ethical Committee, and informed consent was obtained from all subjects examined. Samples were divided two groups after histopathological evaluation of the uterus: control group (Group 1) as normal myometrial tissues and the study groups (Group 2) as leiomyoma tissue. TWIST, SERPINB5, and SERPIN1 were studied as 3 genes for uterine leiomyoma etiopathogenesis.

#### **RNA** Isolation

Tissue samples (1 cm<sup>3</sup>) were homogenized in TRizol Reagent (TRizol Reagent; Invitrogen, New York, USA) using a homogenizer (T10 Basic Ultra-Turrax; IKA®-Werke GmbH & Co. KG, Staufen, Germany) according to the modified protocol published by Chomczynski (13, 14). Homogenized samples were subjected to isopropanol precipitation. RNA pellets were then purified using the Qiagen RNeasy mini-column kit (Qiagen RNeasy mini-column kit; Qiagen Sciences Inc. Valencia, Spain) according to the manufacturer's recommendation indicated in the kit procedures. RNA concentration was measured using a plate reader (Synergy HT Multi-Mode Microplate Reader; BioTek Synergy HT, Winooski, USA). The 260/280 ratios of all samples were above 1.9. RNA integrity was detected on an agarose gel (1%), and the ratios of 18S and 28S rRNA fragments were analyzed.

#### **Real-time RT-PCR Arrays**

One microgram total RNA per sample was converted into cDNA in a reverse transcriptase (RT) reaction. cDNA was synthesized from 1.5 µg of RNA sample using the SABiosciences RT<sup>2</sup> First Strand Kit. Gene expressions of myometrium and uterine leiomyoma tissue were assessed using a RT<sup>2</sup> Profiler<sup>TM</sup> PCR Array Human Cancer Pathway Finder (Cat. No. PAHS-033A) kit (RT<sup>2</sup> Profiler<sup>TM</sup> PCR Array Human Cancer Pathway Finder, PAHS-033A kit; SABiosciences Corporation, Frederick, MD, USA) with RT<sup>2</sup> Real-TimeTM SybrGreen PCR Master mix (RT<sup>2</sup> Real-TimeTM SybrGreen PCR Master mix, PA-012; SABiosciences Corporation,



Figure 1. Result of polymerase chain reaction (PCR) array experiment, indicating the positions of several noteworthy genes based on their 3-fold differences in expression between normal myometrium and leiomyoma tissue (gene expression was calculated by the formula  $2-\Delta\Delta ct$  using the  $\Delta\Delta ct$  method (threshold))

Frederick, MD, USA). Hypoxanthine phosphoribosyltransferase 1 (HPRT1), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and  $\beta$ -actin (ACTB) genes were the housekeeping genes used for normalization. Results were calculated as fold-increase values of gene expression, as instructed by the kit supplier.

#### **Statistical Analysis**

Data obtained from densitometry were expressed as mean±SD. Relative changes as fold-change values in gene expression were calculated by the formula  $2^{-\Delta\Delta ct}$  using the  $\Delta\Delta c_t$  method (threshold) (Statistical Package for Social Sciences for Windows, version 7.5; SPSS, Inc., Chicago, IL, USA). Statistical analysis was conducted using the Mann-Whitney U-Test. Statistical significance was established at p values of less than 0.05.

#### Results

We examined the gene expression profiles exhibited by leiomyoma and normal myometrium tissue. Mean age of the patients was 46.58±4.75 years. Mean value of gravidity for patients was 3.17±1.80. For this study, TWIST, SERPINB5, and SERPIN1 were studied as 3 genes. Figure 1 displays the results from the PCR array experiment, indicating the positions of several noteworthy genes based on their 3-fold differences in expression between normal myometrium and leiomyoma tissue by the formula  $2^{-\Delta\Delta ct}$  using the  $\Delta\Delta c_{t}$  method (threshold). The TWIST gene demonstrated at least a 3-fold difference, but the others did not. Upregulation was observed for the TWIST gene in the leiomyoma sample when compared to normal uterine myometrium. The SERPINB5 and SERPIN1 genes appeared to be downregulated in the leiomyoma sample. The results for all genes are presented in Table 1. According to this, in the TWIST gene, statistical significance was found between groups

Genes	Gene Bank	Function	p value	Fold-change (Group 2/Group 1)		
TWIST	NM_000474	Twist homolog 1 (Drosophila)	0.001	4.84		
SERPINB5	NM_002639	Serpin peptidase inhibitor, clade B (ovalbumin), member 5	0.136	-1.33		
SERPIN1	NM_000602	Serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1	0.197	-1.19		
TWIST: twist homolog 1; SERPINB5: serine peptidase inhibitor; SERPIN1: plasminogen activator inhibitor 1						

#### Table 1. The expression of TWIST, SERPINB5, and SERPIN1 genes in uterine leiomyoma and normal myometrium tissue

(p<0.001). The downregulation was not statistically significant for the SERPINB5 and SERPIN1 genes (p>0.05).

#### Discussion

Uterine leiomyomas are very common in women, but knowledge of the tumor biology is very limited. Clinical and molecular studies have demonstrated that ULs are not a single entity but a clinically and genetically heterogeneous disease (2). ULs are composed of large amounts of extracellular matrix containing collagen, fibronectin, and proteoglycan (15). There is increasing evidence that multiple genomic adjustments may play a role in the initial growth of leiomyoma and that molecular and cellular mechanisms, rather than hormonal factors alone, play a major role in their subsequent growth. TWIST has a role in mesoderm-associated organogenesis; the exact molecular mechanisms by which TWIST controls mesenchymal tissue formation remain largely undefined (16). TWIST is postulated to perform its central regulatory roles in organogenesis at least partially via its control over basic fibroblast growth factor ( $\beta$ FGF) and transforming growth factor beta (TGF- $\beta$ ) signaling (17). In addition to its essential role in modulating mesenchymal tissues critical for organogenesis, TWIST is also expressed in and associated with many types of tumors, including breast cancer, hepatocellular carcinoma (HCC), prostate cancer, and gastric cancer (7, 18-20). TWIST upregulates vascular endothelial growth factor (VEGF) and N-cadherin expression in HCC, and this condition suggests that TWIST may also play an important role in HCC angiogenesis (19). TWIST also enhances VEGF production in prostate cancers (20). VEGF is an angiogenic factor; its enhanced production may accelerate angiogenesis associated with the metastasis of these tumors (19, 20). *βFGF* and its receptors are expressed in normal myometrium and leiomyoma (21). Expression of βFGF and its receptors in leiomyoma is greater than in myometrium (21). TWIST also enhances βFGF expression (22). In our study, we found that TWIST gene expression was increased approximately 5-fold in leiomyoma uterine tissue when we compared with normal uterine myometrial tissue. Based on the information in the literature, TWIST may play role in the formation of ULs by enhancing angiogenesis and growth. SERPINB5 is expressed in many tissues, including prostate, mammary gland, skin, stomach, and uterus (7, 11, 23). SERPINB5 and SERPIN1 have been identified

as potent angiogenesis inhibitors. However, the molecular mechanism responsible for their anti-angiogenic property is unclear. The loss of SERPINB5 expression is significantly correlated with increased expression of VEGF in tumor cells (23). As a tumor suppressor, SERPINB5 functions to inhibit tumor cell migration and invasion and induces tumor cell apoptosis (23). In our study, SERPINB5 and SERPIN1 expression in leiomyoma tissue was decreased when compared to normal uterine myometrium. But, the reduction was not statistically significant. More comprehensive studies with larger numbers of cases may be useful to have a more accurate decision. The limited number of cases in our study can be an issue. For this reason, it is difficult to comment on the role of these two genes in the formation of uterine leiomyoma.

The etiopathogenesis of UL is not fully explained. ULs are not a single entity but a clinically and genetically heterogeneous disease. We showed TWIST gene upregulation for UL tissue when compared to normal myometrial tissue. In spite of the fact that the development of uterine leiomyomas is estrogen- and progesterone-dependent, myometrial cells could be triggered by the TWIST gene for UL development. This situation may be considered a promising sign in preventing the development UL. More comprehensive studies can be done on the role of the TWIST gene and treatment modalities for UL through this gene.

*Ethics Committee Approval: Ethics committee approval was received for this study from Gazi University Scientific and Ethical Committee.* 

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

Peer-review: Externally peer-reviewed.

Author contributions: Concept - M.B., M.S.B., E.U.B.; Design - M.S.B., M.B.; Supervision - G.Y., R.A.; Resource - M.B., E.U.B., B.C.; Materials - M.B., E.U.B., B.C.; Data Collection&/ or Processing - E.U.B., M.B.; Analysis&/or Interpretation - G.Y., Ö.K.Y., M.S.B.; Literature Search - Ö.K.Y., S.M.B.; Writing - M.S.B., R.A.; Critical Reviews - M.B., G.Y., Ö.K.Y.

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

*Financial Disclosure:* This study was financially supported by Gazi University Faculty of Medicine.

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# Natural history of prenatal isolated muscular ventricular septal defects

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#### Abstract

**Objective:** To evaluate the natural history of isolated muscular ventricular septal (m-VSD) defects during gestation and up to 1 year postnatally, as well as the association with chromosomal anomalies.

**Material and Methods:** Between August 2007 and July 2012, 76 fetuses with isolated m-VSDs represented the study population. The following variables were evaluated: site and size of the m-VSDs, presence of chromosomal anomalies, pregnancy outcome, and spontaneous closure rate from diagnosis up to 1 year postnatally.

**Results:** Of the 76 cases with m-VSD, 1 fetus died after birth and 31 cases were lost to follow-up after birth. Thus, a total of 44 fetuses reached their first year of postnatal life, and these cases were available for analysis. Three (6.8%) of 44 defects closed spontaneously in utero, 33 (75%) closed within 1 year, and 8 (18.2%) remained patent. Overall, spontaneous closure occurred more frequently in the apical defects, but no significant difference was found for spontaneous closure between the mid-muscular and apical defects (p>0.05). Also, 83.8% (36 of 44) of defects  $\leq$ 3 mm closed during gestation or the first year of life.

**Conclusion:** We infer that m-VSDs have a high spontaneous closure rate during the first year of life. Also, small m-VSDs frequently close spontaneously. (J Turk Ger Gynecol Assoc 2014; 15: 96-9)

Key words: Isolated, muscular ventricular septal defect, spontaneous closure

Received: 15 January, 2014 Accepted: 11 March, 2014

#### Introduction

Ventricular septal defect (VSD) is a common congenital heart defect (CHD), which accounts for up to 40% of cardiac anomalies (1). The prevalence of VSD varies among studies due to differences in methods of diagnosis and age of participants. The recent increased prevalence in neonates is attributable to changes in diagnostic methods and screening modalities, such as frequent use of fetal echocardiography (2).

An isolated VSD is defined as a defect in the interventricular septum without other sonographic abnormalities, but these defects are also found in association with other structural cardiac defects and complex malformations, such as transposition of the great arteries, congenitally corrected transposition, aortic coarctation or interruption, tetralogy of Fallot, and univentricular atrioventricular connection. Therefore, a detailed echocardiographic examination is warranted upon VSD detection.

The prevalence and natural history of prenatally isolated muscular VSDs (m-VSDs) have not been extensively reported. Little information is known regarding the rate of spontaneous closure during the pre- and postnatal period. The aim of this study was to evaluate the natural history and outcome of prenatally detected isolated m-VSDs during gestation and the first year of life.

#### **Material and Methods**

A historical cohort study was performed on the results of fetal echocardiography conducted between August 2007 and July 2012 at İstanbul Prenatal Centre and Süleymaniye Maternity Hospital. Patients were referred for a routine second trimester anomaly scan and fetal echocardiography. All cases with a VSD as a component of complex CHDs, non-cardiac malformation, and known chromosomal abnormalities were excluded. The local ethics committee approved the study protocol.

Ultrasound scans were performed with the Voluson 730 system (GE Healthcare, Milwaukee, WI, USA) using either a 2-7 MHz or 4-8 MHz transabdominal transducer. The left and right ventricular outflow tracts, major arteries, four-chamber view, three-vessel view, and basal short-axis view were evaluated using gray-scale echocardiography. Color Doppler imaging in at least 2 different planes was used to assess the interventricular septum and bidirectional flow across the defect

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Figure 1. Cohort of the study

while perpendicular as possible to the ultrasound beam. Color velocity was reduced to a low Nyquist limit to capture lowspeed jets, since fetal right and left heart pressures are similar. Shunting across the septum was confirmed by pulsed-wave Doppler study.

Monthly follow-up antenatal visits were scheduled to evaluate the defect throughout gestation. As shown in Figure 1, 1 neonate from the original cohort of 76 isolated m-VSDs died 54 days after delivery due to carotid artery stenosis and cardiac failure. Thirty-one cases were lost to follow-up after birth. Thus, in the remaining 44 cases with isolated m-VSD, complete follow-up during the first year of life was available for analysis.

The following data were retrieved from a computerized database: indication for fetal echocardiography, gestational age at diagnosis, site and size of defect, presence of chromosomal anomalies, intrauterine closure, pregnancy outcome, and neonatal follow-up until 12 months of life. The defect site was categorized as mid-muscular or apical. No anterior or posterior subtypes were detected. Fetal karyotyping following amniocentesis was performed in 18 (23%) of 76 cases. Reliability of VSD diagnosis was confirmed by a postnatal pediatric cardiologist echocardiographic examination. All neonates were followed up until documented echocardiographic closure of the defect or 1 year of age.

Statistical analysis was performed with SPSS 17.1 for Windows (SPSS, Chicago, IL, USA). Fisher's exact test was used to evaluate inter-group differences. The level of statistical significance was set at p equal to 0.05.

#### Results

During the 5-year period, 534 cases of CHD were detected in 23,500 pregnancies referred for fetal echocardiographic examination. Overall, the CHD prevalence was 2.27% in this referralbased study group. There were 264 cases of VSD and 76 cases of isolated m-VSD, representing 49.43% and 14.23% of all CHDs, respectively. The mean gestational age at diagnosis of isolated m-VSD was 23.1 (range 19-37) weeks. Indications for fetal echocardiography were fetal growth restriction in 4 cases (5.2%), suspicion of CHD in 1 case (1.3%), and a second trimester anomaly scan in 71 cases (93.5%). Sixty-three (82.8%) cases had a mid-muscular defect, and 13 (17.2%) had an apical defect. The defect size was  $\leq$  3.0 mm in 74 cases (97.3%), and >3.0 mm in 2 cases (2.7%). Karyotyping was performed in 18 (23.6%) pregnancies, while in the remaining 58 cases, the karyotype was clinically assessed postnatally. No chromosomal anomalies were identified.

