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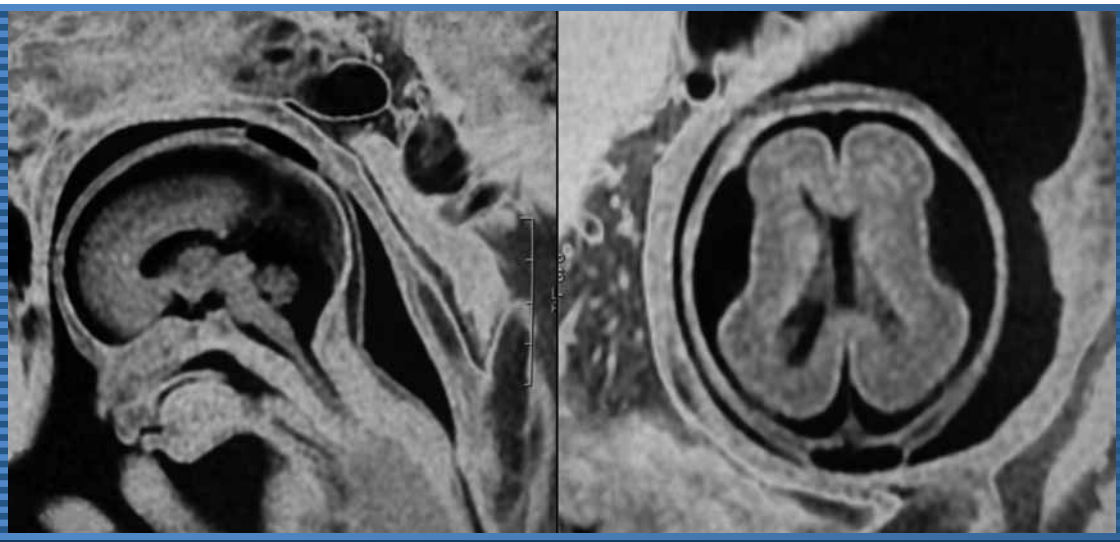

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Journal of the Turkish-German Gynecological Association is the official publication of the Turkish-German Gynecological Education and Research Foundation and Turkish-German Gynecological Association and is published quarterly on March, June, September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

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

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Contents

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Editorial

Dear colleagues,

I am delighted to present you to the third issue of the "Journal of the Turkish German Gynecological Association (JTGGA)" in the publishing year of 2013. Since the last few years, our objective was to collect more research studies and articles from Turkey and the international gynecology and obstetrics community. In this regard, I am glad that we have reached our objective as we are getting much more submissions in comparison with the previous years.

As a result of years of intensive work and great progress in the quality of our journal, I am very proud to inform you that our journal has been approved to be indexed by **PubMed Central (PMC)**. As you already know, PMC is a free full-text archive of biomedical and life sciences journal literature at the U.S. National Institutes of Health's National Library of Medicine (NIH/NLM). We are in the final phase of the technical process and you will be able to display the JTGGA in the online library of the PMC quite soon.

In this issue, we are dealing with very interesting research articles and case reports. You will read an attractive paper showing the importance of simulation training box for surgical skill improvement. This study confirmed the positive effect of low cost box model training on laparoscopic skill acquisition as assessed using LapSim. Novice surgeons should obtain practice on box trainers and teaching centers should make efforts to establish training laboratories. Another review is published to evaluate the effects of the drugs on metabolic parameters in women with PCOS. As you already know, Polycystic Ovary Syndrome (PCOS) is the most common endocrine disorder among women of reproductive age. It is mentioned in the article that, the first step in the treatment of the symptoms related to PCOS includes lifestyle changes and weight loss, if the patient is obese. Medical treatment included Combined Oral Contraceptives (COC), spironolactone, finasteride, flutamide, metformin and combinations of these treatments. Most of the anatomical, physiological and neurochemical genderrelated differences in the brain occur prenatally. You will find a review that aim to evaluate the anatomical, physiological and neurochemical differences in the female and male brains and to assess the effect of prenatal exposure to sex steroid hormones on the developing brain. Besides, some interesting articles on ART techniques and gynecological oncology field can be found in this particular issue. Please also enjoy solving a challenging quiz.

I would like to remind you that **2nd International Research Awards on Obstetrics & Gynecology** will be granted with a total of **$ 10.000** to three researchers or research groups who have been able to carry out the best researches in the field of Obstetrics & Gynecology or related subjects and submit them to the Journal of Turkish German Gynecology Association (JTGGA) by the online submission system at **www.jtgga.org**. The deadline for Manuscript Submissions is ***February 21st, 2014***.

The website of our foundation – Turkish German Gynecological Education and Research Foundation (TAJEV) has been renovated with a more contemporary format with additional content. I strongly suggest you to have a glance at the **www.tajev.org** for general information and up-to-date advances about our foundation, an actual congress calendar of the national and international Ob&Gyn meetings and events, and much more. The web site of our journal is also about to be changed to a more user friendly-format with richer content in the near future.

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

Best regards,

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

Assessment of early decline in the percentage of β-hCG values between days 0 and 4 after methotrexate therapy in ectopic pregnancy for the prediction of treatment success

Ektopik gebelikte uygulanan metotreksat tedavisinden sonra 0 ile 4. günler arasında β-hCG değerlerindeki yüzde düşüşlerinin tedavi başarısını belirlemedeki yeri

Ebru Çelik, Ilgın Türkçüoğlu, Abdullah Karaer, Pinar Kırıcı, Sevil Eraslan

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Abstract

Objective: To evaluate percentage changes in serum beta-human chorionic gonadotropin (β-hCG) values between days 0-1, 0-4 and 0-7 as an indicator of methotrexate therapy success in extra-uterine pregnancies.

Material and Methods: Women with ectopic pregnancy treated with single-dose methotrexate therapy between January 2011 and August 2012 were assessed. Recorded data were reviewed electronically from patient files. All women (n=93) with an ectopic pregnancy treated medically with intramuscular methotrexate (50 mg/m^2) were included. The percentage changes in serum β-hCG levels from day 0 to 1, day 0 to 4 and day 0 to 7 were calculated for each case.

Results: The median β-hCG values decreased between days 0 and 4 by 55.8%, and 89.6% of these cases were treatment successes. The median initial serum β-hCG values were lower in women with successful treatment, but this was not statistically significant (p=0.11).

Conclusion: A decline in serum β-hCG values between days 0 and 4 appears to be the best predictor. It would be beneficial to determine whether a woman with an ectopic pregnancy treated with single-dose methotrexate administration will be treated successfully.

(J Turkish-German Gynecol Assoc 2013; 14: 125-9)

Key words: Ectopic pregnancy, human chorionic gonadotrophin, methotrexate.

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Özet

Amaç: Ektopik gebeliklerin tedavisinde methotraksat tedavisinin başarısını belirlemede beta-human koryonik gonadotropin (β-hCG) değerlerinin 0-1, 0-4 ve 0-7 günleri arasında yüzde değişimlerini değerlendirmek.

Gereç ve Yöntemler: Ocak 2011 ile Agustos 2012 tarihleri arasında ektopik gebelik nedeniyle tanı alan ve tek doz metotreksat tedavisi alan 93 olgu çalışmaya dahil edildi. Elektronik ortamda kaydelilen hastaların dosyalarında bulunan tüm bilgiler incelendi. 0-1, 0-4 ve 0-7 günler arasındaki serum β-hCG seviyelerindeki değişim yüzdeleri her olgu için hesaplandı.

Bulgular: Metotreksat tedavisinin başarılı olan olguların %55.8 ve %89.6'da ortanca β-hCG değerleri 0 ve 4. günler arasında azalmış olarak bulundu. Tedavi öncesindeki ortanca β-hCG seviyesi tedavinin başarılı olduğu olgularda daha düşük saptanmasına rağmen iki grup arasında istatistiksel olarak bir fark saptanmadı (p=0.11).

Sonuç: Serum β-hCG değerlerindeki 0 ile 4. günler arasındaki düşme olması metoteksat tedavisinin başarısını tahmininde en iyi gösterge olarak ortaya çıkmaktadır, ve tek doz metotreksat ile tedavi edilen ektopik gebeliklerin nihai tedavinin başarısını belirlemek yararlı olacaktır. (J Turkish-German Gynecol Assoc 2013; 14: 125-9)

Anahtar kelimeler: Ektopik gebelik, human koryonik gonadotropin, metotreksat

Geliş Tarihi: 8 Nisan 2013 **Kabul Tarihi:** 26 Mayıs 2013

Introduction

Ectopic pregnancy (EP), with high maternal morbidity and mortality, is a real health problem. Its incidence is approximately 2% of all pregnancies (1). Early diagnosis of ectopic pregnancy and the option of conservative treatment have become possible by the use of sensitive human chorionic gonadotropin (hCG) and high-resolution transvaginal ultrasonography (2, 3). Methotrexate (MTX), a folic acid antagonist, is a common alternative treatment to surgery and has been confirmed to be effective, safe and cost-effective (4). Recruitment of eligible patients for MTX treatment is a key point to improve therapeutic response and reduce treatment failure. The single-dose MTX protocol is the most frequently used protocol, with a reported success rate of 65% to 94%, as is associated with minimal toxic side effects, few complications and better compliance of patients (5-7).

Several variables, including gestational sac size, fetal cardiac activity, free fluid in the peritoneal cavity, serum progesterone and β-hCG levels, have been used as indicators for the predic-

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tion of MTX treatment success. The serum β-hCG level before MTX administration has been reported as a particular factor contributing significantly to treatment failure (8). Previously, it has been found that at least a 15% reduction in the β-hCG concentration on the seventh day compared to the fourth day after MTX administration is the best predictor of treatment success, with a positive predictive value of approximately 93.0% (9). A rise in the β-hCG titer on the fourth day after MTX compared to the pretreatment titer occurs in 50 to 70% of patients after MTX injection. However, this rise is not associated with the persistence of trophoblastic tissue (6). Furthermore, absolute measurements of β-hCG titers on day 4 after MTX administration have not been identified to be an anticipating factor of success of single-dose therapy (10).

Given the discrepancy between the results of previous publications, the aim of the current study was to determine whether percentage changes in serum beta-human chorionic gonadotropin (β-hCG) values between days 0-1 and 0-4 are an indicator of methotrexate therapy success in ectopic pregnancy.

Material and Methods

The current study was a cross-sectional retrospective study, conducted on 93 consecutive ectopic pregnancies managed medically with methotrexate at İnönü University School of Medicine, Turgut Özal Medical Centre, between January 2011 and August 2012. Ethical approval was obtained from the institutional ethical committee of İnönü University. Recorded data were reviewed electronically from the files of patients. All women with an ectopic pregnancy treated medically with intramuscular methotrexate (50 mg/m^2) met the following inclusion criteria: 1) no clinical suspicion of active intra-abdominal bleeding at the time of presentation 2) documented ultrasound diagnosis of extra-uterine pregnancy 3) the absence of trophoblastic tissue in the uterine cavity by pathological examination 4) subjects with β-hCG values on days 0, 4 and 7 appropriately measured after medical treatment. Exclusion criteria included 1) incomplete records and patients who were lost to follow-up evaluation, 2) hemodynamic instability, 3) signs of acute abdomen and 4) abnormal hematologic, renal or hepatic laboratory values.

Only 93 of women who were managed with single-dose methotrexate therapy were eligible for the study; 38 had expectant management, 116 underwent surgery and 6 patients given medical therapy were excluded due to incomplete records. The demographic characteristics (age, gravidity, parity and abortus), physical examination (ectopic mass size calculated as the mean of the largest diameter and the diameter perpendicular to it) and laboratory values before and after treatment (serum β-hCG levels on days 0, 1, 4 and 7 after MTX injection) were collected by reviewing the medical charts of patients from the hospital. The success of medical treatment was defined as at least a 15% decrease in the serum β-hCG level on day 7 compared to day 4 after MTX treatment. Successfully treated patients were followed weekly by assessing serum β-hCG levels until this decreased to a value <5 mIU/mL. Treatment failure was defined as the need for either a second methotrexate injection or surgical removal of the ectopic pregnancy mass due to less than a 15% decrease in the β-hCG level on day 7 compared to day 4.

The serum β-hCG titer at the time of MTX administration was defined as the baseline value. Thus, day 1, 4 (hCG4) and 7 (hCG7) measurements were carried out after methotrexate administration. The percentage change in the serum β-hCG level from day 0 to 1, day 0 to 4 and day 0 to 7 were calculated for each case as: (the latter β-hCG level minus the former β-hCG level) divided by the former β-hCG level and then multiplied by 100.

Statistical analysis

Differences between mean and median values were compared by Student's t-tests for the continuous variables. Categorical variables, presented as numbers and percentages, were compared using the Chi-square test between the groups. Results are expressed as median and interquartile range (IQR) or percentage and number, as appropriate. The cutoff points for defining altered percentages of β-hCG levels that predict the success of MTX treatment were determined by performing receiver operator characteristic (ROC) curve analysis. A probability value <0.05 was considered statistically significant throughout the statistical analysis.

Results

The demographic features and clinical findings are presented in Table 1. The median and IQR of age was similar in both groups ($p=0.48$). There were also no significant differences between the groups in terms of obstetric history such as gravidity, parity and abortion ($p=0.69$, $p=0.70$, and $p=0.67$, respectively). In the successful group, the mean size of the mass was smaller than in the failure group ($p=0.05$).

Methotrexate treatment in patients with ectopic pregnancy was successful in 71 cases (76.3%), but the medical treatment failed in 22 cases (23.7%). Of these 22 cases, 14 cases underwent surgery due to acute abdominal pain or rupture of the ectopic gestational sac, and a second dose of methotrexate was administered to eight cases. The median β-hCG values rose between days 0 and 1 in 54.3% of cases, between days 0 and 4 in 63.4%, and between days 0 and 7 in 85.1% of cases. In cases with decreased β-hCG levels between days 0 and 4, the success rate of treatment was 88.2%. Figure 1 presents the course of mean serum β-hCG values for each group.

Although the median and IQR of the initial β-hCG values measured before MTX administration was lower in the successfully treated group in comparison with patients with treatment failure, the difference was not statistically significant ($p=0.11$). A considerable difference in the median β-hCG value was noted on the first day ($p=0.03$), the fourth day ($p=0.01$) and the seventh day after MTX administration ($p=0.007$). In the success group, 47 cases (66.2%) had an initial β-hCG value below 2000 mIU/mL, while in the treatment failure group, 13 cases (59.1%) had levels below 2000 mIU/mL ($p=0.54$). The median and IQR of serum β-hCG concentrations and percentage changes are presented in Table 2.

Table 1. Comparison of demographic features and β-hCG variables in the two groups

Baseline characteristics	Methotrexate treatment (n=93)		p value
	Success group (n=71)	Failure group (n=22)	
Age, years	29 (25-33)	30.5 (27-32)	0.48
Gravidity	2 (1-3)	2 (1-4)	0.69
Parity	1 (0-2)	1 (0-2)	0.70
Endometrial thickness, mm	9.1 (5.7-13.5)	10.0 (7.1-13.0)	0.75
Extrauterine mass, mm	17.5 (14.0-25.0)	24.0 (16-34)	0.05*
Free fluid on transvaginal ultrasonographya, n (%)	26 (36.6%)	10 (45.4%)	0.42
Fetal heart activity present, n (%)	2 (2.8%)	2 (9.1%)	0.25

Values are stated as the median and IQR as otherwise was noted. [a]This information was available for only 86 of 93 patients. [b]This information was available for only 83 of 93 patients. *P value is statistically significant

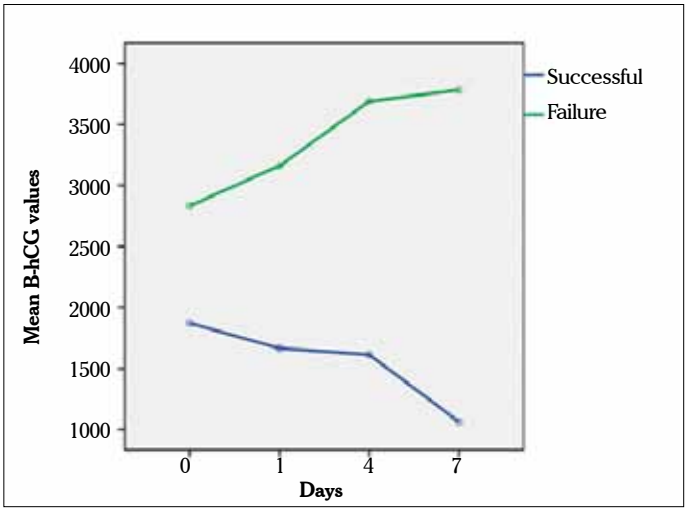

Figure 1. The mean values of serum β-hCG plotted against days after methotrexate injection in the two groups.

In the successful MTX group, the percentage decrease in serum β-hCG values between days 0 and 1, days 0 and 4 and days 0 and 7 were 54.3%, 63.4% and 85.1% of cases, respectively. The entire data set was divided into three groups: 1) the percentage changes in serum β-hCG values between days 0 and 1, 2) the percentage changes in serum β-hCG values between days 0 and 4, and 3) the percentage changes in serum β-hCG values between days 0 and 7. The results of the ROC curve analysis indicate that Group 1 had an area under the curve of 0.63 (95% confidence interval (CI), 0.49-0.76; p=0.09), with a sensitivity of 52.9%, specificity of 65.0%, positive predictive value (PPV) of 45.6% and a negative predictive value (NPV) of 55.2% in terms of identifying which patients were successfully treated with a single dose of MTX. The results of the ROC curve analysis indicate that Group 2 (≥1.36%) had an area under the curve of 0.69 (95% CI, 0.58-0.82; p=0.007), with a sensitivity of 61.8%, specificity of 70.0%, PPV of 47.6% and NPV of 53.1% in terms of identifying which patients were successfully treated with a single dose of MTX. The results of the ROC curve analysis indicate that Group 3 (≥0.18%) had an area under the curve of 0.79 (95% CI, 0.68-0.91; p<0.001), with a sensitivity of 83.8%, specificity of 60.0%, PPV of 58.9%, and NPV of 41.7% in terms of identifying which patients were successfully treated with a single dose of MTX. Furthermore, using the ROC curves, the calculated threshold percentage change between day 0 and 4 of -9.088% had a sensitivity of 67.6%, specificity of 70%, NPV of 49.8% and PPV of 50.8% in terms of identifying which patients failed treatment. The analysis of logistic regression of the value of this cutoff showed an increased risk of treatment failure with an OR of 4.94 (95% CI 2.67-9.13). The results of the ROC curves are presented in Figure 2.

Discussion

The overall success rate of methotrexate treatment in this study was 76.3%, which is essentially in accordance with that reported by the two previous reports (11, 12). In the present study, a 63.4% probability of treatment success was found to be related to a rise in serum β-hCG values between days 0 and 4, which is similar to that indicated by Nguyen et al. (11). However, others have noted lower success rates in that population (13).

Several studies have attempted to ascertain indicators of success after medical treatment of EP (2, 14, 15). Different markers and features of EP have been studied to predict the outcome of treatment approaches (6, 16, 17). Yet, the best predictive marker of medical treatment success for EP has clearly been identified. A recent meta-analysis suggested that the initial β-hCG level is the most important predictor of MTX treatment success in EP (18). Although it did not reach a statistically significant level, the median initial β-hCG concentration was correlated with the treatment success rate. Undoubtedly, women with a declining trend in the β-hCG level benefited from methotrexate treatment for EP. The results of this study are in accordance with previous reports (11, 19-21). Stika et al. (13) demonstrated that the pretreatment β-hCG concentration was higher in women with treatment failure than those who were successfully treated with a single dose of MTX. A recent study including 238 patients also found that the initial β-hCG value is the most important predictor of the outcome of treatment (22).

The findings of this study mainly confirm the results of Skubisz et al. (12), who found that an early decline in serum the β-hCG

Table 2. Comparison of serum β-hCG values in the two groups

Baseline characteristics	Methotrexate treatment (n=93)		p value
	Success group (n=71)	Failure group (n=22)	
Initial β-hCG levels	1341.5 (647.0 – 2294.8)	1595.0 (570.7 – 3587.3)	0.11
β-hCG levels on the first day after MTX administration, mIU/mL	1413.5 (637.8 – 2164.0)	1702.5 (782.3 – 3380.5)	0.03*
β-hCG levels on the fourth day after MTX administration, mIU/mL	870.5 (483.5 – 2220.0)	2268.5 (526.3 – 6137.5)	0.01*
β-hCG levels on the seventh day after MTX administration, mIU/mL	537.0 (202.5 – 1514.3)	2299.5 (431.3 – 7274.8)	0.007*
The percentage difference in β-hCG levels between day 0 and 1	1.09 (-11.12 - 22.34)	-4.07 (-15.24 - 7.62)	0.02*
The percentage difference in β-hCG levels between day 0 and 4	12.77 (-19.06 - 49.57)	-31.16 (-60.73 - 12.34)	0.002*
The percentage difference in β-hCG levels between day 0 and 7	42.31 (19.44 - 79.64)	-18.26 (-68.16 - 19.44)	0.001*

Values are stated as median and IQR. *P value is statistically significant. MTX: Methotrexate

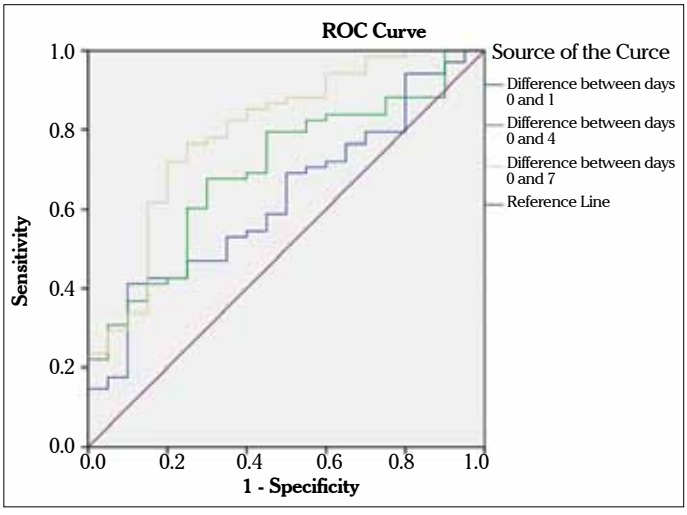

Figure 2. The ROC curves of percentage changes in serum β-hCG values between days 0-1, 0-4 and 0-7.

value between days 0 and 4 was associated with a high rate of treatment success (88%). However, in the success group, 66.2% of patients had an initial β-hCG value below 2000 mIU/mL, and these cases would have undergone spontaneous resolution if they had been treated with expectant management. Considering our similar success rate (89.6%), we support the notion that an early decline in the serum β-hCG value is correlated with likely treatment success. In view of this similar rate, it appears to be unnecessary to measure β-hCG levels on the first day after MTX administration.

A previous study conducted by Agostini et al. (19) evaluated the use of early β-hCG percentage changes between days 1 and 4 after methotrexate administration as an indicator of treatment success. That study noted that an hCG index value of 0.2 (20% decline) was associated with a very high probability of eventual treatment success, with a positive predictive value of 97%. However, the present study indicated a lower success rate (75.1%) in women with a decline in β-hCG percentage between days 1 and 4 after MTX administration. The explanation for this discrepancy may be that the sample size of the present study small.

One advantage of this study is that the data are applicable. The overall success rate of single-dose methotrexate therapy in this study was 76.3%, which is similar to that reported in the literature, 65-96% (5-7, 11). Our study mostly confirms the results of previous studies (19, 21, 22) that noted a rate of treatment success of 88-100% in women with a decline in β-hCG between days 0 and 4, which is similar to our result (88.2%). Taken together, an early decline in serum β-hCG appears to be associated with a very high likelihood of successful treatment outcome. However, considering the 88.2% success rate, not 100%, it would be logical to monitor serial β-hCG values subsequent to MTX administration until the ectopic pregnancy is completely resolved.

In conclusion, β-hCG difference variables may contribute to the prediction of persistent disease. Early detection of resistant disease can be managed promptly. We have found that a decline in serum β-hCG between days 0 and 4 is associated with a high rate of treatment success. The change in the serum β-hCG value between days 0 and 4 appears to be a reliable early indicator of the likely eventual outcome of medical therapy and contributes to identifying which patients have been successfully treated with a single dose of MTX.

Ethics Committee Approval: *This study received ethics approval from İnönü University Ethics Committee on 07.12.2010 (reference number: 2010/128).*

Informed Consent: *N/A*

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Author contributions: *Concept – E. Ç., I.T., A.K.; Design - E.Ç., I.T., A.K.; Supervision - E.Ç., I.T., A.K.; Resource - E.Ç., I.T., A.K.; Materials - E.Ç., I.T., A.K.; Data Collection&/or Processing - E.Ç., P.K., S.E.; Analysis&/or Interpretation - E.Ç., I.T., A.K.; Literature*

Search - E.Ç., P.K., S.E.; Writing - E.Ç.; Critical Reviews - E.Ç., I.T., A.K.

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Does Aquaporin-1 expression have clinical significance in serous epithelial ovarian cancer?

Aquaporin-1 ekspresyonunun seröz epitelyal over kanserinde klinik önemi var mıdır?

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Abstract

Objective: To assess the relationship between Aquaporin-1 (AQP1) expression and clinicopathological variables in serous epithelial ovarian cancer (EOC).

Material and Methods: Serous EOC cases treated in our institution between January 2007 and December 2009 were included in the study. A semi-quantitative immunohistochemical method was used to determine AQP1 expression levels, intratumoral microvessel density (IMD) and AQP1/IMD ratios. The relationship between these parameters and clinicopathological variables were assessed. P values less than 0.05 was considered statistically significant.

Results: A total of 55 cases of serous EOC were included in the study. AQP1 was strongly expressed in the membranes of microvessels and small vessels within all tumor tissues. In a few cases, AQP1 expression was also observed in the membrane of interstitial cells and in individual tumor cells. A positive correlation was detected between preoperative CA125 levels and the expression of AQP1 (R: 0.277, $p<0.05$). AQP1 expression was similar between FIGO stage I-II and FIGO stage III-IV cases ($p > 0.05$). A significant relationship did not exist between AQP1 expression and FIGO stage, lymph node metastasis or ascites volume ($p>0.05$).

Conclusion: In this study, AQP1 expression did not have a significant association with important clinicopathological variables in serous EOC. Future studies are needed to determine AQP1 expression in other histological types of EOC.

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Key words: Aquaporin-1, intratumoral microvessel density, serous epithelial ovarian cancer

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Özet

Amaç: Seröz epitelyal over kanserlerinde (EOK) Aquaporin-1 (AQP1) ekspresyonunun klinik ve patolojik değişkenlerle ilişkisini araştırmaktır.

Gereç ve Yöntemler: Kurumumuzda Ocak 2007 ile Aralık 2009 tarihleri arasında tedavi edilen seröz EOK olguları çalışmaya dahil edildi. AQP1 ekspresyonları ile intratümöral mikrodamar dansitesi (IMD) ve AQP1/IMD oranlarının belirlenmesinde semikantitatif immünhistokimyasal bir metod kullanıldı. Bu parametreler ile klinik ve patolojik değişkenlerin ilişkisi incelendi. P değeri 0.05'den küçük olduğunda istatistiksel olarak anlamlı kabul edildi.

Bulgular: Çalışmaya toplam 55 seröz EOK olgusu dahil edildi. Tüm olgularda, tümör dokusu içindeki mikrodamarlar ve küçük damarların membranlarında güçlü AQP1 ekspresyonu saptandı. Birkaç olguda, interstisyel hücre membranları ve tümör hücrelerinin içinde de AQP1 ekspresyonu gözlendi. Preoperatif CA125 düzeyleri ve AQP1 ekspresyonu arasında pozitif korelasyon saptandı (R: 0.277, $p<0.05$). FIGO evre I-II ve evre III-IV olgular arasındaki AQP1 ekspresyonu benzerdi ($p>0.05$). AQP1 ekspresyonu ile FIGO evresi, lenf nodu metastazı ve asit volümü arasında anlamlı bir ilişki saptanmadı ($p>0.05$).

Sonuç: Çalışmamızda seröz EOK'de AQP1 ekspresyonu ile önemli klinik ve patolojik değişkenler arasında anlamlı bir ilişki saptanmamıştır. EOK'nin diğer histolojik tiplerindeki AQP1 ekspresyonunun belirlenebilmesi için gelecek çalışmalara ihtiyaç vardır.

(J Turkish-German Gynecol Assoc 2013; 14: 130-5)

Anahtar kelimeler: Aquaporin-1, intratümöral mikrodamar dansitesi, seröz epitelyal over kanseri

Geliş Tarihi: 15 Nisan 2013 **Kabul Tarihi:** 16 Mayıs 2013

Introduction

Epithelial ovarian cancer (EOC) is the most lethal gynecologic cancer, with a five-year overall survival of approximately 31 to 53%, considering all stages (1-3). Generally accepted clinicopathological variables that may have an impact on survival are tumor histology, disease stage, patient age, performance status of the patient, presence of ascites, residual tumor burden after surgery and preoperative CA-125 level (4-16).

Aquaporins are a group of homologous water channel proteins, which are expressed in various tissues (17-19). Aquaporin-1 (AQP1) has important functions in various organs such as the kidney, central and peripheral nervous system, eye, lacrimal glands, salivary glands, lungs, pleura, gastrointestinal system, female and male reproductive system, inner ear and skin.