The mean gestational age and birth weight at delivery were 38.3 (range 31.0-41.0) weeks and 3197 (range 1000-5040) gr, respectively. As previously shown in Figure 1, 1 case of carotid artery stenosis was diagnosed in an infant who died at 54 days of age. Forty-four cases were investigated to determine the evolution of isolated m-VSDs during intrauterine and postnatal life. The m-VSD closure rates are presented in Table 1. Three (6.8%) of 44 defects closed spontaneously in utero, 33 (75%) closed within 1 year, and 8 (18.2%) remained patent. Three (9.3%) of 32 mid-muscular defects closed spontaneously in utero, and 23 (71.8%) closed within the first year. None of the apical defects closed in utero, and 10 (83.3%) closed within the first year. While overall spontaneous closure occurred more frequently in the apical defects, no significant difference was found for spontaneous closure between the mid-muscular and apical defects (p>0.05, OR 1.153, 95% CI 0.198-6.679). In 8 neonates whose defects were not closed spontaneously during the first year of life, regular follow-up with an annual echocardiographic examination was planned.

The spontaneous closure rates according to the size of the defect are given in Table 2: 83.8% (36 of 44) of defects  $\leq$ 3 mm closed during gestation or the first year of life, and only 16.2% (8 of 44) of m-VSDs  $\leq$ 3 mm remained patent during the follow-up period. We were unable to correlate the defect size with the spontaneous closure rate, because most m-VSDs were small in size. Only 1 case with a diameter >3 mm completed the follow-up, and this defect remained open at 12 months of life.

Site of the m-VSD	Closure in Utero	Closure During the First Year of Life	No Closure
Mid-muscular	3 (9.3)	23 (71.8)	6 (18.9)
Apical	-	10 (83.3)	2 (16.7)
Total	3 (6.8)	33 (75)	8 (18.2)
m-VSD: muscular ventricular septal defect. Data presented as number (percentage)			

Table 1. Closure of isolated m-VSDs by site

Table 2. Closure of isolated m-VSDs by size of the defect

Size of the m-VSD	Closure in Utero	Closure During the First Year of Life	No Closure
≤3 mm	3 (6.9)	33 (76.7)	7 (16.4)
>3 mm	-	-	1
Total	3 (6.8)	33 (75)	8 (18.2)
m-VSD: muscular ventricular septal defect. Data presented as number (percentage)			

#### Discussion

This study is one of the largest cohorts evaluating the natural history of prenatally detected isolated m-VSDs. Midmuscular m-VSDs were approximately 5-fold more prevalent than apical defects. Most of the m-VSDs (93.5%) were diagnosed during the second trimester anomaly scan, highlighting the importance of targeted detailed ultrasound examinations to improve detection of CHDs.

In this study, spontaneous closure occurred prenatally and during the first year of life in 6.8% and 75% of the cases, respectively. Spontaneous closure is the most exciting aspect of the natural history of these defects. Resolution of an m-VSD could be part of antenatal development. Defects in the muscular septum close with the growth and hypertrophy of the surrounding muscular septum. Also, it has been proposed that small m-VSDs may be looked upon as a protracted process of closing multiple normally occurring channels in the inter-ventricular septum (3). The advent of color Doppler echocardiography has facilitated the evaluation of the perinatal course of these defects (4).

The size and site of the m-VSD are significant in its natural history. One study showed that velocity from right-to-left flow across the defect was negatively correlated with spontaneous closure rate for early closure of small ( $\leq 3$  mm) m-VSDs (5). Midmuscular VSDs frequently spontaneously close while also closing earlier than other types of muscular VSDs (6). In pediatric series in which the children were followed up from birth, spontaneous closure was seen in 81.8-84.8% of m-VSDs during the first year of life. Closure occurred most frequently at the midmuscular location, while apical defects persisted more often (7, 8). Age at first echocardiographic examination and the length of the follow-up period may influence the spontaneous closure rate (9, 10). It has been demonstrated that 12.5-31% of m-VSDs can undergo spontaneous closure during fetal life

(11, 12). Results may vary due to the different classifications of VSDs. Our results show a lower rate of prenatal closure. Prenatal spontaneous closure occurred basically in the mid-muscular VSD group, and no significant difference between the defect locations was found.

The rate of chromosomal anomalies associated with fetal isolated m-VSDs is controversial. Two large series reported a rate of 28.6-38.2% chromosomal anomalies associated with m-VSDs but included cases with extracardiac anomalies and associated known chromosomal anomalies (12, 13). Another study determined a 6.2% prevalence of aneuploidy associated with isolated VSD, but the study included a limited number of cases (11). No chromosomal anomalies were identified in our study, suggesting that isolated m-VSD is a benign finding during pregnancy.

We acknowledge that our study also has several limitations. Firstly, the incidence of apical muscular VSDs is low, which limits the evaluation of the natural history of these defects. Secondly, 42.1% of cases were lost to follow-up during the postnatal period, limiting the conclusions. On the contrary, our results indicate that isolated m-VSDs have no increased risk of chromosomal anomalies and have a high spontaneous closure rate during the first year of life. Also, small m-VSDs frequently close spontaneously. This information may be valuable to counsel parents at diagnosis.

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

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

Peer-review: Externally peer-reviewed.

Author contributions: Concept - A.G., H.F.Y.; Design - O.E.; Supervision - A.G., H.F.Y.; Resource - O.S., A.G.; Materials - A.G., H.F.Y.; Data Collection&/or Processing - O.S., S.K., A.G., O.E.; Analysis&/or Interpretation - O.E.; Literature Search - O.E.; Writing - O.E.; Critical Reviews - H.F.Y., A.G.

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

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

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# Does bleeding affect fetal Doppler parameters during genetic amniocentesis?

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#### Abstract

**Objective:** The aim of this study was to investigate the relationship between fetal Doppler parameters and bleeding at insertion points during amniocentesis.

**Material and Methods:** This prospective study was conducted between July 2010 and February 2011. A total of 215 amniocentesis procedures were performed during this period. Five patients with Down syndrome were excluded from the study. The remaining 210 patients were divided into Group 1 (bleeding at insertion site) and Group 2 as a control group. One needle type was used for all patients. Umbilical artery resistance index (UARI), umbilical artery pulsatility index (UAPI), middle cerebral artery resistance index (MCAPI), and middle cerebral artery peak systolic velocity (MCAPSV) were measured immediately and before and after amniocentesis.

**Results:** Bleeding at the insertion point during amniocentesis did not significantly change the UARI (34% increase for Group 1 and 46.5% increase for Group 2, p=0.238), the MCARI (52% increase for Group 1 and 45% increase for Group 2, p=0.622), or the MCAPSV (37% increase for Group 1 and 49% increase for Group 2, p=0.199). UARI, MCARI, MCA PI, and MCAPSV were not significantly altered following amniocentesis in Groups 1 and 2. There was a significant increase in UAPI following amniocentesis only in Group 2.

Conclusion: Bleeding during genetic amniocentesis did not change umbilical artery and middle cerebral artery Doppler parameters.

(J Turk Ger Gynecol Assoc 2014; 15: 100-3)

Key words: Amniocentesis, bleeding, fetal Doppler

Received: 06 March, 2014

Accepted: 18 April, 2014

#### Introduction

Amniocentesis is the most commonly performed invasive prenatal diagnostic procedure. Although technically simple, it may result with in pregnancy loss. The total pregnancy loss rate due to amniocentesis is the sum of the procedural and the background loss rates (1). Procedure-related fetal losses have been found to be associated with a procedure at 18 weeks or beyond, a procedure performed for abnormal second-trimester biochemical screening test, a bloody tap, and a female fetus but not to the number of punctures or transplacental amniocentesis (2-4). Although considered a complicating factor, transplacental needle passage during amniocentesis has not been shown to induce changes in fetal Doppler parameters when compared to a group with nontransplacental amniocentesis (5, 6).

In the present study, we aimed to investigate the relationship between fetal umbilical artery (UA) middle cerebral artery (MCA) Doppler parameters and bleeding at the insertion point during transplacental amniocentesis.

#### Material and Methods

This prospective study was conducted between July 2010 and February 2011. A total of 215 amniocentesis procedures were performed during this period. Five patients with Down syndrome were excluded from the study. The remaining 210 patients were divided into Group 1 (bleeding at the insertion site). Group 2 was the control group. One needle type was used for all patients. Bleeding from the insertion site was noted when jet flow from the placenta into the amniotic cavity was detected by ultrasonography. Umbilical artery resistance index (UARI), UA pulsatility index (UAPI), MCA resistance index (MCARI), MCA pulsatility index (MCA PI), and MCA peak systolic velocity (MCAPSV) were measured immediately and before and after amniocentesis. The study was conducted using a color Doppler instrument (ProSound  $\alpha$ 10, Aloka, Tokyo, Japan) with a 5 MHz convex probe. For an accurate measurement, the fetal head was in the transverse plan. MCA vessels were detected with color Doppler ultrasound overlying the anterior wing of the sphenoid bone. An angle of insonation of  $<15^{\circ}$  was used (near 0°). For fetal MCA



peak systolic velocity, the highest velocity was used. All of the invasive procedures and Doppler evaluation were performed by a single operator. The study was approved by the ethics and educational issues coordinating committee of the institution where the study was conducted. All of the patients signed written consent that their data could be used with appropriate ethical committee approval prior to genetic amniocentesis.

#### **Statistical Analysis**

Statistical analysis was performed using SPSS (R) version 17 (SPSS; Chicago, IL, USA). An independent samples t-test was performed for parametric variables between groups. A paired samples t-test was performed for parametric variables within groups. A chi-square test was performed for non-parametric variables between groups. A P value less than 0.05 was considered significant.

#### Results

Characteristics of the study population are shown in Table 1. Maternal age and gestational age at amniocentesis were similar among groups. The distribution of nulliparity and indications for amniocentesis were also not significantly different between groups. Patients in Group 1 had significantly more frequent anterior placentation. UA and MCA Doppler characteristics are shown in Table 2. UARI and UAPI before and after amniocentesis were not different between patients in Groups 1 and 2. Among patients in Group 1, 34% had an increase in UARI and 43.5% had an increase in UAPI. Increased UARI and UAPI were observed in 46.5% and 57.5% of patients in Group 2, respectively. The ratio of patients with increased UARI and UAPI did not differ significantly between groups.

Middle cerebral artery pulsatility index was similar in both groups before and after amniocentesis. MCAPSV was similar between groups before and after amniocentesis. The number of fetuses with increased MCAPSV was also similar in Groups 1 and 2.

Changes in UA and MCA Doppler parameters within each group after amniocentesis are shown in Table 3. UARI, MCARI, MCA PI, and MCAPSV were not significantly altered following amniocentesis in Groups 1 and 2. There was a significant increase in UAPI following amniocentesis only in Group 2.

#### Discussion

The findings of the present study indicate that ultrasonographic evidence of bleeding as a result of transplacental needle passage during mid-trimester genetic amniocentesis does not influence Doppler parameters in the present study cohort.

It is debated whether transplacental needle passage significantly increases the rate of complications. Although some earlier reports did find an increase in fetal loss rates (7, 8), many other studies have not found such an association (9-11). Although convincing evidence is not present in terms of fetal loss, it has been shown that transplacental needle passage may lead to certain procedural complications, such as aspiration of hemorrhagic amniotic fluid or fetomaternal hemorrhage (FMH) (10).

Table	1.	Clinical	data	ot	the	study	popu	lation
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	Group 1 (n:62)	Group 2 (n:153)	р
Maternal age [mean (y)±SD]	33.1±6.0	$33.7 \pm 6.7$	0.52
Gestational age [mean (weeks)±SD]	18.1±1.5	17.8±1.8	0.24
Nulliparity [n (%)]	25 (40.3)	45 (29.4)	0.11
Indication of amniocentesis [n (%)]			0.59
Advanced maternal age	15 (24.2)	44 (28.8)	
Sonographic markers	6 (9.7)	10 (6.5)	
Positive maternal screening	38 (61.3)	91 (59.5)	
Previous pregnancy with aneuploidy	3 (4.8)	8 (5.2)	
Placental location [n (%)]			0.001*
Anterior	59 (95.2)	50 (32.7)	
Posterior	0	89 (58.2)	
Fundal	1 (1.6)	10 (6.5)	
Lateral	2 (3.2)	4 (2.6)	
Y: years; SD: standard deviation $p < 0.05$			

Table 2. Comparison of umbilical artery and middle cerebral artery Doppler parameters before and after amniocentesis between two groups

	Group 1 (n:62)	Group 2 (n:153)	р
Umbilical artery RI before amniocentesis	$0.77 \pm 0.08$	$0.78 \pm 0.07$	0.57
Umbilical artery RI after amniocentesis	$0.76 \pm 0.09$	$0.79 \pm 0.08$	0.46
Umbilical artery PI before amniocentesis	1.43±0.29	1.45±0.27	0.81
Umbilical artery PI after amniocentesis	$1.39 \pm 0.29$	1.51±0.36	0.43
No. of fetuses with an increase in umbilical artery RI [n(%)]	21 (34)	71 (46.5)	0.24
No. of fetuses with an increase in umbilical artery PI [n(%)]	27 (43.5)	88 (57.5)	0.07
MCA PI before amniocentesis	$1.61 \pm 0.36$	1.71±0.39	0.21
MCA PI after amniocentesis	$1.62 \pm 0.4$	$1.70 \pm 0.43$	0.63
MCA PSV before amniocentesis	$25.6 \pm 5.3$	$24.5 \pm 4.4$	0.26
MCA PSV after amniocentesis	24.2±5.3	$24.3 \pm 4.2$	0.58
No. of fetuses with an increase in MCA PSV [n(%)]	23 (37)	75 (49)	0.19
RI: resistance index; PI: pulsatility index; MCA: middle cerebral artery; PSV: peak systolic velocity			

It has been previously hypothesized that hemorrhage into the amniotic cavity occurs in most cases when transplacental amniocentesis is performed (12). Visualization of blood jet from the placenta into the amniotic cavity provides ultrasono-

Table 3. Changes in umbilical artery and middle cerebral artery Doppler parameters before and after amniocentesis within groups

	Group 1 (n:62)	Group 2 (n:153)
UARI change	$-0.01 \pm 0.08$	$0.07 \pm 0.07$
UAPI change	$-0.04 \pm 0.31$	$0.05 \pm 0.31$ *
MCARI change	$-0.02 \pm 0.12$	$0.01 \pm 0.1$
MCA PI change	$0.01 \pm 0.48$	$-0.01 \pm 0.42$
MCAPSV change	-0.7±4.3	$-0.2 \pm 4.8$
UA: umbilical artery; PI: pulsatility index; MCA: middle cerebral artery; PSV: peak systolic velocity *p<0.05		

graphic evidence of transplacental hemorrhage (13). Accurate estimation of the level of transplacental bleeding into the amniotic cavity may not be possible through ultrasonography alone. Testing for fetal erythrocytes in maternal circulation may be useful to detect the presence of FMH in this case. However, this may still be of limited value to evaluate the impact of bleeding on the fetus. In the present study, most of the cases of transplacental bleeding occurred in anteriorly located placentas. However, ultrasonographically detected bleeding did not occur in all cases of anteriorly located placentas.

Middle cerebral artery peak systolic velocity is used to evaluate fetuses with anemia due to various etiologies (14). While it is primarily used to detect severity of fetal anemia in red cell alloimmunization, it can be used to diagnose fetal anemia due to different causes. Several authors have demonstrated the validity of MCAPSV in detecting fetal anemia from other causes, such as congenital parvovirus infection and non-immune hydrops fetalis (15, 16). Most authors report a sensitivity of nearly 100% in experienced centers, although this may vary depending on the experience of the operator or the severity of the fetal anemia (17, 18). While the increase of MCAPSV is well established in chronic situations leading to fetal anemia, the role of Doppler studies in acute fetal blood loss is less clear. Results of two previous studies indicate that acute FMH also leads to an increase in MCAPSV. Baschat and associates have detected increased MCAPSV in a case of severe acute FMH, confirmed by the Kleihauer-Betke test (19). In a later study, Yamaguchi and associates demonstrated increased PSV of MCA in an actively bleeding fetus due to spontaneous rupture of a sacrococcygeal teratoma (20). To the best of our knowledge, no study has previously investigated the effect of transplacental bleeding on MCAPSV. Our results indicate that bleeding at amniocentesis did not significantly affect MCAPSV in our study population.

Most previous reports have stated that amniocentesis has no significant effect on umbilical artery Doppler parameters (5, 6, 21), while others have found transient changes or changes attributable to maternal anxiety (22, 23). Two main conclusions can be inferred based on the results of these studies. The amount of fetomaternal bleeding is significantly below the limit that triggers fetal hemodynamic responses. In addition, the physiological reaction to placental puncture in the fetoplacental unit is sufficient to contain the acute insult. Our results are mostly consistent with previously published data, as we were

unable to detect any significant changes, except for increased in UAPI in Group 2. As no bleeding or transplacental passage occurred in this group, we speculate that factors other than placental perforation and bleeding (such as transient uterine activity) might cause increased UAPI.

In conclusion, ultrasonographic evidence of bleeding is commonly encountered during transplacental mid-trimester amniocentesis. This complication is almost always self-limited and has no significant short-term consequences in terms of middle cerebral and umbilical artery Doppler parameters.

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

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

Peer-review: Externally peer-reviewed.

Author contributions: Concept - C.İ., E.T.; Design - C.İ., E.T.; Supervision - E.T.; Materials - E. T., A.P., H.K., T.Ç.; Data Collection&/or Processing - C.İ., E.T., H.K., C.Y.; Analysis&/or Interpretation - C.İ., T.Ç., A.P., C.Y.; Literature Search - C.İ., E.T., H.K., C.Y.; Writing - C.İ., E.T., H.K., Cem Yalçınkaya.; Critical Reviews - E.T.

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

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

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# HOXA11 and MMP2 gene expression in uterosacral ligaments of women with pelvic organ prolapse

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### Abstract

**Objective:** Pelvic organ prolapse (POP) is a common disorder that negatively impacts the quality of life in many women. Uterosacral ligaments (USLs) are supportive structures of the pelvic organs that are often attenuated in women with POP. The HOXA genes regulate the development of the uterosacral ligaments. We compared expression of HOXA11 and MMP2 in USLs of women with and without POP.

**Material and Methods:** A prospective sequential cross sectional study was conducted in ZTB Women's Health Research and Education Hospital. We compared expression of HOXA11 and MMP2 in USLs of women with (n:18) and without (n: 15) POP.

Total RNA was isolated from patient (n:18) and control (n:15) uterosacral ligament tissues with TriPure isolation reagent according to the manufacturer's instructions. Expression levels of HOXA11 and MMP2 were determined using semiquantitative RT-PCR in a Light Cycler 480 system. Real-time ready catalog assays, which are short FAM-labeled hydrolysis probes containing locked nucleic acid, were used for RT-PCR reactions. **Results:** There was no difference in patients' mean age, parity, body mass indexes, and menopausal status between two groups. Means of RNA expression of MMP2 were  $1.27\pm0.6$  and  $0.75\pm0.4$  in the POP group vs control group, respectively (p:0.007). Means of RNA expression of HOXA 11 were  $2.57\pm2.4$  and  $1.94\pm1.4$  in the POP group vs control group, respectively (p:0.376). The POP group was divided as mild and severe POP; there was no difference in HOXA11 and MMP2 RNA expression between groups (p>0.05).

**Conclusion:** Although there was no difference HOXA11 RNA expression in USLs with the POP group vs control, there was a significant difference MMP2 RNA expression in USLs with the POP group vs control. There are limited studies on this subject, and study results are contradictory. Further investigations with larger numbers of cases are needed to clarify this subject. (J Turk Ger Gynecol Assoc 2014; 15: 104-8)

Key words: HOXA11, MMP2, pelvic organ prolapse

Received: 06 May, 2014

Accepted: 07 May, 2014

### Introduction

Uterosacral ligaments (USLs) are the principal support structure of the uterus and vagina. Weakness of these structures and fascial support of the distal genital tract results in pelvic organ prolapse (POP) (1). In women, POP affects the quality of life negatively. It usually presents with urinary and fecal incontinence, pelvic pain, and sexual dysfunction due to disruption of the pelvic anatomy (2). The reported prevalence of POP is around 40% in the female population. Approximately 11-30% of patients who are managed surgically experience recurrent prolapse and are reoperated (3).

Despite the great amount of knowledge about the growth and differentiation of the reproductive tract, we have very little information regarding the molecular pathways of the development of the USL. The uterosacral ligaments are condensed bands of tissue formed from the endopelvic fascia attached to the posterior uterine cervix and the anterior face of the sacrum (4).

Studies report differentially expressed extracellular matrix (ECM) proteins in uterosacral ligaments and vaginas from

women with pelvic organ prolapse (POP) (4-10). Gene expression patterns, including Hox/HOX, modulate the ECM (11). Particularly, HOXA11, which regulates collagen and matrix metalloproteinase (MMP) expression, has a primary role in the development of the lower uterine segment and cervix.

Proteinases secreted by connective tissue cells corrupt collagen. The MMPs are a group of proteinases that degrade the extracellular matrix and components of the basement membrane (12). Type IV collagen, a primary component of the basement membrane, is degraded by MMP-2 (13, 14). Thus, it may be postulated that elevation of MMP-2 expression may lead to progression of POP. Recent studies show that HOXA11 expression was decreased in patients with pelvic organ prolapse (15, 16). The aim of the current study was to compare expression of HOXA11 and MMP2 in USLs of women with and without POP.

#### **Material and Methods**

A prospective sequential cross-sectional study was performed with a total of 33 women. This study was approved



by the local ethics committee and institutional review board of the Zekai Tahir Burak Women's Health Education and Research Hospital and the Başkent University Faculty of Medicine (Project no: KA11/47). Written informed consent was obtained from all volunteers. The ethical principles for medical research involving human subjects enunciated in the 18th World Medical Association Declaration of Helsinki were applied. All patients were evaluated for the presence of pelvic prolapse according to the quantification system advocated by the International Continence Society (17). Women with stage II POP or greater were assigned to the POP group. Eighteen women were diagnosed with POP - mild POP (stage 2), (n:9) and severe POP (Stage 3-4), (n:9) - and underwent vaginal hysterectomy, while 15 women were established with benign gynecologic disorders and underwent total abdominal hysterectomy. Women with systemic disease, previous pelvic surgery, hormone use, or obesity were excluded from the study.

Specimens were collected from women undergoing vaginal or abdominal hysterectomy at our institution. Data regarding age and parity were recorded. At the time of surgery, 5 mm samples of the USLs were taken from the proximal ligament at its insertion into the cervix, where the ligament is consistently identifiable.

#### **Real-Time PCR Analysis**

Total RNA was isolated from 18 patient and 15 control uterosacral ligament tissue samples with TriPure isolation reagent according to the manufacturer's instructions (Roche Diagnostics GmbH, Mannheim, Germany). The quality and quantity of RNA were determined with a NanoDrop 2000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA). One microgram of total RNA was reverse-transcribed using the Transcriptor First-Strand cDNA Synthesis Kit (Roche Diagnostics GmbH, Mannheim, Germany). Real-time ready catalog assays, which are short FAM-labeled hydrolysis probes containing locked nucleic acid, were used for RT-PCR reactions (Roche Diagnostics GmbH, Mannheim, Germany). Expression levels

#### Table 2. Review of the literature

of homeobox A11 (HOXA11) and matrix metallopeptidase 2 (MMP2) were determined using semiquantitative RT-PCR with a Light Cycler 480 II system (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's instructions with a pre-incubation step at 95°C for 10 minutes, followed by 45 cycles at 95°C for 10 seconds, 60°C for 30 seconds, and 72 °C for 1 second. Semiquantitative PCR reactions were run in triplicate. The relative expression levels of HOXA11 and MMP2 transcripts were calculated by the threshold cycle (Ct) and the 2-ddCt method using beta-actin (Roche Diagnostics GmbH, Mannheim, Germany) as the housekeeping gene (18).

#### **Statistical Analysis**

SPSS Statistical 17 package (SPSS Inc., IL, Chicago, USA) program was used for statistical analysis of the data. Descriptive statistics were expressed as mean±standard deviation for patient and control groups. According to these values, significance levels were calculated with independent samples t-test for determining MMP9 and HOXA11 gene expression status.

#### Results

Table 1 shows the clinical characteristics and gene expressions of the groups. There were no statistically significant differences in age and parity between groups (p:0.076 and 0.5214, respectively). Means of RNA expression of MMP2 were  $1.17\pm0.5$  and

Table 1. HOXA11 and MMP2 means of all groups

	POP (n:18)	Control (n:15)	р
Mean age	$56.44 \pm 4.5$	$53.46 \pm 12.15$	0.076
Parity	$4.0 \pm 1.7$	$3.9 \pm 2,8$	0.935
HOXA11	$2.57 \pm 2.4$	$1.94 \pm 1.4$	0.376
MMP2	$1.27 \pm 0.6$	$0.75 \pm 0.4$	0.007
POP: pelvic or p<0.05 is sign	rgan prolapse iificant		<u>.</u>

Author	Subject	Tissues (studied)	Parameters	Finding
Connel 2009 (3)	22 patients	USLs	HOXA11 gene expression and cell proliferation in USLs	Decreased cell proliferation and HOXA11 gene expression in patients with POP
Strinic 2009 (37)	40 patients with POP 40 Control	USLs	MMP1 and MMP2	Increased MMP1 expression Unchanged MMP2 levels
Connel 2008 (14)	18 patients with POP 10 Control	USLs	HOXA11 gene expression, MMP 2, MMP9, Collagen 1 and Collagen 3	Decreased HOXA11 expression, Decreased collagen 1 and 3, Increased MMP2 expression
Wieslander 2007 (39)	mice	Vaginal tissue	MMP2, MMP9, MMP12	MMP2 and MMP9 expression increased postpartum
Phillips 2006 (5)	14 patients with POP 14 Control	USLs Vaginal tissue	MMP2 and MMP9	Increased MMP2 in vaginal tissue Unchanged MMP2 levels in USLs
Gabriel 2006 (4)	17 patients with POP 18 Control	USLs	MMP1 and MMP2	Increased MMP2 expression Unchanged MMP1 levels
USLs: uterosacral ligaments				



Figure 1. MMP 2 levels in USLs from women with and without POP

Patients

control

prolapsus

 $0.7\pm0.3$  in the POP and control groups, respectively (p:0.003). Means of RNA expression of HOXA11 were  $2.12\pm1.9$  and  $1.79\pm1.3$  in the POP and control groups, respectively (p:0.487) (Table 1). The distribution of MMP2 and HOXA11 gene expression values is seen in Figures 1 and 2. There was no significant difference of HOXA11 and MMP2 RNA expression between the mild and severe POP groups, mild vs control, or severe vs control groups.

#### Discussion

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The incidence of pelvic prolapse is very high, and it is related to multiparity, age, and obesity but may occur in the absence of these risk factors; the pathophysiology of pelvic prolapse is still obscure (19, 20). However, nulliparous women without any risk factors may also develop POP. Therefore, a genetic predisposition may play a role as well (21-23).

Regulation of proliferation and cell death, which is controlled by the action of various different genes, is necessary for normal development and function of tissues (24). Cellular proliferation and apoptotic responses are regulated by HOXA11 in the developing uterus and the USLs (24, 25). In human and mice studies, this gene has been demonstrated to be functional in adult life and is believed to have a principal role in providing the plasticity of the uterus during different phases of the menstrual cycle and pregnancy (25, 26).

We evaluated 18 women with POP and 15 women with normal pelvic support in this study. There was no difference in patient mean age, parity, or menopausal status between the two groups. We have demonstrated increased MMP-2 expression in the USLs of women with POP, a finding consistent with prior studies. On the other hand, we did not find a statistically significant difference in expression of HOXA11 in patients with POP vs. controls.

Matrix metalloproteinases are secreted as an inactive form (27) and are capable of degrading extracellular matrix essential in normal and pathologic tissue remodeling processes (28-34). Recent evidence suggests that changes in connective tissue composition are at least partially involved in the pathophysiol-



Figure 2. HOXA11 levels in USLs from women with and without POP

ogy of POP (5, 35, 36). There are several studies related to MMP 2 expression in the uterosacral ligaments of patients with POP. Phillips et al. (6) compared tissue markers of collagen metabolism in uterosacral ligaments with those in vaginal tissue in women with uterine prolapse. They found elevated MMP activity in the vaginal skin of women, but they found no significant differences in the uterosacral ligaments of these women. In addition to this, Chen BH et al. (37) investigated quantitative mRNA expression of MMP-1, MMP-2, MMP-9, and their inhibitors - the tissue inhibitors of metalloproteinases TIMP-1, TIMP-2, and TIMP-3 - in vaginal wall tissue from women with stress urinary incontinence compared to continent controls. They did not find any difference in TIMP-2, TIMP-3, MMP-2, or MMP-9 mRNA expression between stress incontinent women and controls. Also, Strinic T et al. (38) investigated MMP-1 and matrix MMP-2 immunohistochemical expression in uterosacral ligament biopsies from women with pelvic organ prolapse (POP) and controls with normal pelvic support. The authors found a significant increase in MMP 1 immunohistochemical expression but no difference in immunohistochemical expression of MMP 2 between women with POP and those without. On the other hand, Gabriel et al. (5) demonstrated that MMP-2 expression was directly related to the presence of POP, rather than to age or parity. In a study by Jackson et al. (36), total collagen content, solubility, and turnover were determined by matrix metalloproteinase activity in the vaginal epithelium of patients with POP. They found that a reduction in total collagen content and solubility was associated with pelvic prolapse. There was also a significant elevation in collagenolytic activity, demonstrated by higher levels of MMP-2 and MMP-9. In the current study, we found that MMP-2 expression was increased in uterosacral ligament tissue from patients with prolapse ligaments. As mentioned above, there are some studies on this subject, and the results are contradictory. Referring to the pathophysiology of these diseases, these changes in the level of MMP2 can be expected.