Considering its function in angiogenesis, the possible role of AQP1 in carcinogenesis, tumor progression and metastasis ability has been an area of interest (20, 21). Its distribution

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and expression intensity on various cells may have an effect on transvascular fluid flow and tumor cell fluid transport in EOC. A previous study has demonstrated that there is a strong positive correlation between AQP1 expression and intratumoral microvessel density (IMD) in EOC (22). In the present study, we evaluated AQP1 expression in serous EOC, in order to determine its association with clinical and pathological variables of prognostic significance.

Materials and Methods

The study was performed at the gynecologic oncology department of Başkent University School of Medicine, Ankara, Turkey, following scientific and ethical approval from the institutional review board. Tumor specimens were collected from 55 cases diagnosed with primary serous EOC between January 2007 and December 2009. All of the cases had undergone primary debulking surgery without previous neoadjuvant chemotherapy. All of the patients underwent primary debulking surgery, and optimal cytoreduction was possible in 39 (70.9%) cases. Data including patient age, disease stage, tumor grade, preoperative CA-125 level, ascites volume, ascites cytology, lymph node metastasis, lymphovascular space invasion and Gynecologic Oncology Group (GOG)/Eastern Cooperative Oncology Group (ECOG) performance status were recorded for each case (23). All of the study specimens were selected from primary tumor tissues within the ovary. Following initial examination of hematoxylin and eosin stained slides, the most appropriate sections were selected for immunohistochemical analysis. AQP1 expression and IMD (CD34 counts) were determined using a semi-quantitative immunohistochemical method.

Formalin-fixed, paraffin-embedded 4 μm-thick tissue sections were de-paraffinized with xylene and rehydrated with graded alcohols. Endogenous peroxidase was blocked with 10% hydrogen peroxidase and antigen retrieval reaction was carried out by boiling in 10 mM sodium citrate (pH 6.0) buffer for 90 seconds. Normal non-immune serum was applied to reduce non-specific binding. Samples were then incubated with AQP1 primary antibody (1:500 dilution, clone: 1/A5F6 monoclonal Ab, GeneTex Inc., CA,USA) and CD34 primary antibody (1:100 dilution, clone: Q Bend/10, mouse monoclonal Ab, ScyTek Laboratories, UT, USA) at room temperature for 60 minutes, and then with appropriate secondary antibodies (PicTure™ Kits, Zymed Laboratories, NY, USA) at room temperature for 30 minutes. Visualization of the reaction with diaminobenzidine was performed and the slides counterstained with hematoxylin. The negative control was carried out substituting phosphate buffered saline (PBS) for the primary antibody. Positive controls were prepared with AQP1 reactive kidney tissue.

IMD scores were assessed by immunostaining for CD34 as described by Weidner et al. (24). Immune-stained sections were initially assessed in low magnification (x40). Within the tumor or adjacent tissue, areas with the highest number of highlighted microvessels, i.e. “hot spots” were identified. Subsequently, the IMD score was determined by counting all vessels at high magnification (x400). Determination of the staining reaction was strictly limited to the hot spot area. Every sin stained lumen was recorded as one countable microvessel. Single positive cells without a visible lumen were also were regarded as a single microvessel. Two independent pathologists performed the immunohistochemical analyses. The average value of the results from both pathologists was used for all subsequent calculations. If more than 30% discordance was present between these two values, the slides were re-evaluated by both pathologists to calculate a final value. AQP1 expression scores within tumor microvessels were evaluated in the same manner. As the AQP1 expression had variance between cases and within the various hot spots, the AQP1/IMD ratio was used to represent the expression levels of AQP1, in an effort to prevent error caused by this variation.

Study data were analyzed using the Statistical Package for Social Sciences (SPSS) version 17.0 (SPSS Inc., Chicago, IL, USA). The associations were determined between clinical and prognostic variables (patient age, FIGO stage, tumor grade, ascites volume, ascites cytology, lymph node involvement status, GOG/ECOG performance status, lymphovascular space invasion (LVSI) status, preoperative CA 125 level) and AQP1 expression, IMD and the AQP1/IMD ratio. T-tests were used to compare values between two groups, and one-way analysis of variance (ANOVA, Kruskal-Wallis post hoc test) was performed when three or more groups were present. Correlations were determined by calculating the Pearson correlation coefficients. P values less than 0.05 were considered statistically significant.

Results

A total of 55 serous epithelial ovarian cancer (EOC) cases were included in the study. The mean patient age was 56.6±10.8 (minimum 31, maximum 83). Fifty (90.9%) cases had advanced stage (FIGO III-IV), whereas five (9.1%) cases had early stage (FIGO stage I-II) disease. In the histopathological examination, 46 (83.6%) cases had grade 3, and 9 (16.4%) cases had grade 2 tumors. The clinical and prognostic parameters of the study cases are presented in Table 1.

Aquaporin-1 (AQP1) protein was strongly expressed in the membrane of microvessels and small vessels in all primary serous EOCs (Figure 1). AQP1 expression was also observed in the membrane of interstitial cells in tumor tissue (Figure 2). In two cases, AQP1 was expressed in tumor cell membranes (Figure 3). AQP1 expression was not observed in the cytoplasm of tumor cells.

Mean AQP1 expression levels, intratumoral microvessel density (IMD) expression levels and AQP1/IMD ratios in relation with clinical and prognostic parameters are presented in Table 2.

There was a positive correlation between the preoperative CA 125 level and AQP1 expression (R: 0.277, p=0.03). There was no statistically significant difference for AQP1 expression between the FIGO stage I-II and FIGO stage III-IV groups, nor between the grade 2 and grade 3 tumor groups (p=0.24). There was also no correlation between AQP1 expression and IMD. A statistically significant difference was not present between cases grouped for ascites volume, ascites cytology, lymph node metastasis, lymphovascular space invasion (LVSI), preoperative CA 125 level or performance status for AQP1 expression, IMD expression and the AQP1/IMD ratio (p>0.05) (Table 2).

Table 1. Clinical and prognostic parameters of the study cases

Parameters	Cases (Total n=55)	Percentage (%)
FIGO stage		
I-II	5	9.1
III-IV	50	90.9
Tumor grade		
Grade 1	0	0
Grade 2	9	16.4
Grade 3	46	83.6
Preoperative CA 125 level (U/mL)		
< 35	1	1.8
35-499	22	40.0
≥ 500	32	58.2
Ascites volume (mL)		
< 500	17	30.9
500-999	10	18.2
≥ 1000	28	50.8
Ascites cytology		
Negative	24	43.6
Positive	31	56.4
Lymph node metastasis		
Absent	18	32.7
Present	37	67.3
LVSI[2]		
Absent	23	41.8
Present	32	58.2
GOG/ECOG[3] performance status		
1	13	23.6
2	10	18.2
3	21	38.2
4	11	20.0

FIGO: International Federation of Gynecology and Obstetrics; LVSI: Lymphovascular space invasion; GOG/ECOG: Gynecologic Oncology Group/ Eastern Cooperative Oncology Group.
P values <0.05 were considered statistically significant

Discussion

Aquaporins were initially identified nearly 20 years ago (25). Most of these proteins are expressed in epithelial and endothelial cells, where they regulate trans-membranous fluid transport (17-19). AQP1 is most strongly expressed in the microvascular endothelium, in normal tissues such as the brain, cornea and intestinal lacteals (19). In a previous study, it was demonstrated that microvessel AQP1 expression in brain tumors was associated with increased water permeability of the blood brain barrier, which ultimately resulted in brain tumor edema in these cases (26, 27). In another study, increased expression of AQP1 was demonstrated in all stages of colon cancer (28).

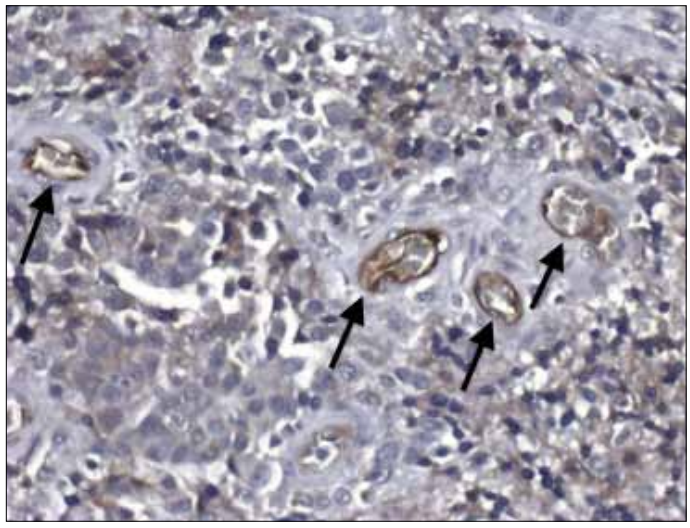

Figure 1. Strong Aquaporin-1 (AQP1) staining in membranes of microvessels within the tumor (*black arrows*; X 400)

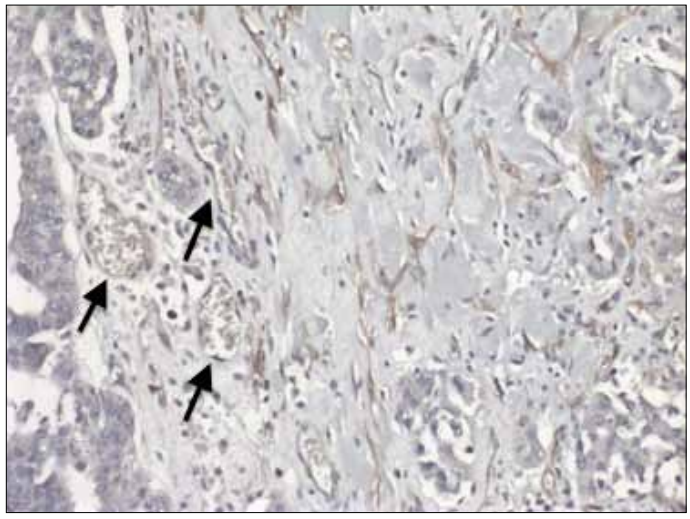

Figure 2. Aquaporin-1 (AQP1) staining in membranes of interstitial cells (*black arrows*; X400)

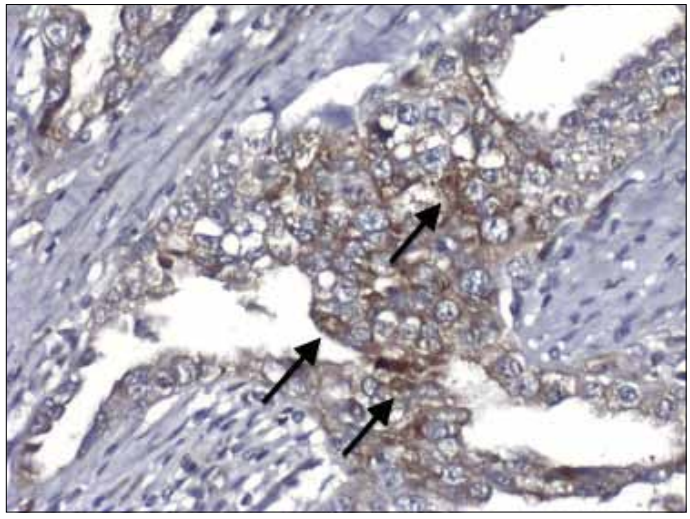

Figure 3. Aquaporin-1 (AQP1) staining in membranes of tumor cells (*black arrows*; X 400)

Table 2. Aquaporin-1 (AQP1) expression, intratumoral microvessel density (IMD) and the AQP1/IMD ratio in primary serous epithelial ovarian cancer with relation to clinical and prognostic parameters

	Cases (n)	AQP1	P*	IMD	P*	AQP1/IMD	P*
FIGO stage							
I-II	5	31.8±27.4		24.6±7.4		1.58±1.87	
III-IV	50	32.7±18.9	>0.05	32.5±19.8	>0.05	1.26±0.90	> 0.05
Tumor grade							
Grade 2	9	41.7±34.0		28.7±11.6		1.76±1.66	
Grade 3	46	30.8±15.2	>0.05	32.3±20.3	>0.05	1.20±0.81	>0.05
Lymph node metastasis							
Absent	18	29.8±18.1		31.1±17.2		1.31±1.30	
Present	37	34.0±20.2	>0.05	32.1±20.2	>0.05	1.29±0.84	>0.05
LVSI							
Absent	23	33.5±18.7		27.6±14.7		1.47±1.17	
Present	32	32.0±20.4	>0.05	34.7±21.6	>0.05	1.17±0.86	>0.05
Ascites volume (mL)							
< 500	17	28.2±16.1		30.9±16.6		1.15±1.07	
500-999	10	33.2±30.1		31.5±15.0		1.30±1.14	
≥ 1000	28	35.1±16.9	>0.05	32.3±22.2	>0.05	1.38±0.94	>0.05
Ascites cytology							
Negative	24	32.2±24.3		35.8±26.6		1.24±1.21	
Positive	31	31.7±13.9	>0.05	28.6±10.0	>0.05	1.30±0.82	>0.05
Preoperative CA 125 level (U/mL)							
< 35	1	22		35		0.62	
35-499	22	30.8±15.1		30.1±16.5		1.30±0.98	
≥ 500	32	34.2±22.4	>0.05	32.7±21.2	>0.05	1.31±1.04	>0.05
GOG/ECOG performance status							
1	13	37.6±31.7		31.0±15.1		1.53±1.50	
2	10	34.3±15.5		36.5±19.4		1.16±0.74	
3	21	30.4±13.4		31.0±24.8		1.26±0.67	
4	11	29.5±14.8	>0.05	29.9±10.4	>0.05	1.20±1.11	>0.05

AQP1: Aquaporin-1, IMD: Intratumoral microvessel density, FIGO: International Federation of Gynecology and Obstetrics, LVSI: Lymphovascular space invasion, GOG/ECOG: Gynecologic Oncology Group/ Eastern Cooperative Oncology Group (ECOG); *p values <0.05 were considered statistically significant

Previous studies have demonstrated that AQP1 protein is strongly expressed in small vessels, but not in the cytoplasm, in nearly all EOC types (22, 29). AQP1 was localized in the microvessel epithelium in these cases. Additionally, the expression of AQP1 was demonstrated on the membranes of interstitial cells of ovarian cancer tissue, and rarely on tumor cell membranes (22, 29). In our study, strong AQP1 protein expression was found in all primary serous EOC microvessels and small vessels. AQP1 expression was also demonstrated in interstitial cell membranes and tumor cells in a few cases. AQP1 was not expressed in the tumor cell cytoplasm. These findings are consistent with previous reports and support the theory that AQP1 has a role in transvascular water flow and fluid transport of tumor cells in EOC patients (22, 29). Our findings also suggest that malignant ovarian tumors have increased vascular permeability, like other tumor types (30).