With respect to gene expression of HOXA11, there are some studies about USL and POP in the literature. Firstly, Connell et al. (15) compared expression of HOXA11, collagen type 1, colla-

gen type 3, MMP2, and MMP9 in USLs of women with and without POP. They demonstrated that expression of HOXA11 and both collagens dramatically decreased, while MMP2 increased in women with POP. In a second study, Connell et al. (4) demonstrated a decrease in the expression of HOXA11 and cellularity in women with POP. The authors suggested that functional development or repair of the USL might be deteriorated by deficiency of HOXA11 signaling after trauma in susceptible women. In another study, Ma Y et al. (16) reported the development of a mouse model of in vivo HOXA11 silencing within the female genital tract with abrogated gene effects on interstitial collagens and the gelatinase class of matrix metalloproteinases. They concluded that the results suggest a mechanism for the weakening of pelvic floor support in women, in that decreased HOXA11 gene expression can be associated with decreased collagen and increased MMP2 expression in the uterosacral ligaments of women with pelvic organ prolapse. In contrast, we did not find any decrease in expression of HOXA11 in USLs of patients with POP. Although Connel et al. (4, 15) showed an association between HOXA11 gene expression and the POP in their studies, we were unable to get the same result. Studies about the gene expression of HOXA11 and MMP2 are summarized in the Table 2. As can be seen from the table, there are no sufficient data and consensus on this issue. For this reason, to clarify this topic, new studies are needed. Also, gene expression may vary between communities; thus, the results of this study may be different for every population.

This study has several limitations. First of all, our study population has a limited number of patients versus other studies in the literature. Secondly, we studied only uterosacral ligaments of patients for two parameters. This study could be done with other parameters (such as MMP9, collagen 1 and 3, or HOXA 10). In conclusion, we demonstrated that MMP2 expression was significantly higher in patients with POP. On the other hand, HOXA11 expression was not different between the groups. As mentioned above, many studies exist on this subject, but there are contradictory results. For this reason, controlled studies with larger series are needed.

**Ethics Committee Approval:** Ethics committee approval was received for this study from the Local Ethics Committee and Institutional Review Board of Zekai Tahir Burak Women's Health Education and Research Hospital and Başkent University Faculty of Medicine.

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

#### Peer-review: Externally peer-reviewed.

Author contributions: Concept - N.Y., F.I.S.; Design - N.Y., F.I.S., Y.K.T.; Supervision - N.Y., F.I.S.; Resource - N.Y., F.I.S.; Materials - N.Y., G.O.; O.A.; B.B.; Data Collection&/or Processing - N.Y., Y.K.T., S.Y.; Analysis&/or Interpretation - Y.K.T., F.I.S., ; Literature Search - N.Y., Y.K.T., S.Y.; Writing - N.Y., S.Y.; Critical Reviews -N.Y., F.I.S.

Acknowledgements: The authors wish to thank Gözde Özer for the statistical analysis and Leyla Mollamahmutoglu for the study

#### design.

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

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

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# Cervical premalignant lesions and their management

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### Abstract

Cervical cancer is the tenth most common cancer in women in developed countries that have national screening programs, while it is in the second line in underdeveloped countries. According to Ministry of Health registry data, cervical cancer is the eighth most common cancer among female cancers in Turkey. Today, the most effective screening for cervical cancer is to obtain smears from the cervix. Therefore, periodic screening programs are of great importance in identifying preinvasive lesions to prevent their progression to invasive cancer. Today, with the use of human papilloma virus (HPV) vaccine, screening programs have brought new insights into the prevention of cervical cancer. Management of preinvasive lesions has to be known by each obstetrics and gynecology specialist. Redundant procedures and treatments can be avoided by directing patients correctly at this step. Cancer phobia should not be created. Ablative or destructive treatments should not be done without histological diagnosis; hysterectomy, which has an equal risk of recurrence, should not be recommended.

(J Turk Ger Gynecol Assoc 2014; 15: 109-21)

Key words: Cervix, preinvasive lesions, management, human papilloma virus

Received: 17 October, 2013 Accepted: 17 October, 2013

#### Introduction

Cervical intraepithelial lesions or invasive tumors are caused by infection with human papilloma virus (HPV). More than 100 different strains of HPV are known today, and those considered of high risk are involved in the etiology of cancer. About 99.7% of HPV infections are sexually transmitted and are rarely transmitted from mother to the neonate through an ascending route during delivery.

Every 2 minutes, a woman dies because of cervical cancer worldwide, which is the leading cause of deaths in women; 52,000 new cervical cancer cases are seen annually in the European Union, and 27,000 people die annually due to this disease (1). Based on these findings, cervical cancer is the second most common cause of cancer deaths after breast cancer among women in the European Union. Cervical cancer is the tenth most common cancer in women in developed countries that have national screening programs, while is in the second line in underdeveloped countries. According to Ministry of Health registry data, cervical cancer is the eighth most common cancer among female cancers in our country (2).

Today, the most effective screen for cervical cancer is to obtain smears from the cervix. Therefore, periodic screening programs are of great importance in identifying preinvasive lesions to prevent their progression to invasive cancer. Today, with the use of HPV vaccine, screening programs have brought new insights in to the prevention of cervical cancer. Management of preinvasive lesions has to be known by each obstetrics and gynecology specialist. Redundant procedures and treatments can be avoided by directing patients correctly at this step. Cancer phobia has not been created. Ablative or destructive treatments should not be done without histological diagnosis; hysterectomy, which has an equal risk of recurrence, should not be recommended.

#### **Relation between HPV and Cervical Cancer**

Human papillomavirus belongs to the Papillomaviridae family. To date, more than 100 different HPV types have been identified. This virus is a small (8 kilobases), double-stranded DNA virus that infects skin and mucosal epithelial surfaces, leading to proliferative lesions, and is specific to species. So, HPV infects only humans. The causal relation with cancer is more pronounced than the relation between smoking and cancer. Currently, the high-risk group is identified during HPV testing, and its typing can be performed, as needed. The most effective methods for HPV testing are hybrid-capture (HC) II assay and polymerase chain reaction (PCR). Both methods detect whether there exist an average of 13 high-risk HPV types or not. An HPV-positive result of these tests means that there exists at least one of the high-risk HPV types, and an HPV-negative result means that a high-risk HPV type does not exist. The classification of HPV types according to risk stratification is presented in Table 1.

Squamous cell disorders are the most prominent pathology after HPV infection of the cervix. Glandular cell abnormalities are rarely seen. Spontaneous regression of low-grade squamous intraepithelial lesion (LGSIL) or HPV infection is noted in 60% of cases. Only about 15% of these patients appear as a high-grade squamous intraepithelial lesion (HGSIL) in the



#### Table 1. HPV subtype classification

High-risk	16, 18, 45, 31, 33, 52, 58, 35, 59, 56, 51, 39, 68, 73, 82
Moderate-risk	26, 53, 66
Mild-risk	6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, CP6108
HPV: human pap	pilloma virus

#### Table 2. Prognosis of untreated CIN lesions

	Regression	Persistence	Progression to CIN 3	Invasive Cancer
CIN 1	60%	40%	10%	1%
CIN 2	40%	40%	20%	5%
CIN 3	33%			>12%
CIN: cervical intraepithelial neoplasia				

presence of cofactors within 3-4 years. These cofactors include high-risk HPV types; cigarette smoking; immunosuppression; cervical infections, such as chlamydia and Herpes simplex virus (HSV); oral contraceptive pill use; multiparity; and genetic factors. Spontaneous regression rates decrease after HGSIL development, and 30-70% of these cases progress to invasive cancer within approximately 10 years. In other words, 15% of women with HPV develop cervical intraepithelial neoplasia (CIN) within 7 years. Regression and progression rates of CIN are shown in Table 2. Development of invasive cancer is established at a rate of 1-3% after high-risk HPV transmission, and the required time period is approximately 25-40 years.

Human papilloma virus testing was found to be positive in 96.6% of patients with cervical cancer. HPV types that are mostly associated with the development of cervical squamous cancer are HPV 16 (53.5%), HPV 18 (17.2%), HPV 45 (6.2%), and HPV 31 (2.9%). These rates and types other than HPV 16 and HPV 18 can differ by countries. However, HPV 16 and HPV 18 are identified as being mostly associated with 70.7% of cervical cancers. HPV 18 is mostly associated with cervical adenocarcinoma. In a retrospective study evaluating data from 10,575 patients with invasive cervical cancer conducted by de Sanjose et al. (3), it was reported that HPV 16 and HPV 18 were positive in 71% of patients. In the same study, HPV 16, HPV 18, and HPV 45 were identified as positive in 94% of cervical adenocarcinoma cases. In the study of Usubütün et al. (4), they identified HPV types in cervical cancer in our country, and they found the ratios of types as follows: HPV 16 was 64.7%, HPV 18 was 9.9%, HPV 45 was 9.9%, HPV 31 was 3%, and HPV 33 was 2.2%.

#### A. HPV testing

Human papilloma virus (HPV) is believed to initiate tumorigenesis in cervical carcinoma. HPV is also associated with some anal, vaginal, vulvar, oral, and skin cancers other than cervix. It is suggested to be associated with 4% of all cancers throughout the body (5). According to western society statistics, 50-80% of women are infected with HPV at least once in their lives (6). While the infection rate is higher in young patients (<25 years), it declines in the 30s and 40s and shows a slight increase in the postmenopausal period (7, 8). Oncogenic HPV type is present in 10% of the 20-65-year-old population. The latent infection prevalence is 8-15% (9). Transient HPV infection is not of importance in cancer development, whereas HPV DNA positivity is a risk factor for cervical cancer (10).

Since colposcopy is recommended for HPV 16-positive cases, HPV tests specific for HPV 16 and HPV 18 are available in addition to oncogenic HPV testing. For women with ASC-US cytology and (-) HPV result, colposcopy and biopsy are unnecessary, and follow-up cytology at regular intervals is acceptable. If HPV is positive, immediate colposcopy should be performed as a secondary management. A positive (+) test result in a patient with ASC-US smear suggests that the patient has HGSIL at more than 90% probability. The negative (-) predictive value of the testing is higher than 90% (11, 12). The sensitivity of HPV DNA testing in HGSIL HPV (+) patients is 98%.

Also, 20-40% of precancerous lesions can not be detected by colposcopy, even by the most experienced specialist. HPV testing in this patient group will be a guide in patient follow-up. While a negative test result supports the lack of detection of lesion by colposcopy, a positive test result will suggest the presence of risk and show that the colposcopy result is a false negative (13).

#### Abnormal Cervical Smear Management Squamous cell abnormalities

#### A. Unsatisfactory cytology

When all cytological methods are included, the probability of unsatisfactory cytology is less than 1%. This result is unreliable in the detection of epithelial abnormalities (14, 15). The most common reason for this unsatisfactory cytology is the insufficient squamous cell count (16). Management of unsatisfactory cytology is presented in Figure 1.

# B. Cytology reported as negative but with absent or insufficient endocervical and transformation zone

Cytology that is reported as negative but with an absent or insufficient endocervical and transformation zone has adequate cellularity for interpretation but lacks endocervical or metaplastic cells, suggesting that the squamocolumnar junction may not have been adequately sampled. Recent publications report that this cytological abnormality has ranged from 10% to 20% and is higher in older women (17, 18). The management of this cytological abnormality, which was recently recommended for early repeat cytology, has changed as indicated in Figure 2.

#### C. Negative cytology with a positive HPV test

Recently, co-testing is the preferred screening strategy for women aged 30-64 years and is not indicated for younger women (19). Despite negative cytology, women with HPV are at higher risk for later CIN 3 than women with negative HPV tests (20). Management of cases with negative cytology but with a positive HPV test is presented in Figure 3.

#### D. ASC-US

Atypical squamous cell (ASC) lesions that are unclassified and with undetermined significance are called ASC-US. ASC-US terminology was not approved in a Bethesda 2001 consensus meeting and has been modified. Accordingly, under the head-



Figure 1. Management of unsatisfactory cytology



Figure 2. Management of cytology reported as negative but with absent or insufficient endocervical and transformation zone



Figure 3. Management of HPV-positive cases with negative cytology





ing of atypical squamous cells, ASC-US and atypical squamous cells can not exclude HGSIL (ASC-H) and will be reported by differentiating malignancy potential. There are two pathways for follow-up of ASC-US. One is to repeat smear testing after 1 year; the second is to test for high-risk HPV.

Yearly follow-up route has the advantages of low cost, easy application, and common use, but 30% of HGSIL can be missed. HPV testing is more likely to diagnose HGSIL and is highly reliable, with 95% negative predictive value. High sensitivity in

colposcopy can be achieved among referred cases. However, it is expensive and uncommon. Low-cost reagents that can determine the type have been marketed since 2001. Several studies about ASC-US have been published recently. The consensus for the ASC-US method algorithm is shown in Figures 4 and 5.

#### E. LGSIL

When minimal cytological abnormalities are detected in a woman, HPV DNA testing should be performed if possible. If



Figure 5. Management ASC-US and LSIL for ages between 21-24 years



Figure 6. LSIL management

HPV DNA testing is negative, patient should be followed up with yearly control. If HPV DNA testing is positive, patients are referred for colposcopy and managed according to the results. If HPV DNA testing is not possible, colposcopic examination will be performed; if it is normal, the patient will be called periodically with yearly controls until the smear becomes normal. If an abnormal lesion is detected by colposcopy, immediate treatment is necessary. The related algorithm is presented in Figures 5-7.

#### E. ASC-H

ASC-H confers higher risk for CIN 3 over time than ASC-US or LGSIL. Reflex HPV testing is not recommended in ASC-H due to high HPV prevalence. In addition, the 5-year cancer risk among



Figure 7. LSIL management in pregnancy



Figure 8. ASC-H management

women with HPV-negative ASC-H is 2%, which is too high to justify observation (Figure 8, 9).

#### F. HGSIL

It should be treated. Laser or loop, which has equal success, can be applied. Cold-knife conization is one the methods. Unsuccessful results are due to non-compliance with the protocol. The skill and knowledge of the gynecologist are more important for the success than the method (21-24). Endocervical assessment is important here. HPV testing can be useful in the follow-up; however, it will not be as important as it is for LGSIL.

Colposcopy constitutes one of the main steps. Biopsy will be necessary in the presence of abnormal colposcopic findings, and the treatment of the lesion will be based accordingly (Figure 9, 10). In 2013, the ASCCP published 2012 consensus results about CINs based on the histological diagnosis following the consensus regarding the management of cervical cytological abnormalities (25). The recommendation for CIN 1 is followup without treatment. However, treatment is also an acceptable option. In the follow-up without treatment, in addition to HPV DNA testing, one of the options, which are cytology and colposcopy, can be used. The related flow-chart is presented



Figure 9. ASC-H and HSIL management between 21-24 years of age





Figure 11. CIN 1 (with ASC-US or LGSIL cytology, HPV16/18(+) or persistent HPV) management



Figure 12. CIN 1 (with ASC-H or HGSIL cytology) management

in Figures 11-13. For the treatment, ablative therapies or excisional procedures (LEEP or cold conization) can be selected. Excisional procedures are necessary for recurrent CIN 1, and it is preferred for the diagnosis of co-existing high-grade lesions, as it provides histological diagnosis. If colposcopy is unsatisfactory, diagnostic excisional biopsy is necessary. However, for pregnant women, adolescents, and patients taking immunosuppressive agents, with unsatisfactory colposcopy, follow-up without treatment is recommended. The treatment schema for CIN 2 and 3 confirmed by biopsy is shown in Figures 14 and 15. Although ablative therapy is an acceptable option in this group of patients with adequate colposcopy, excisional therapies should be preferred for recurrent cases. As excisional procedures may cause complications, such as excessive bleeding and risk for preterm delivery in pregnant women with CIN 2 and 3, it should be performed only in patients with suspicion of invasive cancer. Twice-a-week 5-fluorouracil (5-FU) cream is reported to be the most effec-



Figure 13. CIN 1 management of cases at 21-24 years of age



Figure 14. Management CIN 2-3 cases between 21-24 years of age



Figure 15. CIN 2-3 management of pregnant cases who are not between 21-24 years of age

tive treatments in HIV (+) patients using immunosuppressive agents (25). Follow-up is more recommended for adolescents with CIN 2, as it is in CIN 1.

#### Glandular cell abnormalities

#### A. Atypical glandular cells (AGC)

Atypical glandular cells have been associated with polyps and metaplasia but also with neoplasias, including adenocarcinomas of the endometrium, cervix, ovary, fallopian tube, and other sites. Although the cancer risk is lower in women younger than 35 years of age, the risk of CIN 2 and higher lesions is increased at all ages (26). AGC cytology is most commonly associated with squamous lesions, including CIN 1. For women with all subcategories, except atypical endometrial cells, colposcopy with endocervical sampling is recommended in all subcategories. Endometrial sampling is recommended in women 35 years of age and older or with abnormal bleeding in all subcategories. The treatment algorithm is presented in Figures 16 and 17.

#### B. Adenocarcinoma in situ

In adenocarcinoma in situ (AIS), endocervical glandular cells are replaced by nuclear stratification, hyperchromasia, irregularities, and long columnar cells with increased mitotic activity (27-29). Cell proliferation leads to overcrowded and punched glands. However, the normal branching pattern of the endocervical glands is reached as well. Most neoplastic cells resemble those of the endocervical mucinous epithelium. Endometrioid and intestinal cell types appear less often. Squamous CIN is observed in 50% of the women with cervical AIS. So, some of the AIS lesions represent the findings in the samples obtained for squamous neoplasia treatment. As AIS is near or above the transformation zone, traditional cervical samples may not be effective in AIS sampling. Sampling via Cytobrush may enhance AIS screening. If the AIS focus is small, the findings of cervical biopsy and endocervical curettage may be negative. A more comprehensive cervical examination in conization form is necessary for such cases. With such a sample, it is also possible to exclude invasive adeno-



Figure 16. AGC management



Figure 17. Post-AGC case management

carcinoma. The term "microinvasion" should not be used in adenocarcinoma definitions. Once it spreads to glands, there is no clear technique to determine the true "depth of invasion," because the invasion may have originated from the mucosal surface or from exterior surface of the glands. "Passing" through the basal membrane can not be described completely; therefore, the tumor is either adenocarcinoma in situ or invasive adenocarcinoma.

With the recent increase in endocervical invasive adenocarcinoma, adenocarcinoma in situ has been noticed more. There is evidence that adenocarcinoma in situ can progress to cancer. Boone (30) reported 52 cervical adenocarcinoma cases where 18 endocervical biopsy results were interpreted as negative 3-7 years before cancer developed. Adenocarcinoma was detected among five of them.

Muntz (31) reported 40 cases with AIS who also had cervical conization. Coexisting squamous cell carcinoma was observed in 23 (53%) of these 40 patients. In 10 of the 22 patients who had undergone hysterectomy, margins of coni specimens were positive and 70% had remaining AIS. Additionally, two patients



#### Figure 18. AIS management

had invasive adenocarcinoma. Among the 12 patients with negative margins, focal adenocarcinoma in the hysterectomy specimen was seen in one of them. In 18 women, only conization with negative margins was detected, and the disease did not recurr after 3 years on average. So, positive margins in conization specimens were significant findings for these patients.

Pyonor et al. (32) reported the results of another more interesting study of 28 patients with AIS. Among the 8 patients with positive margins who had undergone repeat conization or hysterectomy, residual AIS was detected in 3 and invasive adenocarcinoma was detected in 1. Of the 10 patients with negative margins who had undergone hysterectomy or repeat conization, 4 had residual AIS. Invasive adenocarcinoma was detected in 1 patient, whose cone margin had not been examined. Among 15 patients treated with repeat cervical conization and close follow-up, recurrent glandular lesions, detected as a result of conization, were found in 7 patients (47%), and in 2 of them, invasive adenocarcinoma was detected. Furthermore, 48% of the patients were not suspected to have glandular lesions based on their endocervical curettage and Pap smear test results obtained before conization.

Adenocarcinoma in situ has to be regarded as a significant adenocarcinoma cancer precursor. The entire endocervical canal is under risk, and examination of lesion by cytology and curettage procedures may be unreliable. Repeat conizations should be performed in patients with positive cone margins. If fertility can be disregarded, hysterectomy should be done, even in the case of a negative margin due to the risk of recurrence (Figure 18).

The NCCN Guidelines Panel for Cervical Cancer Screening endorses the 2012 updated consensus guidelines for the management of abnormal cervical cancer screening tests and cancer precursors (25).

#### **Evaluation of Intraepithelial Lesions During Pregnancy**

Cervical cancer is the most common malignancy during pregnancy. It is relatively rare in developed countries. It is due to the low cancer incidence, depending on low birth rate and routine cervical cytological screening programs. Its incidence is 1-13/10,000. This equals the incidence of 1 cervical cancer in 2000-2500 pregnancies and 1 CIN 3 in 750 pregnancies. Pregnancy was present in about 1% of the women with cervical cancer at the time of diagnosis. Most of the cases are asymptomatic. Therefore, the diagnosis is often delayed. Patients who experience vaginal bleeding during pregnancy and those with post-coital bleeding should be screened for cervical cancer, and speculum examination should definitely be performed in these patients. Pregnancy does not affect the prognosis of cervical cancer (33, 34).

The cervix is soft and hypertrophic during pregnancy and usually moves towards the columnar epithelial porsio. Therefore, unsatisfactory cytology and colposcopy are rare. In the study of Baldauf et al. (35) regarding the management of 140 pregnant women with abnormal smear results, it was found that colposcopy was adequate in 86% of the patients, compatible with 69% biopsy diagnosis, 18% false positive, and 13% false negative rates. Biopsy was performed in 115 patients, and bleeding was noted in only 1 patient. They concluded that colposcopy and accompanying biopsy are reliable procedures in the management of abnormal smears.

Further, 86% of the cervical abnormalities during pregnancy are LGSIL, and most of them are caused by HPV. The remaining 14% are HGSIL. Hormonal and vascular changes during pregnancy do not interfere with the natural progress of invasive cervical cancer. There is no evidence showing faster progression of CIN to invasive cancer during pregnancy.

In conclusion, it should be noted that the goal during pregnancy is not to diagnose and follow up preinvasive lesions or not to treat these lesions. The main goal during this period is to detect the presence of invasive lesions, especially during the first half of the pregnancy.

#### Ethics Committee Approval: N/A.

Informed Consent: N/A.

Peer-review: Internally peer-reviewed.

**Author contributions**: The authors contributed equally during the preparation of this manuscript.

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

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

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# Rectus abdominalis muscle metastasis from uterine leiomyosarcoma: An unusual case and review of the literature

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#### Abstract

Uterine leiomyosarcoma is an aggressive malignancy. Spread to the lung, thyroid, liver, brain, pancreas, heart, duodenum, breast, vagina, submandibular gland, and bone has been reported. We describe a case of metastatic uterine leiomyosarcoma to the rectus abdominalis muscle as the first case in the literature. A 39-year-old nulligravid woman presented with a history of pelvic pain. Physical examination discovered about a 6-cm mass in the suprapubic region. She had previously undergone a hysterectomy for uterine leiomyosarcoma. Operative findings had revealed a mass measuring  $4\times5\times6$  cm located in the rectus abdominalis muscle. Abnormal mitotic figures and necrosis were evident, and uterine leiomyosarcoma was diagnosed. Uterine leiomyosarcomas are malignancies of the smooth muscle arising from the myometrium. Skeletal muscle is an uncommon site of metastasis by hematogenous spread. In conclusion, we have described a case of skeletal muscle metastasis (first case of rectus abdominalis muscle metastasis) secondary to uterine leiomyosarcoma. (J Turk Ger Gynecol Assoc 2014; 15: 122-4) **Key words:** Uterine leiomyosarcoma, metastasis, skeletal muscle, rectus abdominalis muscle

Received: 06 May, 2013 Accepted: 06 October, 2013

#### Introduction

Leiomyosarcomas are malignant tumors of smooth muscle, and it constitutes 5% of soft tissue sarcomas (1, 2). It is diagnosed in 1.3% of all uterine malignancies (3). The disease is most commonly detected in 30-40-year-old age groups, and the prevalence is much higher in black women and European-American Jews than other women (4).

The symptoms of disease are associated with the tumor size and location. However, vaginal bleeding and abdominal pain are mostly common. (5). The tumor primarily metastasizes hematogenously. Spread to the lung (6), thyroid (7), liver (6, 8), brain (9), and bone (6, 10) has been reported. To the best of our knowledge, there are two cases of skeletal muscle metastasis of uterine leiomyosarcoma reported in the literature (5, 11).

In this case report, we describe a case of metastatic uterine leiomyosarcoma to the rectus abdominalis muscle as the first case in the literature.

#### **Case Presentation**

A 39-year-old multigravid woman presented with a history of pelvic pain and mass. She had hypertension and had no family history of cancer and other chronic diseases. Previously, she had undergone myomectomy for leiomyoma that was confirmed by pathological examination. After the surgery, she had in vitro fertilization (IVF) treatment, but she had no successful pregnancy. After IVF treatment failure, she underwent another surgery for uterine myoma, and pathologic investigation revealed leiomyosarcoma, and she was referred to our hospital.

She underwent total abdominal hysterectomy, bilateral salphingo-oopherectomy and bilateral pelvic paraaortic lymph node dissection and appendectomy at our hospital in December, 2005. There was no abnormality in the pelvis or the other abdominal organs and anterior abdominal wall in the immediate exploration. All the lymph nodes were confirmed as reactive lymph nodes by pathological examination. The patient was again admitted to our hospital with a complaint of a suprapubic mass and pelvic pain in March 2006. Physical examination discovered a round, well-circumscribed, movable, firm, and painless mass of about 6 cm in the suprapubic region. Moreover, transvaginal ultrasonography (Picker 9100, Hitachi Medical Ltd, Tokyo, Japan) showed a hypoechoic mass measuring 61x39 mm at the widest diameters anterior to the urinary bladder (Figure 1). This mass could also be a local inoculation; however, the odds for a hematogenous dissemination are higher. Operative findings of a second



surgery had revealed a myomatous mass measuring 4x5x6 cm located in the rectus abdominalis muscle. The mass could not totally extracted from the rectus abdominalis muscle, and histopathological examination revealed that the polypoid tissue was highly cellular and composed of pleomorphic spindle cells with hyperchromatic nuclei (Figure 2). Abnormal mitotic figures and necrosis were evident, and uterine leiomyosarcoma was diagnosed (Figure 3).

Her follow-up abdominal magnetic resonance imaging (MRI) (Intera 1,5 T, Philips Medical Systems, France) 1 month after surgery revealed a 3.5x2.5x2 cm mass in the left rectus abdominalis muscle, and chest X-ray showed 90x35 mm of opacity. With those findings, she underwent 25 cures in total of 50 gr external abdominal radiotherapy and 6 cures of chemotherapy of Adriamycin 100 mg (Pfizer, New York, USA), Haloxan 3 gr (Baxter, Deerfield, USA), and mesna 3 gr (Bedford Laboratories, Bedford, USA). The patient was informed, and informed consent was obtained. The patient was still alive and decided to have her follow-up in another hospital; so, we do not have any information about the fate of the mass anterior of the bladder, and she was lost to follow-up.

#### Discussion

Leiomyosarcoma of the uterus originates from the myometrium (5). Uterine leiomyosarcomas constitute 25%-35% of uterine sarcomas and 1% of uterine malignancies (12). It usually occurs de novo, but in some cases, a history of prior irradiation may be present (5). The tumor spreads most commonly by hematogenous route to the lung (6), thyroid (7), liver (6, 8), brain (9), and bone (6, 10). Bronchogenic carcinoma, breast carcinoma, melanoma, and gastrointestinal and urinary tract tumors commonly metastasize to skeletal muscle, but it is an unexpected site of metastasis of leiomyosarcoma (5). It has a poor prognosis because of its high recurrence and metastasis (13). Salazar et al. (12, 14) reported 5-year survival of leiomyosarcomas of stage I of 53% and stage II-IV of 8%, respectively.

Up to date, 5 cases of skeletal muscle metastasis of leiomyosarcoma have been reported (5, 11, 15-17). O'Brein et al. (5) presented a case of a 68-year-old patient with a complaint of painless mass in the anterior aspect of the thigh. She had undergone a hysterectomy 3 years ago for dysfunctional uterine bleeding. Leiomyosarcoma was confirmed by pathological examination. The patient had radiotherapy and chemotherapy. After 2 years of surgery, a metastatic solitary lesion was found in the liver and treated with radiofrequency ablation. Following 1 year of a recurrence-free period, the patient presented with an enlarging mass in her thigh region. MRI scan revealed 2 discrete masses in the rectus femoris and gracilis muscle. Histological examination of both lesions showed intermediate-grade leiomyosarcoma.

Nusrath et al. (11) presented a case of a 65-year-old female with a mass in the left cheek. It was enlarging for 3 weeks. Physical examination showed a discrete, tender mass in the left masseter muscle and an enlarged preparotid lymph node. The fine needle aspiration result was suspected squamous cell carcinoma. A wide local excision was performed, and histopathology and immunohistochemical stains revealed a tumor that was positive for desmin antibodies with mitotic figures. Computed tomography (CT) scan of the chest was performed and was negative for any tumors. A further excision was performed, and



Figure 1. Transvaginal ultrasound image: "A hypoechoic mass measuring 61x39 mm at the widest diameters anterior to the urinary bladder"



Figure 2. A photomicrograph of the pathological specimen (H&E, x40): Metastatic leiomyosarcoma infiltrating the skeletal muscles

the patient was followed up with CT scans of the neck region. But, the patient had a complaint of suprapubic discomfort during the follow-up period. An ultrasound was performed and showed a pelvic mass originating from the uterus or ovary. Laparotomy was performed. At the laparotomy, they found a large unresectable retroperitoneal mass that was adherent to the small bowel, which on biopsy showed a leiomyosarcoma; so, chemotherapy was given to the patient.

Aslan et al. (15) presented a case of a 76-year-old female with a gross mass in the right temporal region. Two years before presentation, she had been operated on for a primary uterine leiomyosarcoma. She had not received any post-operative radiotherapy or chemotherapy. Physical examination revealed a gross mass, nearly 10 cm in maximum diameter, in the right temporal region of the scalp. She was operated on, and the



Figure 3. A photomicrograph of the pathological specimen: Uterine leiomyosarcoma (H&E, x200): Cellular eosinophilic spindle cell tumor with nuclear atypia and mitosis

mass was totally excised together with the temporalis muscle. On histopathological evaluation, it was composed of spindleshaped atypical mesenchymal cells, which showed 10-12 mitoses/10 high-power fields with marked pleomorphism and central cigar-shaped nuclei arranged in fascicles. The tumor morphology was interpreted as high-grade (III) leiomyosarcoma.