The number of microvessels may be different within different tumors and different vascular areas that are evaluated. This may lead to incorrect AQP1 expression results. Therefore, the AQP1/IMD ratio may reflect AQP1 expression better in EOC specimens, as IMD reflects the microvessel count within the tumor (22). In our study, IMD was determined by CD34 staining. In previous studies, anti-CD34 antibodies were reported to be superior to anti-CD31 and factor VIII related antigen to identify poorly differentiated endothelial cells (31, 32). The growth and metastasis of a tumor depends mostly on neovascularization (24, 33, 34). IMD was reported to be higher in malignant ovarian tumors with respect to borderline tumors and also higher in borderline tumors with respect to benign tumors (35, 36).

AQP1 expression and IMD together with AQP1/IMD ratio had no significant relationship with FIGO stage, lymph node metasta-

sis, ascites volume and tumor grade in this study. In addition, relationships with other clinicopathological parameters such as age, performance status and preoperative CA125 levels were not detected. There was also no correlation between AQP1 expression and IMD. These findings were in contrast with a previous study in which AQP1 expression in microvessels of EOC had a significant association with ascites volume, FIGO stage and lymph node metastases. However, no relationship was reported between AQP1 expression and histological type or tumor grade (22). In our study, a positive correlation was found between AQP1 expression and the preoperative CA 125 level. The possible mechanisms underlying this finding should be further assessed in future trials.

In summary, AQP1 expression levels did not have a significant relationship between FIGO stage, ascites volume and lymph node metastasis in our study. According to these findings, the expression of AQP1 in serous EOC does not appear to contribute to the formation of ascites or impact on the prognosis of the disease. Future studies with larger sample sizes and other histological types are needed to clarify the clinical importance of AQP1 expression in EOC.

Ethics Committee Approval: *Ethical and scientific approval was obtained from Başkent University Medical School institutional review board.*

Informed Consent: *Informed consent was obtained from the participants of this study participants.*

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Author contributions: *Concept – M.K.T., C.B.; Design - M.K.T., C.B., A.A., O.O., A.N.H.; Supervision A.A.; Resource – A.A.; Materials – A.A., P.D., O.O., A.N.H.; Data Collection&/or Processing – M.K.T., M.D.K., E.B.; Analysis&/or Interpretation – M.K.T., E.B.; Literature Search – M.K.T.; Writing – M.K.T., E.B.; Critical Reviews – A.A., C.B., P.D.*

Conflict of Interest: *The authors declare that there are no conflicts of interest.*

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Effect of follicular fluid NO, MDA and GSH levels on *in vitro* fertilization outcomes

Folikül sıvısındaki NO, MDA ve GSH seviyelerinin in vitro fertilizasyon başarısına etkisi

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Abstract

Objective: To investigate the biochemical markers such as nitric oxide (NO), malondialdehyde (MDA) and reduced glutathione (GSH), indicators of the oxidative status of the follicle, to predict the outcome of *in vitro* fertilization.

Material and Methods: Follicular aspirates of dominant follicles were collected during oocyte retrieval. Biochemical analyses of NO, MDA and GSH were performed on all aspirates.

Results: When the successful and unsuccessful pregnancy groups were compared in terms of NO, MDA and GSH, follicular fluid MDA was significantly higher (p=0.001) and follicular fluid NO level was significantly lower (p=0.039) in the pregnant group. Correlation analysis between oxidative stress and IVF parameters showed that MDA had a positive weak correlation with the number of grade 1 embryos (r=0.271, p=0.033) and fertilization rate (r=0.263, p=0.039). ROC curve analysis found that malondialdehyde has an area under the curve of 0.74 and can predict pregnancy with high sensitivity.

Conclusion: As malondialdehyde was significantly different in pregnant and non-pregnant women and had a good sensitivity profile in predicting pregnancy, it may be considered a marker for predicting IVF success. (J Turkish-German Gynecol Assoc 2013; 14: 136-41)

Key words: Follicular fluid, malondialdehyde, nitric oxide, glutathione, *in vitro* fertilization, pregnancy

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Özet

Amaç: Folikül oksidatif durumunun belirteci olan nitrik oksit (NO), malondialdehit (MDA) ve redükte glutatyon (GSH) gibi kimyasal belirteçlerin *in vitro* fertilizasyon başarısına etkisinin araştırılması.

Gereç ve Yöntemler: Oosit toplama sırasında dominant foliküllerin aspire edilen sıvıları toplandı. Tüm aspire edilen sıvılarda biyokimyasal analiz ile NO, MDA ve GSH seviyelerine bakıldı.

Bulgular: Gebelikle sonuçlanan ve gebe kalamayan olguların NO, MDA and GSH seviyeleri karşılaştırıldığında, folikül sıvısı MDA seviyesi gebe kalanlarda anlamlı olarak yüksek bulunurken (p=0.001), NO seviyesi ise gebe kalanlarda anlamlı olarak düşük bulundu (p=0.039). Oksidatif stres ve in vitro fertilizasyon parametreleri arasında korelasyon analizi yapıldığında, MDA ile grade 1 embriyo sayısı arasında (r=0.271, p=0.033) ve fertilizasyon oranı arasında (r=0.263, p=0.039) zayıf pozitif bir korrelasyon bulundu. ROC eğrisi analizinde MDA'nın çizgi altında kalan alanı 0.74 hesaplanarak gebe kalan olguları öngörmede yüksek bir duyarlılığı olduğu bulundu.

Sonuç: Malondialdehit gebe kalan olguların follikül sıvılarında gebe kalamayanlardan anlamlı olarak yüksek bulunmasının yanı sıra, gebeliği öngörmede iyi bir duyarlılığa sahipti ve IVF olgularında gebeliği öngörmede bir belirteç olarak kullanılabilir.

(J Turkish-German Gynecol Assoc 2013; 14: 136-41)

Anahtar kelimeler: Folikül sıvısı, malondialdehit, nitrik oksit, glutatyon, *in vitro* fertilizasyon, gebelik

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Introduction

Infertility is defined as the inability to conceive for at least one year despite having regular sexual intercourse without using any contraception method. There are different causes of infertility. Nearly 40-50% of infertility problems are estimated to be of female origin and approximately 30% of cases are of male origin. A further 20-25% of couples suffer from unexplained factors (1). The pathophysiology of unexplained infertility is still a scientific question (2). In order to find some answers for unexplained cases, several investigators are trying to develop new, non-invasive biochemical markers that may affect gamete and embryo quality. Oxidative stress is being investigated as a causative marker in this manner. Studies on the pathophysiology of unexplained infertility have indicated that oxidative stress may be involved as an underlying factor (3, 4).

The maintenance of homeostasis in cells requires a complex interaction between prooxidants and antioxidants. Oxidative stress occurs as a result of a shift in this balance between prooxidants and antioxidants towards excess free radical formation (5). Oxidative stress affects all important components of cells, including lipids, proteins, carbohydrates and DNA. One of the most important harmful effects of free radical attack is the oxidation of unsaturated fatty acids, known as lipid peroxidation. One of the end products of lipid peroxidation

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is malondialdehyde (MDA). Since it is a stable end product, it can be used as a cumulative measure of lipid peroxidation (6). Nitric oxide (NO) is an inorganic, short-lived free radical gas that is synthesized from L-arginine via NO synthases. It has various physiological functions such as suppression of pathogens, vasodilatation and neurotransmission. It is a highly diffusible molecule and forms stable oxidized metabolites known as nitrites and nitrates (7). It has been reported that nitric oxide locally modulates granulosa cell function (8) and is involved in follicular maturation and ovulation in women (9, 10).

Evolutionarily, aerobic organisms have developed a biochemical defense system against the oxidative effects of reactive oxygen species. Thiol glutathione (GSH) functions as the most important endogenous antioxidant for the maintenance of the prooxidant-antioxidant balance in humans. GSH is a tripeptide containing a free sulfydryl group on a cysteine residue. It is found in high concentrations in the cytoplasm, nucleus and mitochondria (11). Oxidative stress is believed to affect reproductive functions (12). The effect of oxidative stress on the reproductive potential of men has been investigated extensively worldwide. However, there are limited reports about the possible effects on the female reproductive system (13-15).

Follicular fluid (FF) is the biological environment that supports the development of the oocyte and the subsequent embryo that is generated. It is a product of secretions of the granulosa and theca cells that surround the follicular wall. It may give perhaps the most important information about the effect of hormonal fluctuations which have an impact on oocytes. The composition of follicular fluid includes various substances such as cytokines, growth factors, antioxidants and vasoregulatory molecules. These mediators may a have direct effect on the maturation ability and the quality of oocytes (16).

The aim of this study was to investigate biochemical markers of oxidative stress such as NO and MDA and the antioxidant GSH in the follicular fluids of women undergoing ovarian hyperstimulation, intracytoplasmic sperm injection (ICSI) and embryo transfer, and to compare these parameters between two groups that were classified as successful pregnancy and unsuccessful pregnancy as an outcome of their assisted reproduction treatment.

Materials and Methods

Subject selection

Sixty-two infertile women aged between 25-32 years, who were admitted to the Kocaeli University IVF Unit and started to IVF treatment between September 2008 and October 2009, were included in this study. All of them had an etiology of unexplained infertility (no indication of a hormonal, ovulatory, tubal, uterine or sperm problem among the couples) and all were non-smokers. All of the subjects were free of any systemic disease (hypothyroidism, hyperthyroidism, diabetes, hyperprolactinemia) and had their first IVF treatment cycle. All of the women underwent ovulation induction with the long agonist (N=19), short antagonist (N=37) and microdose flare-up (N=6) protocols based on timing, hormonal conditions and ovarian reserve status of the women on the discretion of the clinician, followed by oocyte collection, ICSI and embryo transfer. ICSI was the preferred method for fertilization since it is associated with higher fertilization rates compared to conventional *in vitro* fertilization. Informed consent was obtained from each patient before participating in the study. Ethical approval was given by the Kocaeli University Ethics Committee.

Sample collection and processing

FF samples were taken from the dominant follicles of the patients during the oocyte pick-up procedure. No flushing was done during ovum pick-up in order to prevent culture media contamination. The samples were centrifuged at 2000 rpm for 10 minutes, then the clean supernatants (free of erythrocytes) were aliquoted and stored at -20°C for later use.

MDA measurement

Follicular fluid MDA measurement was done according to the protocol described by Ohkawa et al. (17). MDA reacts with thiobarbituric acid (TBA) to give a red compound which has a maximum absorbance at 532 nm. TBA reagent was prepared by mixing 0.2 mL SDS (8.1%), 1.5 mL acetic acid (20%, pH=3.5) and 1.5 mL TBA (0.8%) together, then 0.2 mL of each thawed follicular fluid sample was mixed with this 3.2 mL TBA reagent and 0.8 mL distilled water. The mixture was incubated in a boiling water bath for 1 h and then cooled on ice.

After cooling, the mixture was mixed with 5 mL N-butanol/pyridine (15:1 v/v) and 1 mL distilled water and centrifuged at 2500 rpm for 5 minutes. The absorbance of the upper butanol phase was read at 532 nm against a blank and the results were calculated as μM MDA.

GSH measurement

Follicular liquid samples were supplemented with an equal volume of 5% (v/v) metaphosphoric acid, and centrifuged at 3000 g for 10 min at 4°C. GSH concentrations in the samples were measured by the method described by Yakubu et al. (18). The GSH concentrations in the samples are expressed as μmol/L.

NO measurement

Direct measurement of NO is difficult since it is an unstable product. NO was measured indirectly by measuring the total nitrite and nitrate concentration in the sample using the Griess method as described by Archer et al. (19). After deproteinization of the sample, all the nitrate in the sample was reduced to nitrite by incubation with cadmium. Afterwards, Griess reagent (1% sulfanylic acid+0.1% N-(1-naphtyl) ethylene diamine) was added into the sample and the absorbance was measured at 545 nm. The NO concentrations are expressed as μmol/L.

The collection of embryology laboratory data

All the embryological data (number of oocytes retrieved, embryo quality, pregnancy status) were obtained from patient consultations and evaluation forms during the study. The embryo grading assessment was done according to fragmentation degree and blastomere size. Embryos with even blastomeres and <10% fragmentation were categorized as grade A embryos, while those with even blastomeres and <26%

fragmentation (cell number 2 or 5 for day 2 OR 4 for day 2 and ≥9 for day 3) and 11-25% fragmentation (cell number of 4 day 2 OR 4 for day 2 and 7-8 for day 3) were categorized as grade B embryos and with those uneven blastomeres and 26-35% fragmentation were categorized as grade C embryos according to ASEBIR embryo assessment criteria (20). Grade D embryos were kept out of the statistical analysis. All women were transferred a single, grade A embryo on day 3. Women who had a blood human chorionic gonadotropin level above 10 mIU/mL 12 days after embryo transfer were classified into the successful pregnancy group, while the others were considered the unsuccessful pregnancy group.

Statistical Analysis

SPSS version 13.0 was used for statistical analysis. Non-parametric Mann-Whitney U tests were used for group comparisons. The pregnancy status of women after IVF treatment was considered the primary outcome measure. Spearman correlation analysis was used for correlations. $p<0.05$ was accepted as statistically significant.

Results

The mean ages of the patients in the successful pregnancy (Group 1) and unsuccessful pregnancy (Group 2) groups were 32.32±4.94 and 31.42±3.53, respectively (p=0.898). IVF outcomes of the patients in both groups are given in Table 1. When the groups were compared in terms of the oxidative stress parameters shown in Table 2, statistically significant differences were found in FF MDA and FF NO levels (p=0.001 and p=0.039, respectively); however no significant difference was found between groups in terms of FF GSH (p=0.076). Correlation analyses between oxidative stress and IVF parameters showed that MDA had a positive weak correlation with the number of grade A embryos (r=0.271, p=0.033) and fertilization rate (r=0.263, p=0.039) (Table 3). ROC curve analysis revealed that

Table 1. IVF outcomes in both groups

Outcome variables	Group 1 (N= 27)	Group 2 (N= 35)	p value
# of oocytes	12.15±6.91	11.83±9.66	0.447
# of mature oocytes	9.30±5.71	8.91±8.12	0.381
Fertilization rate (%)	81.84± 3.74	70.77±4.86	0.137

*Analyses were performed by the non-parametric Mann-Whitney U test.