Cappellani et al. (16) treated a 61-year-old woman for mesocolon leiomyosarcoma by radical resection of left nephrectomy and left hemicolectomy. Three years after the surgery, a leiomyosarcoma of the duodenal wall was diagnosed, and she underwent pancreatoduodenectomy. Three months later, a mass was observed rapidly growing to a diameter of 4 cm over 1 month. The histological examination revealed metastasis from high-grade leiomyosarcoma. The patient underwent chemotherapy treatment. The patient experienced progression of disease with multiple pulmonary and encephalic metastases 5 months later.

Courtney et al. (17) presented a case of a 52-year-old female with a metastatic uterine leiomyosarcoma to the left flank 10 years following total abdominal hysterectomy for a left primary adnexal mass. Leiomyosarcomas rarely metastasize to the flank or skeletal muscle. The majority of the recurrences occurs within 8 to 16 months after hysterectomy. The case offers an atypical recurrence of this malignancy.

In the present case, the patient had a diagnosis of leiomyoma of the uterus and had undergone myomectomy. Pathology had shown a benign lesion. But, in a short period, she again had surgery for a similar complaint, but at that time, the histological result was malignant. There may be a rapid progression to leiomyosarcoma or misdiagnosis of the previous mass excised. In conclusion, we present a case of metastasis of uterine leiomyosarcoma to the rectus abdominalis muscle for the first time in the literature. Therefore, skeletal muscle metastasis should be kept in mind in the management of such malignancies.

#### Ethics Committee Approval: N/A.

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

Peer-review: Externally peer-reviewed.

Author contributions: Concept - B.Y., T.G., H.A., S.A.; Design - B.Y., H.A.; Supervision - T.G., B.Y.; Resource - H.A., S.A.; Materials - B.Y., H.A., S.A.; Data Collection&/or Processing - B.Y., H.A.; Analysis&/or Interpretation -B.Y., H.A.; Literature Search - B.Y., H.A., S.A.; Writing - B.Y., H.A.; Critical Reviews - T.G., B.Y.

**Conflict of Interest:** No conflict of interest was declared by the authors. **Financial Disclosure:** The authors declared that this study has received no financial support.

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# Safe vaginal delivery in a renal transplant recipient: A case report

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### Abstract

Here, we aimed to present a case of safe vaginal delivery in a renal transplant recipient and to mention the possible additional risks of caesarean section in such cases. A 40 year-old patient (G4P3), who had undergone renal transplantation 5 years ago, was admitted to our clinic at  $39^{2/7}$  weeks of pregnancy. The transplanted kidney at right iliac fossa was sonographically normal except for grade 1 hydronephrosis; the proximity of vascular anastomoses between the transplanted kidney and iliac vessels to the lower segment of the uterus was remarkable. There was no contraindication for vaginal delivery and it was believed that there would be a possible risk of injury to the transplanted kidney with caesarean delivery. The patient delivered a healthy baby weighing 3540 grams. There is a risk of injury to the renovascular and ureter anastomoses in renal transplant recipients during caesarean delivery. Normal vaginal delivery without abdominal compression is the safest method of delivery in these patients. If a situation that can necessitate internal iliac artery ligation or caesarean hysterectomy such as placenta accreata is expected, surgery should be performed in a centre where the renal transplant surgeon can oversee the surgery. (J Turk Ger Gynecol Assoc 2014; 15: 125-7)

Key words: Anastomosis, delivery, injury, renal, transplant

Received: 14 September, 2013 Accepted: 09 October, 2013

#### Introduction

Pregnancies in women with chronic renal insufficiency are accepted as high risk pregnancies, because there are some possible complications that can occur during pregnancy and can affect the mother, the foetus or both. The most important of these are the development or aggravation of pre-existing hypertension and/or proteinuria, preeclampsia, intrauterine growth retardation, premature delivery and its associated risks. There can also be some deterioration of the renal condition.

The above risks are possible in renal transplant recipients. However, in this case, there were some additional concerns for the obstetrician, such as the risks associated with the immunosuppressive medications that the patient was required to use during pregnancy and the decision that should be taken about the mode of delivery. Most of the previous reports about the mode of delivery mention that the transplanted kidney does not usually obstruct the birth channel and vaginal delivery can be accomplished in most cases (1, 2). These reports also mention that caesarean section should be reserved for obstetric indications. A case series of pregnant women with a renal transplant, however, shows that most of the deliveries occurred by caesarean section (3). Most of the deliveries in this series were premature deliveries. Here, we present an uncomplicated pregnancy in a renal transplant recipient who was followed by normal vaginal delivery. The suspected surgical risk for the transplanted kidney from the caesarean operation was higher because of the proximity of the kidney and the renovascular anastomoses to the caesarean surgery site.

#### **Case Presentation**

A 40 year old woman in her 4th pregnancy was admitted to our clinic at a gestational age of 39 weeks 2 days. She had undergone kidney transplantation to the right iliac fossa from a living related donor 5 years previously. Before transplantation, she had been receiving antihypertensive treatment for 5 years and eventually developed end-stage renal disease. One month after initiating haemodialysis, she underwent the transplantation surgery. Since then, she had used several immunosuppressive medications at differing doses, but upon admission to our clinic, she was using Tacrolimus (Prograf; Astellas Ireland Co. Ltd., Killorglin, Co. Kerry, Ireland) and Azathioprine (İmuran; Glaxo Smith Kline, İstanbul, Turkey). She had continued these two drugs throughout her pregnancy. After transplantation, her renal function tests were all normal, and she had no hypertension. Her past and current obstetric history were uneventful. She had no hypertension, no deterioration in the renal functions and foetal development, and amniotic fluid and Doppler evaluation of the umbilical artery were all within the normal range. At 39 weeks and

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Figure 1. The transplanted kidney in the right iliac fossa (thick arrow) which is very close to the foetal head in the vertex presentation (thin arrow)



Figure 2. The proximity of the vascular supply of the new kidney to the lower segment of the uterus is remarkable

2 days of gestation, the estimated foetal weight was 3716 grams. The ultrasound evaluation of the transplanted kidney performed with a Siemens SONOLINE Antares ultrasound machine by using a 1-4 MHz transducer (Siemens Medical Solutions USA Inc., Malvern, PA) demonstrated a normal kidney in the right iliac fossa, except for grade 1 hydronephrosis. However, the proximity of the vascular anastomosis between the transplanted kidney and the iliac vessels to the lower segment of the uterus was remarkable at ultrasound (Figure 1, 2). From the previous surgical records, it was confirmed that the renal vein had been anastomosed to the external iliac vein and the renal artery had been anastomosed to the internal iliac artery at the right side while the kidney had been placed to the right iliac fossa. Since the overall evaluation of the foetus and mother showed no contraindication for vaginal delivery and since caesarean delivery could pose a risk of injury to the transplanted

kidney, we followed the patient for vaginal delivery after administering vaginal dinoprostone. She had an uncomplicated vaginal delivery and gave birth to a healthy female baby weighing 3540 grams. The baby had no congenital defects. Informed consent was obtained from the patient prior to publishing this case report and accompanying images.

#### Discussion

As we mentioned before, most of the previous reports support the suggestion that a transplanted kidney has no obstructive effect on the birth channel, and no injury to the kidney is expected during vaginal delivery. However, Shrestha et al. (4) reported a case of caesarean section in a renal transplant recipient during which the transplanted kidney was injured and had to be surgically corrected. During renal transplantation, the new kidney is most commonly placed to the iliac fossa and the vessels of the new kidney are most commonly anastomosed to the iliac vessels. The ureter of the new kidney is anastomosed to the urinary bladder by ureteroneocystostomy. In our case, however, the kidney had been placed to the right iliac fossa, the renal vein had been anastomosed to the external iliac vein as an end-to side anastomosis and the renal artery had been anastomosed to the internal iliac artery as an end-to end anastomosis. In a routine caesarean section, the phannenstiel incision is generally used for entrance to the abdominal cavity and a lower segment uterine incision is used for the uterus. In the case of Shrestha et al. (4), the lower pole of the kidney was accidentally transected during entrance to the abdominal cavity through a phannenstiel incision. A blunt injury to a kidney in this localisation also seems possible during traction of the abdominal muscles to the lateral sides, which is a common manoeuvre applied by surgeons after the peritoneal space is entered. Also, this traction can be harmful for the anastomoses of both the vessels and the ureter of the transplanted kidney which are located very close to the site of this traction. Vaginal delivery, which does not pose these risks, seems safer for these patients because of these possibilities. Care should be taken during vaginal delivery to avoid manoeuvres that can cause blunt injury to the transplanted kidney, such as applying compression to the uterus from the abdominal wall to ease the delivery of the foetus.

Although the risks of hypertension, preeclampsia, intrauterine growth retardation and premature delivery are higher in these patients, the occurrence of such problems depends on the general medical condition of the patient. If the transplanted kidney functions very well and if the patient has no hypertension or diabetes, and if there is no clinical finding of rejection of the transplanted kidney, the pregnancy most probably progresses in an uneventful manner. The renal function tests of our patient after transplantation were all normal. The duration of time that elapses after transplantation is also important. It is supposed to be better if the female transplanted with a new kidney waits at least two years before pregnancy (5). In this way, a stable condition will be achieved for renal functions and immunosuppressive medications, and risks such as rejection, infection and hypertension will be minimised (6). Since the time elapsed after transplantation was long for our patient and since she was clinically stable, her pregnancy reached term without any complications.

Renal transplant patients can have uncomplicated pregnancies if the general medical condition of the patient is good. Normal vaginal delivery without abdominal compression is the safest method of delivery in suitable patients. Choosing to perform a caesarean section in any condition which is unusual is common among obstetricians because of a belief that it will be safer. However, we wanted to state that pregnancy in renal transplant patients is one of the important exceptions to this belief. In renal transplant patients, the renovascular and ureter anastomosis are situated in such a place that caesarean section can have a risk of injury to these anastomosis as well as to the transplanted kidney. If a situation that can necessitate internal iliac artery ligation or caesarean hysterectomy such as placenta accreata is expected, the surgery should be performed in a centre where the renal transplant surgeon can oversee the surgery, since these procedures can be risky for the anastomoses of the transplanted kidney.

#### Ethics Committee Approval: N/A.

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

Peer-review: Externally peer-reviewed.

Author contributions: Concept - T.Ö., M.E.D.; Design - T.Ö., M.E.D.; Supervision - T.Ö., M.E.D., E.D., H.T.; Resource - T.Ö., M.E.D., E.D., H.T.; Materials - T.Ö., M.E.D., E.D., H.T.; Data Collection&/or Processing - T.Ö., M.E.D., E.D., H.T.; Analysis&/or Interpretation - T.Ö., M.E.D.; Literature Search - T.Ö., M.E.D.; Writing - T.Ö., M.E.D.; Critical Reviews - T.Ö., M.E.D., E.D., H.T.

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

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

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# What is your diagnosis?

A 23-year-old patient was referred during her second pregnancy at 38 weeks of gestation. The referring physician detected a hyperechogenic mass over the vertebra and suspected it to be a spinal lipoma with closed spina bifida. Her medical and obstetric history was unremarkable. Prenatal evaluation was done using a Voluson 730 Pro with a convex volumetric transducer (RAB 4-8 MHz) probe. During her ultrasound evaluation, intracranial findings of spinal dysraphism and vertebral irregularity were absent, and nothing remarkable was observed, except for a left paravertebral hypoechogenic skin mass. The mass was 36x35x18 mm in size. Power Doppler investigation showed high vascularization in the mass (Figure 1). 3D ultrasound, enhanced with power Doppler, permits one to visualize the highly vascular skin lesion (Figure 2). With the aid of 3D ultrasound, the location of the mass was estimated to be between T10-T12 (1) (Figure 3). There was no sign of cardiac failure due to the hypervascular mass. What is your diagnosis?



Figure 1. a, b. Prenatal 2D (left) (a) gray scale and (right) (b) power Doppler imaging of the lesion



Figure 2. 3D power Doppler angiography of the lesion shows highly vascularized, paravertebral skin mass



Figure 3. The localization of the mass with 3D ultrasound imaging



#### Answer

On the same day, she delivered a 3150-g male infant vaginally. He had a 4x3x2 cm skin hemangioma on the left thoracolomber paravertebral region (Figure 4). The mass was confirmed to be a hemangioma with a postnatal ultrasound evaluation. Fortunately, the newborn had neither thrombocytopenia nor anemia. The newborn was otherwise noted to be healthy. The lesion spontaneously and completely regressed, and no complications occurred during the follow-up period.

Hemangiomas are benign vascular tumors commonly located in the skin and subcutaneous tissue. They are estimated to occur in 0.3% of Caucasian infants (2). Their size is important, because as their size increases, complications, like thrombocytopenia, microangiopathic hemolytic anemia, and Kasabach-Merritt syndrome, may occur (3). Despite these possible important complications, they are rarely diagnosed prenatally. This is probably because during prenatal evaluation, the skin is often omitted.

Major risk factors for the development of hemangiomas are advanced maternal age, placenta previa, preeclampsia, multiple pregnancies, and fair-skinned, female, and premature infants (4). Chorionic villus sampling is also found to increase the incidence of skin hemangiomas (5). Although they are commonly seen alone, they can also be a part of various syndromes, like PHACE syndrome (6). So, careful investigation should also be undertaken for other systems (eg, cardiovascular, facial).

Cutaneous lesions can be located anywhere on the body. Their differential diagnosis should be made, because depending on their location, they can be misdiagnosed as lipoma, fibroma, or even encephalocele. When they are located on the skull, they can easily be confused with an encephalocele. This significantly changes the prognosis and parental counseling. In these cases, visualization of the underlying bone continuity may be used to differentiate.

Fetal cutaneous lesions should also be evaluated with either power or color Doppler in order to determine its vascularization. Highly vascular lesions are mostly hemangiomas, whereas low vascularity suggests masses, like lipomas or fibromas. With the addition of the 3D ultrasound, the localization, size, and volume of the lesion can be determined. These factors are important in counseling the patient about the prognosis of the lesion.

Magnetic resonance imaging (MRI) is another diagnostic method in hemangiomas. However, in most cases, MRI is not needed to establish the diagnosis. MRI can be used, especially for the lesions located on the scalp in order to exclude an encephalocele (7).

When skin hemangiomas are diagnosed prenatally, patients



Figure 4. Postnatal photography of the hemangioma

should be counseled that this is a common and spontaneously regressing benign tumor. They can even begin regressing prenatally (8). However, their sizes are important. If large hemangiomas are diagnosed prenatally, the patient should be monitored closely with ultrasound and functional echocardiography. Large hemangiomas can cause thrombocytopenia, anemia, and cardiac failure. So, these patients should be encouraged to deliver in a tertiary health-care facility.