Table 2. Comparison of follicular fluid free radical and antioxidant GSH levels between groups

	Group 1 (N=27)	Group 2 (N=35)	p value
FF MDA (μmol/L)	1.16±0.57	0.70±0.49	0.001
FF NO (μmol/L)	33.67±12.23	41.11±15.11	0.039
FF GSH (μmol/L)	8.21±5.32	5.67±3.12	0.076

*Analyses were performed by the non-parametric Mann-Whitney U test, NO: nitric oxide; MDA: malondialdehyde; GSH: glutathione

Table 3. Correlation between oxidative stress and IVF parameters

	# of grade A embryos	# of grade B embryos	# of grade C embryos	Fertilization rate	# of mature embryos
FF MDA					
r	0.271	-0.102	-0.053	0.263	-0.098
p	0.033	0.430	0.680	0.039	0.450
FF NO					
r	-0.217	0.018	0.126	-0.114	-0.089
p	0.091	0.892	0.328	0.378	0.492
FF GSH					
r	0.086	-0.050	-0.018	-0.102	-0.004
p	0.505	0.701	0.888	0.430	0.976

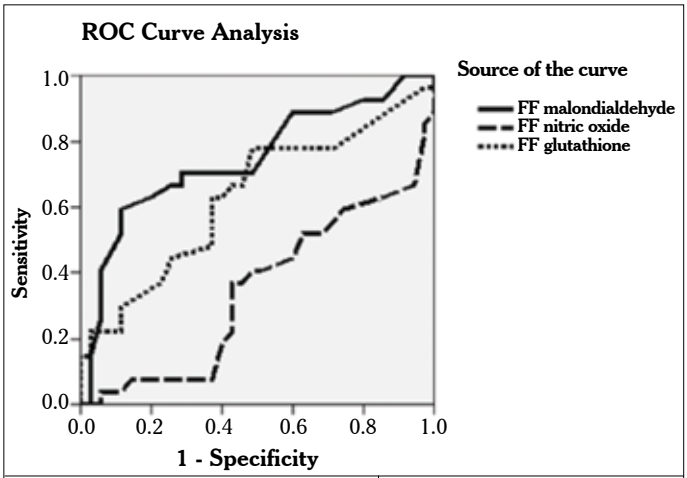
NO: nitric oxide; MDA: malondialdehyde; GSH: glutathione; r: Correlation coefficient; p: Significance
*Correlation analyses were performed by the Spearman correlation test

area under the curve for malondialdehyde was 0.74 for predicting pregnancy followed by glutathione (AUC=0.63) and nitric oxide (AUC=0.34) (Figure 1). These data show that the level of nitric oxide is better at predicting unsuccessful pregnancy outcome.

Discussion

Follicular fluid is a very important microenvironment that plays a crucial role in the development of oocytes. The oocyte is maintained with the components of the follicular fluid while maturing. Therefore, it is highly possible that some biochemical characteristics of the follicular fluid play a critical role on oocyte quality and the subsequent potential to achieve fertilization and embryo development. The biochemical composition of follicular fluid includes proteins, sugars, reactive oxygen species, antioxidants and hormones. Moreover, the oxidant-antioxidant state of follicular fluid and its effects on oocyte and IVF outcomes has been of great interest in recent years (16).

Nitric oxide is an inorganic, free radical gas that has many physiological roles in folliculogenesis, ovulation and luteal function as well as in the cardiovascular, neurological and immune systems. Recent studies have demonstrated that nitric oxide is expressed by human granulosa cells and therefore present in significant amounts in human follicular fluid, and has also been detected in the follicular fluid after gonadotrophin stimulation. This suggests that this factor may be involved in the regulation of ovarian blood flow or in the maturation of human ovarian follicles (21). The observation that NO synthesis increases with follicle development showed that NO may have a role in regulating ovarian function. Rosselli et al. (22) found that increases in NO concentrations showed a correlation with increases in estrogen hormone levels. Moreover, Shukovski and Tsafriri (23) demonstrated that ovulation in rat was inhibited by the administration of NOS inhibitors, thus showing that NO participates in the ovulatory processes. Although these findings provide evidence about the role of NO in regulating ovarian function, it is still unclear if these effects are related to NO generated in the vasculature or generated by various cells in the ovary (24).



Test Result Variable(s)	Area
FF malondialdehyde	0.746
FF nitric oxide	0.347
FF glutathione	0.631

Figure 1. ROC curve analysis of follicular fluid malondialdehyde, glutathione and nitric oxide in predicting pregnancy outcome

The data on the impact of NO on IVF outcome are conflicting. Lee et al. (25) reported that nitrite/nitrate concentrations were significantly lower in the follicular fluid of mature oocytes, and higher fragmentation and lower implantation rates were observed in embryos exposed to high follicular fluid nitrite/nitrate concentrations. In parallel, Barrionuevo et al. (26) showed that follicular NO_3^-/NO_2^- levels have a strong inverse relationship with the fertilization potential of mature oocytes. In the light of these observations, at higher follicular fluid concentrations, NO is thought to be a good predictor of oocyte and embryo outcomes. Conversely, Manau et al. (27) suggested that NO assessments might be tricky since intrafollicular levels are not correlated with IVF outcomes.

Although the controlled generation of NO may have a physiological function as a signaling molecule, uncontrolled NO production may result in embryo toxicity (2, 5). The results of our study show that intrafollicular NO levels were significantly higher in the non-pregnant group compared to the pregnant group. However, when the relationship between NO and IVF parameters such as the number of mature oocytes, fertilization rate and embryo grading was assessed, no significant correlations were observed. Thus, we may conclude that high levels of NO may exert effects on pregnancy status through complex mechanisms of endometrial receptivity (implantation) or may have negative (toxic) effects on embryos that commits them to apoptosis before implantation.

When the deleterious effects of free radicals on cell integrity are considered, it is expected that there has to be a negative correlation between peroxidation levels and IVF outcomes; however, many studies have shown conflicting results in this respect. In a study by Appasamy et al. (28), it was found that follicular fluid ROS levels had a positive correlation with the pregnancy rate in IVF patients. This observation made researchers think that a limited amount of oxidative stress may be essential for embryonic development since it is an indicator of metabolic activity. The results of a study by Pasqualotto et al. (14) showed parallel results, as pregnant women after IVF treatment had higher lipid peroxidation (LPO) products compared to non-pregnant women. However, Oral et al. (12) found no significant relationship between follicular fluid malondialdehyde levels and fertilization rates. Accordingly, Jozwick et al. (29) reported no significant correlation between IVF outcome and different oxidative stress marker concentrations. On the contrary, in a study by Das et al. (30), it was observed that there was a negative correlation between ROS levels and embryo quality.

According to our results, we have found that there was a statistically significant difference in follicular fluid MDA levels between pregnant and non-pregnant women. The follicular fluid MDA levels in pregnant women were significantly higher than those in non-pregnant women. This might be explained by the weak positive correlation of MDA with the number of grade

A embryos and fertilization rate, which are major predictors of successful IVF outcome. Our findings are in concordance with the results of Pasqualotto et al. (14), who found that pregnant women had higher lipid peroxidation levels. Agarwal et al. (2) also showed a positive correlation between pregnancy rates and lipid peroxidation.

In order to keep ROS levels in balance in the follicular fluid, it has been shown that the follicular fluid contains free radical scavengers to protect oocytes and embryos (14, 29). According to the results of some animal studies, oocyte GSH was shown to be important in decreasing disulfide bonds during sperm DNA decondensation and pronucleus formation, zygotic centrosome formation and pronucleus localization (31). In addition, GSH and GSH-forming blocks in oocyte and embryo culture media used in *in vitro* fertilization procedures have improved fertilization rates and embryo development (11).

Most of the studies relating the effect of GSH on oocyte and embryo quality in the literature have studied GSH content inside the cells (31, 32). There are few reports on the GSH content outside the cells, i.e. in follicular fluid, and its relationship with IVF outcome. In one of the studies about GSH content in follicular fluid, Liu et al. (33) found high GSH concentrations in the follicular fluids of small, medium and large sized follicles; however, there was no significant difference between different sizes in terms of GSH levels. They concluded that GSH in the follicular fluid may act as an anti-oxidant to protect oocytes from oxidation during oocyte growth and maturation. Ozkaya et al. (34) also studied the effect of multivitamin and mineral supplementation on GSH levels in the follicular fluid and serum of women with unexplained infertility in a recent study. However, in none of these studies were GSH levels in the follicular fluid correlated with IVF parameters and associated with IVF outcome.

In the present study, no significant correlation was found between follicular fluid GSH levels and fertilization rate and embryo quality. Also, there was no significant difference between pregnant and non-pregnant women in terms of follicular fluid GSH levels. This conclusion may reveal the inadequate efficiency of relatively low GSH levels in follicular fluid as an antioxidant compared to intracellular levels.

ROC curve analysis indicated that the follicular fluid malondialdehyde concentration is a good marker for pregnancy with an AUC of 0.746; glutathione and nitric oxide showed less sensitivity in estimating pregnancy status. This finding might be supported by the positive correlation of MDA with fertilization rate and the number of grade A embryos.

In order to eliminate any possible confounding effect related to age and the number of transferred embryos, the age range of women included in this study was kept very narrow (there was no statistically significant difference in age between the groups) and all were transferred a single, grade A, day 3 embryo. However, our study has some limitations. In our study, the follicular fluids taken from dominant follicles were evaluated as reflecting the entire ovary microenvironment. The oocytes from those follicles were not assessed independently. In this manner, further studies with a larger sample size are needed to perform individual and parametric analyses.

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

Informed Consent: *Written informed consent was received from the participants of this study.*

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The effect of post-wash total progressive motile sperm count and semen volume on pregnancy outcomes in intrauterine insemination cycles: a retrospective study

İntrauterin inseminasyon sikluslarında yıkama sonrası total progresif motil sperm sayısı ve semen volümünün gebelik sonuçlarına etkisi: Retrospektif bir çalışma

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Abstract

Objective: The purpose of this study was to determine the impact of post-wash total progressive motile sperm count (TPMSC) and semen volume on pregnancy outcomes in intrauterine insemination (IUI) cycles.

Material and Methods: The retrospective study included a total of 156 cycles (141 couples) and was performed in our center over a 24-month period. The semen parameters were recorded for each man and each insemination. The semen samples were re-evaluated after the preparation process. Post-wash TPMSC values were divided into four groups; Group 1: $<1\times10^6$; Group 2: $1\text{-}4.9\times10^6$; Group 3: $5\text{-}9.9\times10^6$; Group 4: 10×10^6 and $>10\times10^6$. Post-wash inseminated semen volume was divided into three groups; Group 1: 0.3 mL; Group 2: 0.4 mL; Group 3: 0.5 mL. The effect of post-wash total progressive motile sperm and semen volume on pregnancy outcomes was evaluated.

Results: The pregnancy rates per cycle and per couple were 27.56% and 30.49%, respectively. There was not a significant relationship between the inseminated semen volume and pregnancy rate ($p>0.05$). However, a significant linear-by-linear association was documented between the TPMSC and pregnancy rate ($p=0.042$).

Conclusion: Our findings suggest that the post-wash inseminated semen volume should be between 0.3-0.5 mL. An average post-wash total motile sperm count of 10×10^6 may be a useful threshold value for IUI success, but more studies are needed to determine a cut-off value for TPMSC. (J Turkish-German Gynecol Assoc 2013; 14: 142-5)

Key words: Intrauterine insemination, post-wash total progressive motile sperm count, post-wash semen volume, pregnancy

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Özet

Amaç: Bu çalışmada intrauterin inseminasyon sikluslarında yıkama sonrası motil sperm sayısı ve semen volümünün gebelik sonuçlarına etkisini araştırmak hedeflenmiştir.

Gereç ve Yöntemler: Retrospektif olan çalışma, 24 aylık periyodda merkezimize başvuran toplam 156 siklusu (141 çift) kapsamaktadır. Her inseminasyonda semen parametreleri kaydedildi. Semen örnekleri yıkama sonrası tekrar değerlendirildi. Yıkama sonrası total progresif motil sperm sayısı dört gruba ayrıldı; Grup 1: $<1\times10^6$; Grup 2: $1\text{-}4.9\times10^6$; Grup 3: $5\text{-}9.9\times10^6$; Grup 4: 10×10^6 ve $>10\times10^6$. Yıkama sonrası insemine edilen semen volumü ise üç gruba ayrıldı; Grup 1: 0.3 mL; Grup 2: 0.4 mL; Grup 3: 0.5 mL. Yıkama sonrası total progresif motil sperm sayısının ve insemine edilen semen volumünün gebelik oranlarına etkisine bakıldı.

Bulgular: Siklus ve çift başına gebelik oranları sırasıyla %27.56 ve %30.49 olarak saptandı. İnsemine edilen semen volümü ile gebelik oranı arasında anlamlı bir ilişki saptanmadı. ($p>0.05$) Ancak total progresif motil sperm sayısı ile gebelik oranı arasında "lineer by lineer ilişki" saptandı. ($p=0.042$).

Sonuç: Bulgularımız yıkama sonrası semen volümünün 0.3-0.5 mL arasında olabileceğini desteklemektedir. Yıkama sonrası total motil sperm sayısının en az 10×10^6 olması intrauterine inseminasyon başarısı için sınır değer olabilir. Ancak total progresif motil sperm sayısını sınır değer olarak kabul edebilmek için daha fazla çalışmaya gereksinim vardır. (J Turkish-German Gynecol Assoc 2013; 14: 142-5)

Anahtar kelimeler: Intrauterin inseminasyon, yıkama sonrası total progresif sperm sayısı, yıkama sonrası semen volümü, gebelik

Geliş Tarihi: 24 Mayıs 2013 **Kabul Tarihi:** 02 Temmuz 2013

Introduction

Intrauterine insemination (IUI) is a method that has been used for many years in the treatment of infertile couples. IUI is the first referenced assisted reproductive technique in mild to moderate male infertility. IUI is non-invasive, very simple and less expensive than classical in vitro fertilization and intracytoplasmic sperm 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 number of motile sperm inserted into the uterus (1).

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Most studies have suggested that the success of IUI, and therefore pregnancy rates, decrease if there is not a sufficient number of motile sperm after washing (2-5).

Semen analysis is the first step to accurately diagnose male infertility. Sperm count, sperm motility and the percentage of sperm with normal morphology are the main criteria for the quality of semen. When determining sperm quality, the values established by the World Health Organization (WHO) 2010 are often used (6). However, the cut-off values for TPMSC (total motile progressive sperm count) are still controversial and vary between $0.3x10^6$ and $20x10^6$ (7).Total progressively motile sperm is defined as the product of total sperm count and percent progressive motility.

The purpose of this study was to investigate the effect of post-wash TPMSC and semen volume on pregnancy outcomes in intrauterine insemination cycles.

Materials and Methods

This retrospective study included a total of 156 cycles (141 couples) and was performed in our center over a 24-month period (July 2009 to July 2011). These couples were candidates for IUI because of mild male factor infertility or unexplained infertility. Men with mild male factor infertility had semen analysis where only one of the sperm parameters was below the normal range (6).

All the IUI cycles underwent controlled ovarian stimulation with gonadotrophins. The stimulation treatment was started on the second day of the cycle and was continued until ovulation or observation of an initial rise in luteinizing hormone (LH). Recombinant follicle stimulating hormone (FSH) (Gonal-F; Ares-Serono, Geneva, Switzerland; or Puregon, Organon International Inc., Roseland, NJ, USA) was used. The starting gonadotrophins dosage (37.5–100 IU/day) was adjusted according to the hormone profile, age and infertility duration. The initial dose was continued for six days and organized according to the ovarian response.

Female serum hormone levels (estradiol-17b and LH) were measured and vaginal ultrasonography was performed to assess the size and number of follicles and endometrial maturation. This evaluation was begun on the seventh day and the evaluation was repeated after two or three days, depending on follicular development. Ovulation triggering was achieved by subcutaneous injection of 250 μg of recombinant human chorionic gonadotrophin (hCG; Ovitrelle, Ares-Serono). Insemination was performed 36 hours after the hCG injection. A single dose of recombinant hCG (10000 IU) was given when the mean diameter of the leading follicle reached ≥18 mm. The husband was instructed to abstain from sexual intercourse for 3-5 days before insemination. Semen samples were obtained by masturbation in sterile plastic containers. After liquefaction of the semen (about 30 minutes at room temperature), the initial semen volume, sperm count and progressive motility were evaluated according to WHO criteria. Semen samples were processed using a density gradient (PureSperm®, Nidacon International AB, Sweden) and sperm wash medium (Sperm Wash, Nidacon, International AB, Sweden). PureSperm gradients 90% and 50% were used for the experiment. All procedures were conducted under sterile conditions. Media were brought to 37°C. Using a sterile pipette, 1 mL of the lower layer (90% PureSperm gradient) was transferred into a conical centrifuge tube. Using a new sterile pipette, 1 mL of the upper layer (50% PureSperm gradient) was gently dispensed on top of the lower layer. A liquefied semen sample was 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. Using a transfer pipette, 4 mL of sperm wash medium was added and the re-suspended pellet was centrifuged for 5 minutes at 1500 rpm. The supernatant was then removed and 0.3-0.5 mL of sperm wash medium was gently dispensed on the top of the pellet. The tube was then incubated at an angle of 45° for 30-60 minutes in the incubator at 37°C and 5% CO_2. After the incubation period, the entire supernatant was transferred into a 5 mL tube. IUI was performed using a catheter (Gynetics Medical Products N.V. Hamont-Achel, Belgium) through the cervix and injecting washed sperm directly into the uterus. Total motile sperm count and post-wash motile sperm count were calculated by the formula (volume x motile sperm count x motility).

Post-wash TPMSC was divided into four groups; Group 1: $<1\times10^6$; Group 2: $1\text{-}4.9\times10^6$; Group 3: $5\text{-}9.9\times10^6$; Group 4: 10×10^6 and $>10\times10^6$. Post-wash inseminated semen volume was divided into three groups; Group 1: 0.3 mL; Group 2: 0.4 mL; Group 3: 0.5 mL.

Serum hCG levels were determined two weeks after hCG injection in the absence of menstruation for a diagnosis of pregnancy. A clinical pregnancy was defined as serum positive β-hCG.

Statistical analysis of the data was done using SPSS software (version 16, SPSS, Chicago, USA). The data were analyzed using the independent t-test and the x^2 test. Significance was set at $p<0.05$.

Results

The median age of the women was 30 years (39-42) while that of the males was 33 years (23-65) at the time of IUI. Pregnancy rates per cycle and per couple were 27.56% and 30.49%, respectively. Baseline characteristics in the pregnant and non-pregnant groups are shown in Table 1.

A comparison of the sperm parameters in the pregnant (41 single pregnancies, 2 twin pregnancies) and non-pregnant groups are summarized in Tables 2 and 3.

Table 1. Baseline characteristics in the pregnant and non-pregnant groups

	pregnant	non-pregnant	
	n (range) *	n (range) *	p value
Woman's age (y)	30 (21-40)	31(19-42)	0.75
Man's age (y)	32 (23-41)	33 (25-65)	0.12
Infertility duration (y)	2.5 (1-6)	3 (1-7)	0.40
*Data are presented as median (min-max)			

When the TPMSC was ≥10×10^6, the pregnancy rate was higher than in the groups with TPMSC <1x10^6, 1-4.9x10^6 and 5-9.9x10^6 (40.30%, 27.20%, 19.60% and 21.40%, respectively (Tables 3 and 4). A "linear by linear" association was found between post-wash TPMSC and the pregnancy rate (p=0.042). On the other hand, no significant relationship was found between the post-wash inseminated semen volume (0.3, 0.4 or 0.5 mL) and the pregnancy rate (p>0.05) (Table 4).

Table 2. Sperm parameters in the pregnant and non-pregnant groups

	pregnant	non-pregnant	
Pre-wash	**n (IQR)**	**n (IQR)**	**p value**
Semen volume (mL)	2 (1)	3 (2)	0.38
Sperm count (10^6/mL)	44 (47)	44 (44.5)	0.65
Progressive motility (%)	36 (20)	40 (20.5)	0.05
TPMSC (10^6)	40 (48)	60 (74)	0.01
Post-wash			
Semen volume (mL)	0.4 (0.2)	0.3 (0.2)	0.66
Sperm count (10^6/mL)	24 (35)	15 (22)	0.65
Progressive motility (%)	100 (0)	100 (0)	-
TPMSC (10^6)	9 (11)	6 (7.5)	0.59
*Data are presented as the median, IQR: interquartile range			

Table 3. Comparison of sperm parameters (post-wash TPMSC and semen volume) between groups

	pregnant	non-pregnant	
Post-wash TPMSC (x10^6)	**n (%)**	**n (%)**	**p value**
<1	3 (7)	8 (7.1)	0.08
1-4.9	10 (23.30)	41 (36.3)	0.00
5-9.9	9 (20.90)	33 (29.2)	0.001
10 and >10	21 (48.80)	31 (27.4)	0.16
Post-wash semen volume (mL)	**n (%)**	**n (%)**	**p value**
0.3	20 (46.5)	61 (54)	0.00
0.4	6 (14)	11 (9.7)	0.22
0.5	17 (39.5)	41 (36.3)	0.002
* Parentheses indicate percentages			

Discussion

It is known that semen parameters affect IUI success. Although the World Health Organization's (WHO) reference values for semen analysis are commonly used to assess sperm quality, predictive sperm parameters and threshold values with respect to semen characteristics for successful IUI are still controversial (1). The post-wash inseminated sperm count is considered to be an important prognostic factor (2). In different studies, the cut-off values for the post-wash TPMSC have varied between 0.3x10^6 and 20x10^6 (7). This difference could be based on sperm preparation techniques (swim-up or density gradient wash).

In this study, the pregnancy rate per cycle was 27.56. Baseline characteristics in pregnant and non-pregnant groups were homogeneous (Table 1). Most studies have reported very low pregnancy rates in groups in which the post-wash TPMSC was <1x10^6 (3). In our study, the pregnancy rate for this group was 1.92% (Table 3).

A "linear by linear" association was found between post-wash TPMSC and the pregnancy rate (p=0.042). For the post-wash TPMSC, a lower value of 10×10^6 could be a criterion for IUI success. However, more studies are needed to determine a threshold value for post-wash TPMSC.

Some studies have shown conflicting results when IUI techniques were compared, also in terms of the volume of material deposited in the fundus region of the uterus during the procedure (8-10). Franco Junior et al. (10) evaluated the volume of material deposited during IUI and the site involved by hysterosalpingography and noted that, starting from 0.4 mL, the contrast dye reached the uterus, isthmus and tube ampulla, whereas a volume of 0.2 mL did not reach the tube.

Do Amaral et al. (11) performed 164 cycles of ovulation induction followed by IUI. The patients were divided into two groups according to the technique used. The low volume

Table 4. Pregnancy rates, post-wash total progressive sperm counts and semen volumes

	Group 1	Group 2	Group 3	Group 4	Total
Post-wash TPMSC (x10$_6$)	<1	1-4.9	5-9.9	10 and >10	
Cycles (n)	11	51	42	52	156
Pregnancies (n)	3	10	9	21	43
Pregnancies per cycle (%)	27.20	19.60	21.40	40.30	27.56
	Group 1	**Group 2**	**Group 3**		
Post-wash semen volume (mL)	0.3	0.4	0.5		
Cycles (n)	81	17	58		156
Pregnancies (n)	20	6	17		43
Pregnancies per cycle (%)	24.69	35.20	29.30		27.56
TPMSC: total progressive motile sperm count					

group included 50 cycles and 0.5 mL of inseminated semen with the high volume group included 114 cycles and 3.0 mL of inseminated semen. They obtained a similar clinical pregnancy rate for the two groups (14.0% for the low volume group and 15.7% for the high volume group).

We wanted to investigate the effect of low volumes of inseminated sperm on pregnancy outcomes. When we compared the results of IUI with the volumes 0.3-0.4 mL and 0.5 mL, we detected no advantages. In our study, no significant relationship was found between post-wash inseminated semen volume (0.3, 0.4 or 0.5 mL) and the pregnancy rate ($p>0.05$). Our findings support the notion that post-wash inseminated semen volume can be from 0.3 to 0.5 mL. There are no detailed studies in the literature on this subject.

The lack of analysis regarding sperm morphology and the retrospective nature of the study are the weaknesses of this study. In the next step, our aim is to plan a prospective study including more patients with an assessment of sperm morphology.

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

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

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Sentinel lymph node detection and accuracy in vulvar cancer: Experience of a tertiary center in Turkey

Vulva kanserinde sentinel nod uygulamasının güvenilirliği çalışması: Türkiye'de bir üçüncü basamak hastanenin deneyimi

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Abstract

Objective: To explore the accuracy of sentinel lymph node (SLN) dissection in predicting regional lymph node status by using either only Technetium-99m-labelled (Tc-99m) or in combination with a blue dye in patients with squamous cell cancer of vulva.

Material and Methods: Twenty-one patients who had T1 (≤2 cm) or T2 (>2cm) tumors that did not encroach into the urethra, vagina or anus were included in the study. For the first twelve patients, Tc-99m was used for SLN identification, and the combined technique was used in subsequent patients. Preoperatively, Tc-99m and a blue dye was injected intradermally around the tumor. Following SLN dissection, complete inguinofemoral lymphadenectomy was performed.

Results: We could detect SLN in all 21 patients (100%) by either Tc-99m or the combined method. SLN was found to be histopathologically negative in 13 groins via Tc-99m and 10 groins via the combined method. Twenty-one of these 23 (91.3%) groin non-SLN were also negative, but in two groins, we detected metastatic non-SLN.

Conclusion: Although SLN dissection appears promising in vulvar cancer, false negative cases are reported in the literature. Sentinel lymph node dissection without complete lymphadenectomy does not seem appropriate for routine clinical use, since it is known that groin metastasis is fatal. (J Turkish-German Gynecol Assoc 2013; 14: 146-52)

Key words: Sentinel node, lymph node, vulvar cancer

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Özet

Amaç: Vulva kanserli hastalarda teknesyum-99m (Tc-99m) veya mavi boya (blue dye) kullanılarak yapılan sentinel lenf nodu (SLN) diseksiyonlarının hastaların lenf nodu tutulumların göstermedeki başarısını belirlemek.

Gereç ve Yöntemler: Üretra, vajina ya da anüste tutulumu olmayan T1 (≤2cm) ya da T2 (>2cm) tümörü olan 21 vulva kanserli hasta çalışmaya dahil edildi. Çalışmanın ilk 12 hastasında SLN'nu tespit etmek için Tc-99m kullanırken diğer hastalarda kombine teknik (Tc-99m ve blue dye) kullanıldı. Operasyon öncesinde tümör çevresine intradermal olarak Tc-99m ve blue dye enjekte edildi. SLN diseksiyonunu takiben biri hariç tüm hastalarda tamamlayıcı inguinofemoral lenfadenektomi yapıldı.

Bulgular: Çalışmaya katılan 21 hastanın tümünde bilateral SLN diseksiyonu (toplam 42) yapılırken 39 tanede tamamlayıcı inguinofemoral lenfadenektomi yapıldı. Hastaların tümünde Tc-99m veya kombine metot kulllanılarak SLN tespit edildi. Tc-99m kullanılan hastaların 13 inguinal bölgesinde (groin) ve kombine metot kullanılan hastaların 10 inguinal bölgesinde SLN'u histopatolojik olarak negatif bulundu. Bu 23 inguinal bölgenin 21'inde (91.3%) SLN olmayan lenf nodları da negatifti ancak 2 inguinal bölgede SLN olmayan lenf nodlarında metastaz tespit edildi.

Sonuç: Vulva kanserinde SLN diseksiyonları ümit verici olsa da, literatürde yanlış negatif vakalar rapor edilmiştir. İnguinal bölge metastazları vulva kanserli hastalarda ölümcül olduğundan, tamamlayıcı inguinofemoral lenfadenektomi olmadan yapılan sentinel lenf nodu diseksiyonlarının rutin kullanım için uygunluğu tartışmalıdır.
(J Turkish-German Gynecol Assoc 2013; 14: 146-52)

Anahtar kelimeler: Sentinel nod, lenf nodu, vulva kanseri

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Introduction

Vulvar squamous cell cancer is a disease of postmenopausal women, with a median age at diagnosis of about 65 years. It accounts for about 4% of genital tract cancers. The increase in incidence of vulvar intraepithelial neoplasia and life span has brought vulvar cancer into a place of more importance among genital tract cancers (1). Lymphatic spread to the inguinal and femoral lymph nodes is the major pathway of invasion in early stage vulvar cancer. The status of the regional lymph nodes is clearly the most important prognostic factor in vulvar cancer. Predictive factors of nodal involve-

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ment are the depth of infiltration, the size and the location of the tumor (2). Currently, surgery is the cornerstone of treatment in early stage vulvar cancer. Historically, all patients with vulvar cancer have been treated with radical vulvectomy and en bloc inguinofemoral lymph node dissection (LND) with a single incision. Following this aggressive surgery, morbidities have been significant with wound breakdown, chronic lymph edema in lower extremities and sexual dysfunction (3). In the past 20 years, treatment has become less invasive by performing excision of the tumor and uni- or bilateral inguinofemoral LND according to the laterality of the tumor through separate incisions. Despite these more conservative surgeries, significant morbidity has persisted. Although wound complications are generally short-lived, lymphedema is a chronic problem which requires long-term management (4). Recently, sentinel lymph node (SLN) biopsy has been introduced as an alternative in order to adequately assess for regional lymph node metastasis and to avoid the morbidity of complete inguinofemoral lymphadenectomy.

Recent data in the literature demonstrate that the SLN biopsy technique has become an integral treatment in the management of patients with early stages of breast cancer and melanoma (5, 6). Since the tumor is suitable for injection with a vital blue dye and/or technetium 99m and the lymphatic flow pathway is predictable, vulvar cancer is also a good target for SLN biopsy (4). Since 1994, a great number of clinical trials have been published on SLN detection and accuracy in vulvar cancer (7-17). In these clinical trials, the accuracy of SLN biopsy using a radiocolloid alone or in combination with a blue dye was demonstrated with a high negative predictive value (95%), but false positive cases were also reported.