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## CONGRESS CALENDAR

### **INTERNATIONAL MEETINGS**

9-12 October, 2014	11 <sup>th</sup> Congress of the Mediterranean Association for Ultrasound in Obstetrics and Gynecology Antalya, Turkey www.perinatal.org.tr
20-22 November, 2014	20 <sup>th</sup> Asia Pasific COGI 6 <sup>th</sup> Asia Pacific Congress on Controversies in Gynecology, Infertility & Ultrasound (COGI) Saigon, Vietnam www.cogi.org
4-7 December, 2014	20 <sup>th</sup> World Congress on Controversies in Obstetrics, Gynecology & Infertility (COGI) Paris, France www.cogi.org
8-12 April, 2015	3 <sup>rd</sup> Annual Middle East Society for Gynecologic Endoscopy (MESGE) Congress & 6th Annual Congress of Turkish Society of Gynecological Endoscopy (TSGE) Conjoint Meeting Antalya, Turkey www.mesge2015.org
11-13 June, 2015	7 <sup>th</sup> SERG Meeting İstanbul, Turkey www.sergs2015.org
16-19 June, 2015	11 <sup>th</sup> AAGL International Congress on Minimally Invasive Gynecology & 15 <sup>th</sup> Annual Meeting of the Israeli Society of Gynecologic Endoscopy - ISGE IL Conjoint Meeting Jerusalem, Israel www.aagljerusalem2015.com

### NATIONAL MEETINGS

13-15 June, 2014	Turkish Maternal-Fetal Medicine and Perinatology Society Ultrasonography Course İstanbul, Turkey http://www.tmftprenataltani2014.org
23-24 June, 2014	5 <sup>th</sup> Gynecological Endoscopy Course İstanbul, Turkey www.turkishsfp.com
15-18 October, 2014	7 <sup>th</sup> National Urogynecology Congress İstanbul, Turkey http://urojinekoloji2014.org/
6-9 November, 2014	6 <sup>th</sup> Biannual Meeting of the Turkish Society of Reproductive Medicine Antalya, Turkey http://2014.tsrm.org.tr/
19-23 November, 2014	14 <sup>th</sup> National Gynecologic Oncology Congress Antalya, Turkey www.trsgo.org

# JTGGA CME/CPD CREDITING



## Questions on the article within the scope of CME/CPD

- 1. Squamous cell disorders are the most prominent pathology after HPV infection of cervix. Which of the following is not a cofactor with HPV for cervical intraepithelial neoplasias?
  - a) Smoking
  - b) Chlamydial infections
  - c) Oral contraceptives
  - d) Nulliparity
  - e) Immunosuppression
- 2. Which one of the following answers is not associated cancer with HPV infection?
  - a) Anal cancer
  - b) Oral cavity cancer
  - c) Vulvar cancer
  - d) Cervical cancer
  - e) Colorectal cancer
- 3. Which of the following is the most correct answer for the management of "unsatisfactory cytology"? a) Direct colposcopy
  - b) Repeat cytology at 12 months of initial test
  - c) Colposcopy if HPV test is positive
  - d) LEEP
  - e) Routine screening
- 4. Which of the following is not acceptable for the management of ASC-US cytology?
  - a) Cervical biopsy
  - b) Repeat cytology at 1 year
  - c) HPV testing
  - d) Repeat cotesting at 3 years if HPV is negative
  - e) Colposcopy if HPV is positive
- 5. All the following answers about the management of HSIL between 21-24 years of age are correct, except
  - a) Direct colposcopy
  - b) Immediate loop electrosurgical excision
  - c) Observation with cytology and colposcopy at 6 months intervals if no CIN seen on colposcopy
  - d) Routine screening if two negative cytology tests are observed after colposcopy
  - e) Diagnostic excisional procedure if HSIL persists for 24 months
- 6. Which of the following answers is not acceptable for the management of HSIL?
  - a) Immediate hysterectomy
  - b) Immediate loop electrosurgical excision procedure
  - c) Direct colposcopy with endocervical assessment
  - d) Observation with cytology and colposcopy after negative colposcopic biopsy for ages between 21-24
  - e) Conization if CIN 2-3 observed on colposcopic biopsy at 21-24 years of age

# JTGGA CME/CPD CREDITING



# Answer form for the articles within the scope of CME/CPD

1 <sup>st</sup> Question						4 <sup>th</sup> Question					
A	В	С	D	E		А	В	C	D	E	
2 <sup>nd</sup> Question						5 <sup>th</sup> Question					
A	В	C	D	E		А	В	C	D	E	
3 <sup>rd</sup> Question						6 <sup>th</sup> Question					
A	В	C	D	Е		А	В	C	D	Е	

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

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# Türkçe Özler – Haziran 2014

J Turk Ger Gynecol Assoc 2015; 16: 69-73 • DOI: 10.5152/jtgga.2014.0044

# Tıp öncesi öğrencilerin minimal invaziv cerrahi becerilerinin değerlendirilmesi: Gelecekteki öğrencilerden neler öğrenebiliriz?

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## ÖΖ

**Amaç:** Gelecekteki öğrencilerin bazal laparoskopik ve robotik cerrahi becerilerinin bilinmesi onlara en uygun öğretim stratejilerini geliştirmek için esastır. Bu çalışmanın amacı lise ve üniversite öğrencilerinin temel laparoskopik ve robotik becerilerini belirlemek ve halen kadın hastalıkları ve doğum asistanı olan kişilerle karşılaştırmaktır.

Gereç ve Yöntemler: Kesitsel (Sınıf II-2) pilot çalışma. Üniversite ve lise öğrencilerinin laparoskopik ve robotik cerrahi becerileri simülatörler kullanılarak değerlendirildi ve kadın hastalıkları ve doğum asistanları ile kıyaslandı. Ayrıca, video oyunu oynama ve bilgisayar kullanımına ilişkin anket verileri toplandı.

**Bulgular:** Lise (n=9) ve üniversiteden (n=8) toplam 17 öğrenci, ayrıca 11 asistan çalışmayı tamamladı. Genel olarak, öğrenciler basit egzersizleri asistanlarla kıyaslanabilir şekilde yaptı (p>.05). Ancak, öğrenciler karmaşık egzersizleri tamamlamak için önemli ölçüde daha uzun zaman harcadı (p=.001). Son olarak, öğrenciler asistanlardan daha çok video oyunları oynadı (p<.001).

**Sonuç:** Gelecekteki öğrenciler özgeçmişlerinde farklı bir beceri durumuna sahip olabilirler. Bu fark muhtemelen video oyunları oynamaktan dolayı gelişmiş el-göz koordinasyonu ile ilgili olabilir. Bu pilot çalışmanın sonuçları cerrahi öğretim stratejileri için daha fazla araştırmayı teşvik etmelidir. **Anahtar kelimeler:** Simülasyon, eğitim, robotik cerrahi, laparoskopi, video oyunu

#### Özgün Araştırma

J Turk Ger Gynecol Assoc 2015; 16: 74-77 • DOI: 10.5152/jtgga.2014.65632

# Gebe kadınlara yönelik aile içi şiddet: İstanbul metropolünde prospektif bir çalışma

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## ÖΖ

**Amaç:** Kadınlara, özelliklede gebe kadınlara yönelik şiddet giderek dünya çapında önemli bir problem olarak kabul edilmektedir. Gebe kadınlara yönelik aile içi şiddetle ilgili az sayıda çalışma mevcuttur. Bu çalışmanın amacı gebelik sırasında görülen aile içi şiddetin yaygınlığı ve bunu etkileyen faktörleri belirlemektir.

**Gereç ve Yöntemler:** Bu prospektif çalışma Ocak 2012 ile Nisan 2013 tarihleri arasında, Kadın Hastalıkları ve Doğum Kliniğinde, yürütüldü. Yaş ve sosyo-ekonomik durumdan bağımsız olarak toplam 1.349 gebe kadınla yerel dilde anket yöntemiyle görüşülmüştür.

**Bulgular:** Çalışmaya dahil edilen olgularda aile içi şiddetin sıklığı %2.37 idi. Şiddete maruz kaldığını belirten kadınların yaş ortalaması 29.06 $\pm$ 5.53 yıl idi. Yaş, evlilik süresi, gravida, parite, eğitim düzeyi, eşin eğitim düzeyi ve aylık gelir düzeyinin; kadınların aile içi şiddete maruz kalmaları üzerinde önemli bir etkisi olmadığı bulunmuştur (p>0.05). Kalabalık, geniş bir aile ile birlikte yaşayan gebeler, çekirdek aile içinde yaşayan gebelerle kıyaslandığında aile içi şiddete maruziyet riski belirgin olarak artmıştır (p=0.018).

**Sonuç:** Gebelik sırasında aile içi şiddet potansiyel bir halk sağlığı sorunudur. Eğitim ve ekonomik özerklik ve toplumun davranışları aile içi şiddeti azaltabilir. Kadınları aile içi şiddetten korumak için sağlık personelinin risk faktörlerinin farkındalıkları artırmalıdır.

Anahtar kelimeler: İstismar, aile içi şiddet, gebe kadınlar, Türkiye

#### Özgün Araştırma

J Turk Ger Gynecol Assoc 2015; 16: 78-81 • DOI: 10.5152/jtgga.2014.07355

## Postmenopozal kanaması olan hastalarda endometrial karsinomu öngörmede üç boyutlu power Doppler ultrasonun değeri

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ÖΖ

**Amaç:** Postmenopozal kanamalı ve endometrium kalınlığı ≥5 mm olan kadınlarda 3 boyutlu (3D) ultrason görüntüleme ile ölçülen endometrial hacim veya power Doppler indekslerinin benign ve malign endometrium arasında ayrım yapıp yapamadığını belirlemek.

**Gereç ve Yöntemler:** Mevcut tanının doğruluğu çalışması Ain Shams Üniversitesi Maternite Hastanesinde gerçekleştirildi. Postmenopozal kanamalı ve endometrium kalınlığı  $\geq$ 5 mm olan 84 hastada korpus uteri incelemesi için 3D power Doppler ultrasonografi yapıldı. Endometriumda vaskülarizasyon indeksi (VI), akım indeksi (FI) ve vaskülarizasyon akım indeksi (VFI) ile birlikte endometrium hacmi hesaplandı. Altın standart endometriumun histopatolojik tanısı idi.

**Bulgular:** Çalışmaya dahil edilen 84 kadının 56'sında (%66.7) benign endometrial lezyonlar ve 28'inde (%33.3) malign endometrial lezyonlar vardı. Endometrium kalınlığı, endometrium hacmi ve akım indeksleri (VI, FI ve VFI) benign endometriumu olanlara kıyasla malign endometriumlu hastalarda daha yüksekti. Alıcı işlem karakteristiği eğrisi altında kalan alan (AUC) endometrium kalınlığı için 0.83, endometrium hacmi için 0.73 ve en iyi power Doppler değişkeni olan FI için 0.93 idi. Maligniteyi öngörmede en iyi lojistik regresyon modeli AUC değeri 0.93 olan FI ve endometrial kalınlık değişkenlerini içermekteydi.

**Sonuç:** Benign ve malign endometrium arasındaki ayrım konusunda 3D görüntüleme ile ölçülen endometrial hacmin tanısal performansı 2D ultrasonografi ile ölçülen endometrial kalınlığınkinden daha üstün değildi, ama 3D power Doppler indeksleri endometrial karsinomu öngörmede iyi tanı araçlarıdır.

Anahtar kelimeler: Endometrial karsinom, postmenopozal kanama, power Doppler, 3-boyutlu ultrason

#### Özgün Araştırma

J Turk Ger Gynecol Assoc 2015; 16: 82-85 • DOI: 10.5152/jtgga.2014.36776

## İntrauterin inseminasyon zamanının semen parametrelerine etkisi

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## ÖΖ

**Amaç:** Bu gözlemsel çalışmada yıkama sonrası sperm inkübasyonunun başlangıcı ile intrauterin inseminasyon zamanı arası sürenin semen parametrelerine (konsantrasyon, motilite) etkisi olup olmadığı araştırılmıştır.

**Gereç ve Yöntemler:** Klinikte elde edilen 100 normozoospermik erkeğin semen örnekleri oda sıcaklığında 20 dakika likefaksiyon için bekletildi. Semen örnekleri makroskopik ve mikroskopik olarak incelendi. Gradyent ve sperm yıkama medyumları ile santrifüjden sonra örnekler inkübatöre kondu. Konsantrasyon ve motilite 30, 60 ve 120 dakika sonra kaydedildi.

**Bulgular:** Bonferroni post-hoc test sonuçlarına gore ortalama sperm sayısı (mil/mL), progresif sperm motilite yüzdesi (%) ve total motil sperm sayısı (mil) 30 ile 120 dakika (p=0.000, p=0.000 and p=0.000) ve 60 ile 120 dakika karşılaştırıldığında (p=0.000, p=0.000 ve p=0.001) anlamlı olarak farklıydı. Fakat 30 ve 60 dakika karşılaştırıldığında anlamlı fark yoktu (p=1, p=0.173, p=1).

**Sonuç:** Bu çalışma, semen parametrelerinin uzayan inkübasyon zamanından olumsuz etkilendiğini göstermektedir. Yıkama sonrası sperm inkübasyon başlangıcı ile IUI zamanı arası sürenin 60 dakika ile sınırlandırılması gebelik oranlarını yükseltebilir.

Anahtar kelimeler: IUI zamanı, sperm yıkama, semen parametreleri

J Turk Ger Gynecol Assoc 2015; 16: 86-91 • DOI: 10.5152/jtgga.2014.26932

# Melatoninin gebe olmayan rat uterusunda myoelektriksel aktivite üzerine çift yönlü etkileri

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ÖΖ

**Amaç:** Bu deneysel çalışmada, melatoninin gebe olmayan rat uterusunun myoelektriksel aktivitesi üzerine etkilerinin incelenmesi amaçlandı. **Gereç ve Yöntemler:** 46 dişi rat 6 gruba ayrıldı: (1) kontrol grubu; (0,2 ml %0.9 NaCL intravenöz (IV) verildi, n=6); (2) 0.4 mg/kg melatonin IV verildi (n=8); (3) 4 mg/kg melatonin IV verildi; (4) tek doz 100 mU/kg oksitosin IV verildi (n=8); (5) 0.4 mg/kg melatonin ile birlikte 100 mU/kg oksitosin IV verildi (n=8); (6) 4 mg/kg melatonin ile birlikte 100 mU/kg oksitosin IV verildi (n=8). Ratlara laparotomi yapıldı ve uterin myoelektriksel aktivite kaydedildi. Sinyallerin spektral içeriği üzerinden üzerinden ortalama sinyal tayfı gruplar arasında karşılaştırıldı.

**Bulgular:** Melatoninin doz bağımlı şekilde uterin myoelektriksel aktiviteyi arttırdığı saptandı. Oksitosin sonrası melatonin verilmesi ile ortalama sinyal gücünün baskıladığı görüldü. Melatonin verilen ratlarda serum melatonin konsantrasyonu anlamlı olarak yüksek bulundu.

**Sonuç:** Çalışmamıza gore her ne kadar oksitosin ile uyarılmış uterin elektriksel aktivite melatonin ile baskılansa da, melatonin doz bağımlı olarak uterin myoelektriksel aktiviteyi arttırmaktadır. Bu bulgular, melatoninin anormal uterin aktivite ile karakterize durumların tedavisi için muhtemel yararlı etkilerinin araştırılmasına yönelik ileri çalışmalar yapılmasını hak etmektedir.