We performed a prospective trial to evaluate the diagnostic accuracy of performing SLN biopsy with consecutive complete inguinofemoral lymphadenectomy in predicting the status of the inguinal lymph nodes in patients with early stage vulvar cancer. The aim of the study was to examine the sensitivity of the SLN technique using either preoperative lymphoscintigraphy with a radiocolloid (technetium-99m) alone or as combination of the radiocolloid and a blue dye.

Materials and Methods

Patients with primary squamous cell cancer of the vulva were referred to our hospital as a tertiary center for further treatment between April 2000 and October 2005. Patients with T1 (≤2cm) or T2 (>2 cm) tumors that did not encroach in the urethra, vagina or anus were eligible for the study. All patients had histologically confirmed diagnosis of invasive squamous vulvar cancer with invasion greater than 1 mm in depth and were candidates for inguinal lymph node dissection. Patients with clinically palpable groin lymph nodes were excluded from the study. Patients with a prior vulvar surgery that could disrupt lymphatic drainage were also excluded from the study. None of the patients had undergone excisional biopsy for the primary lesion prior to enrollment in the study. Approval for the study was given by the medical ethics committee of the hospital. After informed consent, we prospectively studied 21 women with operable vulvar cancer. The first 12 cases underwent SLN identification with a gamma probe using a Tc-99m-labelled nanocolloid, and in the subsequent nine patients, the combined technique (Tc-99m and a blue dye) was used.

In the first 12 patients, approximately one hour before the operation, 0.4-0.6 mL of Tc-99m microcolloidal sulfur (Lymphoscint®, Nycomed-Amersham-Sorin, Germany) was injected circumferentially around the tumor via the intradermal route in each quadrant of the tumor. Sentinel lymph nodes were identified using a handheld gamma probe which identifies SLN based on high counts, usually at least 10x the basal count (Europrobe 2000, Eurorad, France); SLN were removed separately. The count rate in vivo and in vitro was examined. After removal of the first SLN, the groin was re-examined for radioactivity, and dissection continued in search of additional SLN and make sure that all SLN were identified and removed.

In the subsequent nine patients, following the procedure described above, 2.0 mL of patent blue V dye (2.5% in aqueous solution containing 0.6% sodium chloride and 0.05% disodium hydrogen phosphate; France) was injected around the tumor in a manner and location similar to the Tc-99m sulfur colloid injection. Compression and massage were applied gently in order to allow the dye to travel through the lymphatics. Fifteen minutes after dye injection, a longitudinal bilateral inguinal incision was carefully made to the point which showed the maximum color change. The bluish afferent lymphatic channel was seen and followed up to the blue sentinel lymph node (SLN). This node was then excised and counts in the node and background counts in the operative field were then checked and compared. If no blue nodes were seen, SLN were detected only by radioactivity. Afterwards, uni/bilateral inguinofemoral lymphadenectomy with local radical excision of the tumor or radical vulvectomy were performed via separate incisions. The primary tumor was excised with a free margin of at least 1-2 cm of normal skin. The removed SLN and the lymphadenectomy specimens were sent for histopathological examination separately.

For routine histopathological examination, all the lymph nodes were bisected parallel to the long axis and totally submitted for histopathological evaluation in two or more blocks. Each block was stained with hematoxylin and eosin. The two patients (number 3 and 9) who had negative SLN and positive non-SLN underwent further histopathological examination. Sentinel lymph nodes of these two patients were serially sectioned and stained with hematoxylin and eosin. The SPSS for Windows version 15.0 software (SPSS Inc., Chicago, IL, USA) was used to perform the statistical analyses of the study.

Results

Patient characteristics

From May 2000 through October 2005, 21 patients with squamous cell cancer of the vulva underwent inguinal SLN dissection and inguinofemoral LND according to the planned procedure. The mean age of the patients was 62.2±11.1 years

(range 45-87 years). The mean tumor size was 24.5 mm (range 8-40 mm). Of all 21 patients, 10 had tumors greater than 20 mm in diameter and the other 11 patients had tumors less than or equal to 20 mm in diameter. The histologic type was squamous cell cancer in all patients. Tumors were located at the midline in ten patients, on the left side in five patients and on the right side in six patients.

Complete inguinofemoral LND was performed in 39 groins in this study. Out of 21 patients, 19 patients had bilateral complete inguinofemoral LND and sentinel lymph node (SLN) dissection together, while one patient (number 10) had SLN dissection with ipsilateral inguinofemoral LND and one had only SLN dissection without complete lymphadenectomy. The latter was the oldest case (87 years) in our study; she was not submitted to general anesthesia for the operation because she had a history of severe heart failure. Therefore, local excision of the tumor from the right labia majora and bilateral SLN dissection were performed under local anesthesia. All of the patients had bilateral SLN dissections. No adverse effect or allergy to the dye was observed in the study.

Sensitivity of the radioisotope and blue dye for SLN detection

For the first 12 patients (patients #1-12), a Tc-99m-labelled nanocolloid was used for SLN identification, and the blue dye was added to the procedure in the subsequent nine patients (patients #13-21). The utilization of only the gamma probe (Tc-99m) allowed for the detection of a SLN in all of the first 12 patients (100%). Out of 24 groin SLN dissections performed in this first group of patients, SLN were found to have tumors in seven groins, SLN were not detected in four groins and SLN were found to be tumor-free in 13 groins. Out of these 13 groins with tumor-free SLN, two groins (patients #3 and 9) were found to have a skip metastasis in non-SLN. The false negative rate for SLNs using Tc-99m was calculated as 2/13 (15.4%) per groin in this study. The tumor and SLN characteristics of the patients in the first group (Tc-99m) are summarized in Table 1.

With the use of the blue dye and radioisotope together, SLN were detected in all of the subsequent nine patients (100%). Out of 18 groin SLN dissections performed in the combined method group, SLN were found to have tumors in two groins, SLN were not detected in six groins and SLN were found to be histolologically tumor-free in 10 groins. In total, 16 groins (10+6) with tumor-free and absent SLN were also found to be free of tumor metastases in the other non-SLN. Therefore no skip metastases to non-SLN were found via the combined method (Tc-99m and a blue dye) by patient and by groin. The tumor and SLN characteristics of the patients in the second group (Tc-99m plus a blue dye) are summarized in Table 2.

SLN characteristics

At least one SLN was detected in all 21 (100%) patients. Sentinel node dissection of 39 groins produced a total of 39 SLN; one SLN was identified in 26 groins, two SLN in three groins, three SLN in one groin, and four SLN in one groin. SLN could not be identified in 10 groins (four groins in the Tc-99m group and six groin in the combined method group), but the non-SLN of these groins were also free of metastases. The SLN were found to be histopathologically negative in 23 groins (13 in the Tc-99m group and 10 in the combined method group). Of all these 23 groins with tumor-free SLN, 21 groins had non-SLN which were also negative, but in two groins (patients #3 and 9), metastatic non-SLN were also detected. The SLN of these patients (patients #3 and 9) were subjected to further histopathological examination with serial sectioning and were again found to be free of metastases. SLN were not identified in 10 groins (four in the Tc-99m group and six in the combined method group). Of all these 10 groins with absent SLN, non-SLN were also found to be histopathologically tumor-free. SLN were found to have tumor metastases in nine groins (seven in the Tc-99m group and two in the combined method group). Of all these nine groins with tumor-positive SLN, only five groins had metastatic SLN with histologically tumor-free non-SLN and four groins had metastases in both SLN and non-SLN.

Table 1. Summary of tumor and sentinel node characteristics undergoing SLN dissection via a combined method (technetium and blue dye)

Case no	Age	Tm size	Tm location	SLN status Right	SLN status Left	Positive Nodes Right	Positive Nodes Left
13	58	2 cm	Midline	Not identified	Neg	None	None
14	72	1.5 cm	Left side	Not identified	Neg	None	None
15	65	3 cm	Midline	Neg	Neg	None	None
16	48	3 cm	Midline	Pos	Pos	SN	SN
17	47	2 cm	Left side	Not identified	Neg	None	None
18	70	1.5 cm	Midline	Neg	Not identified	None	None
19	72	1 cm	Right side	Neg	Not identified	None	None
20	52	1.5 cm	Right side	Neg	Neg	None	None
21	72	3 cm	Right side	Neg	Not identified	None	None

SLN: Sentinel lymph node; Pos: Positive; Neg: Negative

Table 2. Summary of tumor and sentinel node characteristics undergoing SLN dissection via technetium-99m

Case no	Age	Tm size	Tm location	SLN status Right	SN status Left	Positive Nodes Right	Positive Nodes Left
1	45	1.5 cm	Midline	Not identified	Neg	None	None
2	49	0.8 cm	Right side	Neg	Not identified	None	None
3	65	4 cm	Midline	Neg	Neg	None	Non-SN
4	87	4 cm	Right side	Neg	Neg	None	None
5	60	3 cm	Midline	Neg	Neg	None	None
6	49	3 cm	Right side	Pos	Pos	SN-NonSN	SN-NonSN
7	69	4 cm	Midline	Pos	Pos	SN	SN-NonSN
8	65	1.5 cm	Left side	Not identified	Neg	None	None
9	65	2 cm	Midline	Pos	Neg	SN-NonSN	NonSN
10	75	3.5 cm	Midline	Not identified	Pos	None	SN
11	58	4 cm	Left side	Neg	Pos	None	SN-NonSN
12	69	1.7 cm	Left side	Neg	Neg	None	None

SLN: Sentinel lymph node; Pos: Positive; Neg: Negative

Inguinofemoral lymphadenectomy and non-SLN characteristics

In total, 39 groin dissections (completion inguinofemoral lymphadenectomy) were performed and the average number of total lymph nodes sampled were 8,4 nodes per groin. Among the 39 groins dissected, 11 groins were found to have tumor metastases. Of 11 groins, nine groins had tumor positive SLN whereas the other two groins (patients #3 and 9) had false negative SLN with metastatic non-SLN. Of these 11 groins, four contained metastasis to the SLN with negative non-SLN and seven had metastatic SLN and non-SLN together. If we look on the basis of patients, out of the 21 patients who participated in the study, six patients in the Tc-99m and one patient in the combined method group were found to have tumor metastases in the groin. Out of the six patients in the Tc-99m group, five patients had at least one histopathologically positive SLN detected and complete bilateral lymphadenectomy was performed in this group of patients. On the other hand, one patient (patient #3) had tumor-free SLN on both the right and left side but tumor positive non-SLN were found in the left groin after complete lymphadenectomy was done. If we had performed only SLN dissection without complete inguinofemoral LND in this case, she would have had inadequate surgical treatment and probably reduced disease-free survival. The average sizes of the tumors in the patients with groin metastases and the size of the tumor in the patient with a false negative SLN (patient #3) were 2 cm and 4 cm, respectively.

Tumor location and SLN characteristics

Out of 10 cases who had tumors at the midline (clitoris fourchette and perineum), only six (60%) were found to have bilateral sentinel nodes. Among the subsequent 11 cases who had tumors located laterally more than 2 cm from the midline, six had ipsilateral sentinel lymph nodes.

Discussion

According to our literature search, this is the first prospective clinical trial investigating the feasibility and accuracy of SLN dissection in the surgical management of early stage vulvar cancer in Turkey. For the first 12 patients, a radiocolloid was used for SLN dissection and a combined method (radiocolloid and a blue dye) was used in the subsequent nine patients. We successfully detected at least one SLN in each of all 21 patients with vulvar cancer.

Levenback et al. (13) first reported the use of a blue dye for SLN detection in seven of 12 (58%) groin dissections in 1994. They reported SLN identification in 57 out of 76 (75%) cases in a later article (14). Other studies in which Tc-99m was used for SLN dissection have demonstrated that SLN can be detected easily in the majority of patients. In the studies by Merisio et al. (17) and Moore et al. (16), sentinel nodes were detected with the use of Tc-99m in 100% of cases. Many other investigators have used the combined technique (Tc-99m and a blue dye) in order to identify sentinel nodes more accurately. De Hullu et al. (18) and Martinez-Palones et al. (10) reported a 100% sentinel lymph node detection rate with the use of the combined technique. Recently, the results of a GOG protocol investigating whether SLN dissection could replace complete inguinofemoral lymphadenectomy were published, and the SLN detection rate was reported to be 92.5% in 452 patients with squamous cell vulvar cancer (4). Hassanzade et al. (19) analyzed forty-nine studies in the literature in a systematic review; the SLN detection rate per patient and per groin were found to be 94.4% and 84.6%, respectively in this meta-analysis. Since radiocolloid use is associated with a better detection rate compared to the blue dye alone, blue dye might be used if only radiocolloid fails to detect the sentinel node. Recently, fluorescent dyes and near infrared (NIR) optical imaging have been introduced for SLN identification. However due to the

limited penetration of NIR fluorescence, radiocolloids are still essential for the detection of SLN, especially more deeply located ones (20).

Inguinal lymph node status is the most important prognostic factor in patients with early-stage vulvar cancer. Since local inguinal lymph node metastasis is fatal, the determination of lymph node status is very important (21). In a meta-analysis of studies investigating methods of detecting lymph node metastases in vulvar cancer, SLN dissection was found to be the most accurate way of diagnosing lymph node status (sensitivity of 97%). Other alternative approaches to detect lymph nodes, like groin ultrasound with or without fine needle aspiration, computerized tomography, magnetic resonance imaging and positron emission tomography had sensitivities ranging from 45% to 86% (22).

SLN dissection was introduced in order to adequately predict the status of other non-sentinel lymph nodes and to decrease the complications of aggressive surgery. Negative SLN should indicate the absence of tumor metastases in other non-sentinel lymph nodes, but in our study, two patients with negative SLN were found to have non-sentinel lymph node metastases. Although we are sure that the SLN were histologically tumor-free (ultrastaging and immunohistochemical examination were also negative for the presence of tumors in these lymph nodes), we detected skip metastases to non-sentinel lymph nodes. Enhanced histopathological analysis with serial sectioning and immunohistochemistry have been found to be highly accurate for detecting even subclinical micrometastases in the lymphatics (8). While some authors would rather use serial sectioning and immunostaining (i.e., ultrastaging) of SLN to search for micrometastases, the optimal method (hematoxylin and eosin satin versus ultrastaging) remains uncertain (23, 24). At present, no reliable non-invasive method is available to discriminate between patients with and without inguinofemoral lymph node metastases; therefore, in our study, routine inguinofemoral lymphadenectomy was performed in all of our patients.

Out of 23 groins with negative SLN using either only Tc-99m or the combined method, two groins were found to have skip metastases in non-SLN. The false negative rate for SLN for per groin was 2/23 or 8.6%. Out of 21 patients, two patients had false negative SLN in our study; although the SLN were negative in the groin, there were metastatic non-SLN. Approximately 10% (2/21 or 9.5%) of the patients in our study had skip metastases to other regional lymph nodes. One case out of 10 is an important ratio and it is difficult to ignore because lymph node metastasis is fatal in vulvar cancer.