Anahtar kelimeler: Uterin kontraksiyon, melatonin, oksitosin, elektriksel stimülasyon

#### Özgün Araştırma

J Turk Ger Gynecol Assoc 2015; 16: 92-95 • DOI: 10.5152/jtgga.2014.13005

# Uterin Leiomyoma etiopatogenezinde TWIST, SERPINB5 ve SERPIN1 genlerinin rolü

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## ÖΖ

**Amaç:** Bu çalışmanın amacı, uterin leiomyoma etiopatogenezinde Twist homolog 1 (TWIST), serin peptidaz inhibitör (SERPINB5) ve plazminojen aktivatör inhibitörü 1 (SERPIN1) genlerinin rolünü araştırmaktır.

**Gereç ve Yöntemler**: Çalışmaya yaşları 39 ila 58 arasında olan histerektomi yapılan 12 hasta dahil edilmiştir. Histerektomi sonrası ölçülen uterin leiomyoma doku boyutları 20 ila 130 mm arasında idi. Doku örnekleri histerektomi yapılan hastanın normal myometriyum ve leiomyoma dokusundan (1cm<sup>3</sup>) elde edilerek ve -86 ° C' de kullanıma kadar saklanmıştır. Materyaller histopatolojik değerlendirme sonucunda iki gruba ayrıldı; kontrol grubu (Grup 1), normal myometriyal dokusu ve çalışma grubu (Grup 2) uterin leiomyoma dokusu olarak kabul edildi. Uterin leiomyoma etiopatogenezi için TWIST, SERPINB5 ve SERPIN1genleri çalışıldı.

**Bulgular:** TWIST gen ekspresyonu leiomyoma grubunda anlamlı olarak daha yüksekti (p<0.001). SERPINB5 ve SERPIN1 genleri ekspresyonu uterin leiomyoma grubunda azalmış olarak bulundu. Ancak fark istatistiksel olarak anlamlı değildi.

**Sonuç:** TWIST gen aktivitesi uterin leiomyoma dokusunda normal myometriyum dokusuna göre artmıştır. Leiomyoma gelişimi, östrojen ve progesteron reseptörlerine bağlı olması dışında myometriyal hücrelerinde artmış TWIST gen aktivitesi tarafından da tetiklenebilir.

Anahtar kelimeler: Uterin leiomyom, etiopatogenez, TWIST, SERPINB5, SERPIN1

J Turk Ger Gynecol Assoc 2015; 16: 96-99 • DOI: 10.5152/jtgga.2014.0012

# Prenatal tanısı konulan izole musküler ventriküler septal defektlerin doğal seyri

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#### ÖΖ

**Amaç:** Bu çalışmanın amacı, izole musküler ventriküler septal defektin (m-VSD) antenatal dönem ve doğum sonrası bir yıllık takip sürecinde doğal seyri ve kromozomal anomalilerle ilişkisini değerlendirmektir.

Gereç ve Yöntemler: 2007 Ağustos- 2012 Temmuz tarihleri arasında tanı konulan toplam 76 izole m-VSD olgusu çalışmaya dahil edildi. İzole m-VSD olgularında defektin yerleşim yeri ve çapı, tanı sonrası antenatal dönem ve doğum sonrası bir yıllık takipte kapanma oranı, gebelik sonuçları, kromozomal anomali görülme oranı değerlendirildi.

**Bulgular:** 76 olgudan bir tanesi doğum sonrası kaybedilmiş, 33 olgu doğum sonrası dönemde takip edilememiştir. 44 olguda doğum sonrası bir yıllık takipte 3 olguda (6.8%) intrauterin dönemde, 33 olguda (75%) doğum sonrası ilk bir yılda defekt kapanmıştır. Olguların tümü değerlendirildiğinde apikal yerleşim gösteren defektde spontan kapanma daha sık olmasına rağmen, mid-musküler yerleşim gösteren defektle karşılaştırıldığında spontan kapanma oranı bakımından istatistiki bir fark saptanmamıştır (p>0.05). Çapı  $\leq$ 3 mm olan defektlerin %83.8'i (44 olgudan 36 tanesi) tanı sonrası antenatal dönem ve doğum sonrası ilk yılda kapanmıştır.

Sonuç: Doğum sonrası ilk yılda m-VSD'in spontan kapanma oranının yüksek olduğu sonucuna varılmıştır. Ayrıca küçük defektler çoğunlukla spontan kapanmaktadır.

Anahtar kelimeler: İzole, musküler ventriküler septal defekt, spontan kapanma

#### Özgün Araştırma

J Turk Ger Gynecol Assoc 2015; 16: 100-103 • DOI: 10.5152/jtgga.2014.0031

## Genetik amniyosentez sırasında kanama fetal Doppler parametrelerini etkiler mi?

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## ÖΖ

**Amaç:** Bu çalışmanın amacı amniyosentez sırasında iğne giriş yerinden kanama ile fetal Doppler parametreleri arasındaki ilişkinin araştırılması idi. **Gereç ve Yöntemler:** Bu prospektif çalışma, Temmuz 2010 ve Şubat 2011 tarihleri arasında yapılmıştır. Bu dönemde 215 amniyosentez uygulandı. Down sendromlu 5 hasta çalışma dışı bırakıldı. Kalan 210 hasta Grup1 (giriş yerinde kanama) ve Grup 2 (kontrol grubu) olarak ikiye ayrıldı. Bir iğne tipi tüm hastalar için kullanıldı. Umbilikal arter rezistif indeksi (UARI), umbilikal arter pulsatilite indeksi (UAPI), orta serebral arter rezistif indeksi (MCARI), orta serebral arter pulsatilite indeksi (MCAPI) ve orta serebral arter pik sistolik hız (MCAPSV) işlemden önce, ve işlemden hemen sonra ölçüldü.

**Bulgular:** Amniyosentez sırasında iğne giriş yerinde kanama UARI'i değiştirmedi (Grup 2 Grup 1 için % 34 artış ve % 46,5 artış, p = 0.238). MCARI (Grup 1 için% 52 artış ve Grup 2 için% 45 artış, p = 0,622), veya MCAPSV'de de işlem sonrasında gruplar arasında fark yoktu (Grup 1 için % 37 artış ve Grup 2 için % 49 artış , p = 0,199). UARI, MCARI, MCAPI ve MCAPSV için her iki grupta, amniyosentez öncesi ve sonrasında anlamlı fark saptanmamıştır. Sadece Grup 2'de amniosentez sonrası UAPI'de istatistiksel olarak anlamlı bir artış vardı.

Sonuç: Amniyosentez sırasında kanama umbilikal arter ve orta serebral arter Doppler parametrelerini etkilememektedir.

Anahtar kelimeler: Amniyosentez, kanama, fetal Doppler

J Turk Ger Gynecol Assoc 2015; 16: 104-108 • DOI: 10.5152/jtgga.2014.0088

# Pelvik Organ Prolapsuslu Kadınların Uterosakral Ligamanlarında Hoxa 11 ve MMP 2 Gen Ekspresyonu

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## ÖΖ

**Amaç:** Pelvik organ prolapsusu(POP) pek çok kadının yaşam kalitesini olumsuz etkileyen sık karşılaşılan bir problemdir. Uterosakral ligamanlar(USL) POP lu kadınlarda etkilenen pelvik organları destekleyen önemli yapılardır. HOXA genleri USL gelişimini kontrol ederler. Biz çalışmamızda POP olan ve olmayan kadınların USL da HOXA 11, MMP2 eksperyonunu karşılaştırdık.

**Gereç ve Yöntemler**: Zekai Tahir Burak Kadın Sağlığı Eğitim ve Araşlırma Hastanesinde prospektif kesitsel bir çalışma planlandı. Biz çalışmamızda POP olan( n:18) ve olmayan(n: 15) kadınların USL da HOXA 11, MMP2 ekspresyonunu karşılaştırdık. USL da TriPure ile RNA izolasyonu yapıldı. HOXA11 VE MMP2 ekspresyon sevyeleri semikantitatif RT PCR in Ligth Cycler 480 sistemi ile belirlendi. RT PCR reaksiyonu için real time ready catalog assays kullanıldı.

**Bulgular:** Yaş, parite, vucut kitle indexi ve menopozal durum açısından 2 grup arasında fark yoktu. Ortalama MMP 2 RNA ekspresyonu POP grubunda  $1.27 \pm 0.6$ , POP olmayan grupta  $0.75 \pm 0.4$  idi(p: 0.007). Ortalama HOXA11 RNA ekspresyonu POP grubunda  $2.57 \pm 2.4$ , pop OLMAYAN grupta  $1.94 \pm 1.4$  (p: 0.376). POP olan hastalar hafif ve ileri derece POP diye gruplandırıldığında HOXA 11 ve MMP2 ekspresyonunda gruplar arasında fark yoktu (>0.05)

**Sonuç:** POP ve kontrol grubu arasında USL HOXA 11 RNA ekspresyonunda fark olmamasına rağmen MMP 2 RNA ekspresyonunda anlamlı farklılık bulundu. Bu konuda sınırlı ve sonuçları tartışmalı çalışmalar mevcuttur. Daha geniş vaka sayılı çalışmalara ihtiyaç vardır. **Anahtar kelimeler:** HOXA11, MMP2, Pelvik organ prolapsusu

#### Derleme

J Turk Ger Gynecol Assoc 2015; 16: 109-121 • DOI: 10.5152/jtgga.2014.29795

# Servikal premalign lezyonlar ve yönetim

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## ÖΖ

Serviks kanseri; ulusal kanser taraması olan gelişmiş ülkelerde onuncu sırada olmasına rağmen, az gelişmiş ülkelerde ikinci sıradadır. Sağlık Bakanlığı verilerine göre Türkiye'de servikal kanser, kadın kanserleri arasında sekizinci sıradadır. Günümüzde, en etkili serviks kanseri taraması servikal smear almaktır. Bu nedenle, preinvazif lezyonların yakalanması ve invazif kansere ilerlemesinin engellenmesi için periyodik tarama programlarının önemi büyüktür. Tarama programlarını değiştirmeden human papilloma virüs (HPV) aşılarının kullanılması, serviks kanserini önleme açısından yeni bir anlayış getirmiştir. Preinvazif lezyonların yönetimi her kadın hastalıkları ve doğum uzmanının bilmesi gereken bir konudur. Bu aşamada gereksiz işlemlerin ve tedavilerin önlenmesi açısından hastaların doğru yönlendirilmeleri gerekir. Kanser fobisi oluşturulmamalıdır. Histolojik tanı olmadan ablatif veya destrüktif tedaviler yapılmamalıdır ve eşit rektirrens riski nedeniyle de histerektomi önerilmemelidir.

Anahtar kelimeler: Serviks, preinvazif lezyonlar, yönetim, human papilloma virüs

J Turk Ger Gynecol Assoc 2015; 16: 122-124 • DOI: 10.5152/jtgga.2014.53533

## Uterin leiomyosarkomanın rektus abdominalis kasına metastazı: Nadir bir vaka sunumu ve literatürün derlenmesi

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ÖΖ

Uterin leiomyosarkom agresif malign bir tümördür. Akciğer, tiroid, karaciğer, pancreas, kalp, duedonum, meme, vajina, submandibular bez ve kemik metastazları bildirilmiştir. Bizim olgumuz literatürdeki ilk rectus abdominalis metastazı olan uterin leiomyosarkom vakasıdır. 39 yaşında nulligravid pelvik ağrı şikayeti ile başvurdu. Hastanın yapılan fizik muayenesinde suprapubik bölgede yaklaşık olarak 6 cm'lik kitle ele geldi. Hastanın anamnezinde daha once uterin leiomyosarkom nedeni ile operasyon hikayesi vardı. İncelemeler sonucunda rektus abdominalis kası içerisinde  $4x5 \times 6$  cm boyutlarında kitle izlendi. Patolojik incelemede anormal mitozlar ve nekroz odakları izlendi ve uterin leiomyosakom metastazı tanısı aldı. Uterin leiomyosarkom uterin düz kas hücrelerinden köken alan malign bir tümördür. Hematojen yol ile iskelet kası metastazı çok nadir görülür. Biz burada literatürde ilk defa rastlanan, uterin leiomyosarkomu rektus abdominalis kasına metastazını sunduk. **Anahtar kelimeler:** Uterin leiomyosarkom, metastazı, iskelet kası, rektus abdominalis kası

#### Olgu Sunumu

J Turk Ger Gynecol Assoc 2015; 16: 125-127 • DOI: 10.5152/jtgga.2014.45389

## Renal transplantlı bir hastada güvenli vajinal doğum: Vaka takdimi

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ÖΖ

Renal transplantı olan komplike olmamış gebelerde vajinal doğum mümkündür. Bu vaka takdiminde renal transplantlı bir hastada güvenli vajinal doğum olgusunun sunulması ve bu tür vakalarda sezaryenin olası ek risklerinin vurgulanması amaçlandı. 40 yaşında (G4P3), 5yıl önce hipertansiyona bağlı gelişen son dönem böbrek hastalığı nedeniyle renal transplantasyon yapılan hasta 392/7 gebelik haftasında kliniğimize başvurdu. Transplantasyon sonrası böbrek fonksiyonları ve kan basıncı normal seyreden hastanın geçmiş ve şimdiki obstetrik hikayesinde özellik bulunmamakta idi. Fetal gelişim, amniyon sıvısı, umbilikal arter Doppleri de normal sınırlarda idi. Sağ iliak fossadaki transplante böbrek ultrasonda 1. derece hidronefroz dışında normal olarak değerlendirildi. Fakat, transplante böbrek ile iliak damarlar arasındaki vasküler anastomozların uterus alt segmentine yakınlığı dikkat çekmekte idi. Geçmiş cerrahi kayıtlardan renal venin eksternal iliak vene ve renal arterin internal iliak artere sağ tarafta anastomoz edildiği öğrenildi. Vajınal doğum için herhangi bir kontrendikasyon bulunmaması ve sezaryende transplante böbreğe zarar verme ihtimalinin olması nedeniyle hasta vajinal dinoproston uygulamasını takiben normal doğum için takip edildi. Sorunsuz bir vajinal doğumla 3540 gr sağlıklı bir kız bebek doğurtuldu. Renal transplantlı hastalarda renovasküler ve üreter anastomozlarının ve böbreğin yerleşimi dolayısıyla sezaryenle doğumda bu yapılara zarar verme ihtimali olabilir. Abdominal kompresyon uygulanmadan gerçekleştirilecek normal vajinal doğum renal transplantlı hastalardan uygun olanlarda en güvenilir doğum şeklidir. Plasenta akreata gibi internal iliak arter ligasyonu yada sezaryen histerektomi gerektirebilecek bir durum bekleniyorsa, bu müdahaleler transplante böbreğin anastomozları için riskli olabileceğinden, cerrahi renal transplant cerrahının da ameliyata katılabileceği bir merkezde yapılmalıdır.

Anahtar kelimeler: Anastomoz, doğum, hasar, renal, transplant