With the use of radiocolloid only, Merisio et al. (17) reported a case of a falsely negative lymph node in vulvar cancer. This patient was a 70-year-old obese woman with a tumor exceeding 4 cm in diameter. It was said that injection of higher quantities of the radiocolloid might be needed for larger tumors. Martinez-Palones et al. (10) also reported a case with a false negative sentinel node. Terada et al. (8) reported a case of a groin recurrence less than two years after apparently negative sentinel node biopsy. This node was pathologically a false negative; pathologic analysis with step sectioning and immunohistochemistry revealed micrometastases in the sentinel node. Fons et al. (25) also reported a case with detection failure in the sentinel node with a combined method; the sentinel node was detected only in one groin and exploration of the other groin showed a large 2 cm lymph node totally replaced with tumor tissue. Neither the radioactive material nor the blue dye could stain it. In order to minimize the risk of failure in the sentinel node procedure, preoperative selection of patients with magnetic resonance imaging was recommended in this study. Radziszewski et al. (26) reported the rate of false negative sentinel lymph nodes as 17% (8/46), which is inconsistent with the literature. Inadequate surgeon experience with vulvar cancer surgery was said to be the main predisposing factor for the high rate of false negative SLN. In the former study, although surgeons had at least 15 years of experience, the SLN dissection technique had only been performed a few times by each of them. There is a learning curve in performing the SLN detection technique, and de Hullu et al. (18) defined the first 10 patients as the learning phase for the SLN procedure. Early cases within the series can also lead to false negative SLN results; in our study, the two patients with false negative SLN were operated on in 2001 and 2002, respectively, and can be accepted as early cases. The SLN detection rate is also related to the size of the vulvar tumor. Patients with a tumor size exceeding 4 cm have not been accepted as candidates for the SLN dissection technique since the results of the GROINSS-IV study were published in 2010. The sensitivity of SLN dissection in <4 cm vulvar cancer tumors was 7% higher than >4 cm tumors in a meta-analysis (93% and 86%, respectively). Oonk et al. (27) also reported that when the size of a sentinel node metastasis increases, the risk of non-sentinel lymph node metastasis also increases; no cutoff value for size could be determined in order to make us sure of the absence of non-sentinel lymph node metastasis. In our study, the patients with false negative SLN had tumors 2 cm and 4 cm in size, which could also be a contributing factor to detection failure.

The literature also warns us about midline tumors of the vulva, since unilateral SLN detection can cause false negative findings on the contralateral side. The best approach should be inguinal lymphadenectomy on the detection failure groin. In our study, both of the patients with false negative sentinel nodes had midline tumors. There may be technical problems during injection of the radioisotope or blue dye, and this may result in false negative sentinel nodes. Another reason for failure may be speculated that lymph nodes fully replaced with tumor tissue can lead to the stasis of lymphatic flow, such that the real SLN is by-passed and another lymph node is assumed to be the sentinel node.

Recent data on the SLN dissection technique are impressive and suggest that this is a reliable method for the detection of SLN in vulvar malignancy (18, 28). In a multicenter trial, patients with negative SLN were reported to have low groin recurrence and survival. Van der Zee et al. (29) noted that SLN biopsy should be done by a quality controlled multidisci-

plinary team. We could successfully detect SLN in all patients using Tc-99m or a blue dye, but histologically tumor-free SLN do not guarantee that the remaining lymph nodes are also tumor-free. Since false negative cases have been reported both in our trial and in other studies in the literature, the SLN dissection procedure without complete inguinofemoral lymphadenectomy is not appropriate for routine clinic use in our country. Because patients with vulvar cancer generally relapse locally and groin recurrence is almost always fatal, further validation trials are necessary in order to assess the safety and accuracy of the SLN technique, especially in centers with a small number of patients with vulvar cancer.

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Effect of the afterloaded external guidance embryo transfer technique on pregnancy rates in single embryo transfer cycles

Dış kateter yönlendirmesi ile embriyo transfer tekniğinin tek embriyo transferi sikluslarında gebelik oranlarına etkisi

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Abstract

Objective: To investigate effect of the afterloaded external guidance embryo transfer technique on pregnancy rates in single embryo transfer intracytoplasmic sperm injection (ICSI) cycles.

Material and Methods: This retrospective study was performed at the Dr. Zekai Tahir Burak Women's Health Research and Education Hospital. Three hundred and thirteen women who underwent ICSI were included in the study. Subjects were categorized according to the embryo transfer technique; Group 1 (n: 232): easy transfer with a soft catheter, Group 2 (n: 45): after external guidance transfer, and Group 3 (n: 36): difficult transfer with a stylet. Basal parameters, clinical and laboratory IVF outcomes and pregnancy rates were studied.

Results: Infertility etiology, basal follicle stimulating hormone (FSH) levels, antral follicle count, duration of stimulation, total dose of gonadotropin, peak estradiol levels, endometrial thickness, oocyte number, 2 PN, and fertilization rate were similar between the three groups (p>0.05). Despite the decreased pregnancy rate in Group 3, there were no differences in clinical pregnancy rates among the groups (p=0.204).

Conclusion: Embryo transfer is one of the critical steps in assisted reproduction procedures. Using the afterloaded external guidance embryo transfer technique did not improve pregnancy rates.

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Key words: Embryo transfer technique, pregnancy rates, IVF

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Özet

Amaç: Dış kateter yönlendirmesi ile embriyo transferinin tek embriyo transferi yapılan intrasitoplazmik sperm injeksiyonu (ICSI) siklusların-da gebelik oranlarına etkisinin araştırılmasıdır.

Gereç ve Yöntemler: Zekai Tahir Burak Kadın Sağlığı Eğitim ve Araştırma hastanesinde gerçekleştirilen retrospektif çalışmaya ICSI uygulaması yapılan 313 hasta dahil edilmiştir. Hastalar embriyo transfer tekniğine gore gruplara ayrıldı; Grup 1 (n: 232): yumuşak kateter ile kolay transfer, Grup 2 (n: 45): iç-dış kateter uygulaması ile transfer, Grup 3 (n: 36): Sert kateter ile gerçekleştirilen zor transfer. Bazal parametreler, klinik ve laboratuar ICSI sonuçları ve gebelik oranları araştırıldı.

Bulgular: Üç grubun, infertilite sebepleri, bazal folikül stimule edici hormon (FSH) düzeyleri, antral folikül sayıları, stimülasyon süresi, total gonadotropin dozu, pik estradiol düzeyleri, endometrial kalınlık, toplanan yumurta sayısı, 2 PN, fertilizasyon oranları benzerdi (p>0.05). Grup 3'te gebelik oranları daha düşük olsa da klinik gebelik oranlarında fark izlenmedi (p: 0.204).

Sonuç: Embriyo transferi yardımcı üreme tekniklerinde kritik bir adımı oluşturmaktadır. Dış kateter yönlendirmesi ile embriyo transferi uygulaması gebelik oranlarını arttırmamıştır.

(J Turkish-German Gynecol Assoc 2013; 14: 153-6)

Anahtar kelimeler: Embryo transfer tekniği, gebelik oranları, IVF

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Introduction

The pregnancy rate after embryo transfer (ET) depends on clinical and embryonic characteristics. Apart from embryo quality, endometrial receptivity and the age of the patient, pregnancy rate after ET seems to be mostly dependent on the ET technique. Cohen reported that 'bad embryo transfer' is responsible for failed implantation in 30% of cases (1). Although the importance of the ET has been suggested by studies comparing different operators or different ET catheters and techniques, the relationship between the ET technique, catheter type, operator and the clinical pregnancy rate is still controversial (2-7).

Mansour reported the use of a mock ET before starting an IVF cycle in 1990 (2). A mock ET allows the physician to choose the appropriate transfer catheter and anticipate potential problems during ET. However, a mock transfer remote from the actual ET is done under different circumstances and may not be reflective of the actual conditions encountered on the day of ET (3). Sharif et al. (3) proposed to circumvent this problem by performing a mock ET immediately before the actual ET. To avoid additional trauma by the passage of two

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separate catheters, Neithardt began transferring embryos by an afterload technique (afterloaded external guidance), in which an empty catheter is placed at, or just past, the internal cervical os. The inner sheath is withdrawn, and a second inner sheath containing the embryos is passed. This gives the provider the benefit of an immediate mock transfer while minimizing manipulation of embryos and possibly reducing trauma to the endometrium (6).

We performed a retrospective analysis of 313 single ET ICSI cycles to determine the impact of the transfer technique on pregnancy rates.

Material and Methods

From March 2010 to February 2012, at the Dr. Zekai Tahir Burak Women's Health Research and Education Hospital, IVF Department, 313 consecutive single ET ICSI cycles were retrospectively reviewed. This study was approved by the ethics committee and institutional review board of Dr. Zekai Tahir Burak Women's Health Research and Education Hospital. Written informed consent was obtained from all volunteers. A total of 313 otherwise healthy women who complained of primary infertility were eligible. Only fresh cycles were included.

Pituitary down-regulation was achieved and maintained using the long protocol luteal phase administration of a GnRH agonist. The GnRH agonist leuprolide acetate (Lucrin daily; Abbott Cedex, Istanbul, Turkey) was initiated on day 21 of the preceding luteal phase (0.5 mg/d SC) until menses and dropped to 0.25 mg/d until triggering ovulation. Recombinant (rec) FSH (Puregon; Organon, Oss, the Netherlands; or Gonal F; Serono, Istanbul, Turkey) was used for ovulation stimulation. The initial gonadotropin dose used for ovarian stimulation was individualized according to the patient's age, baseline serum FSH concentrations on day 3, body mass index, and previous response to ovarian stimulation. The starting regimen was fixed for the first 3 days (100-225 IU rec FSH/day), and thereafter the dose of gonadotropin was adjusted according to the individual ovarian response. Serum estradiol concentrations and transvaginal ultrasonography were measured routinely every 2-3 days thereafter. Ovulation was triggered by administration of rec HCG (Ovitrelle, 250 μgr, Serono, Istanbul, Turkey) when at least two follicles reached 18 mm in diameter. Oocytes were retrieved at 36 hours after HCG injection and subjected to intracytoplasmic sperm injection regardless of infertility origin. ET was done on day 3.

Embryo transfer technique

All ETs were performed with a full bladder under ultrasound guidance (Aloka SSD-1000, Germany) using a catheter (Rocket Genesis embryo transfer catheter system R57630-00-23, R57591-00-23). The difficulty of the ET was determined according to the opinions of two physicians with the same practice and experience with the transfer technique. The scored difficulty of transfers generated by the two physicians was the same for all the transfers. The ETs were scored as easy transfer with a soft catheter, moderate transfer with external guidance, or difficult transfer with a stylet.

Direct Embryo Transfer

The patient was placed in the lithotomy position. A sterile bivalve speculum was placed in the patient's vagina and the cervix was exposed. Excess mucus and debris were cleared from the ectocervix using sterile cotton swabs dampened with phosphate-buffered saline. The embryos were loaded into the transfer catheter by the embryologist as described elsewhere, and the catheter was passed to the transfer physician. The embryos were then deposited approximately 1.0 cm from the uterine fundus under ultrasound guidance. After approximately 5 seconds, the catheter was gently removed. The embryologist immediately flushed the catheter with media to check for retained embryos, blood, or mucus. Patients remained supine for 10 minutes after the procedure.

Difficult transfers were managed first with the use of external guidance with introduction of the catheter through an advanced sheath rather than with a stylet. We excluded cycles in which a teneculum was used.

Afterloaded External Guidance Embryo Transfer

An empty catheter was passed to the level of the lower uterine segment under ultrasound guidance to a point where the inner catheter entered the endometrial cavity. The inner sheath was slowly removed, leaving the outer sheath just beyond the internal os. A second inner sheath was loaded by an embryologist who then assisted the transfer physician in threading the inner sheath into the catheter. The inner catheter was slowly advanced by the physician, and the embryos were deposited 1.0 cm from the fundus. After approximately 5 seconds, the catheter was gently removed. The embryologist immediately flushed the catheter with media to check for retained embryos, blood, or mucus. Patients remained supine for 10 minutes after the procedure.

Luteal phase support was routinely given as progesterone in the form of Crinone 8% gel (90 mg; Serono) daily for 14 days, until a pregnancy test was performed.

Subjects were categorized according to embryo transfer technique; Group 1 (n: 232): easy transfer with a soft catheter, Group 2 (n: 45): afterloaded external guidance transfer, Group 3 (n: 36): difficult transfer with a stylet. ETs with blood (n: 9) or mucus (n: 3) on the catheter tip were not included in the study. Data were collected for baseline (age, day 3 FSH level, antral follicle count) and stimulation parameters (duration of stimulation, total gonadotropin dose, number of follicles, peak estradiol levels, endometrial thickness), and cycle outcome (oocytes, mature oocytes, embryo transfer type and pregnancy rate). Clinical pregnancies were defined as those with fetal heart activity documented on ultrasound examination 4-5 weeks after embryo transfer.

Data analysis was performed by using SPSS for Windows, version 11.5 (SPSS, Inc., Chicago, IL, USA). We used one-way ANOVA, Kruskal Wallis, and Chi-square tests for the analysis. A P value less than 0.05 was considered statistically significant.

Results

Mean age, baseline FSH, estradiol levels, antral follicle count, duration of stimulation, gonadotropin dose, peak estradiol lev-

els, endometrial thickness, oocyte number, 2 PN, and fertilization rate were not different among the three groups (p>0.05). Despite the decreased pregnancy rate in Group 3, there were no differences in clinical pregnancy rates between the groups (p=0.204). All results are shown in Table 1.

Discussion

The ET technique is equally important as clinical and embryonic characteristics regarding the pregnancy rate in ART cycles. In the last decade, it has been demonstrated by many studies that differences in technique may affect pregnancy rates (2-7). There have been studies comparing the effect of different transfer catheters, different operators or transfer type on outcome. Although Burke reported that the difficult ET did not affect the clinical pregnancy rate, Abusheika found that a difficult embryo transfer technique negatively affects pregnancy rates (8, 9). On the other hand, a meta-analysis by Abou-Setta demonstrated that softer catheters are associated with higher clinical pregnancy rates than firmer catheters by overall comparison (10). Zhan Yao found that variation in pregnancy rates between embryo transfer catheters depends on variation between operators (11). Many investigators reported improved pregnancy rates when uterine contractions were minimized by the use of soft catheters, ultrasound guidance and fixed distance transfers (4, 6, 12). To avoid additional trauma by the passage of two separate catheters, Neithardt began transferring embryos by an afterload technique (afterloaded external guidance) (6). We performed a retrospective analysis of 313 single ET ICSI patients to determine whether there were differences in pregnancy rates based on the transfer technique using external guidance. In this study, we had a rather homogenous group of patients receiving only an agonist protocol with single ET cycles. All transfers were done on day 3. In our study, two senior operators performed all ETs, which in turn minimized the impact of operator skill and experience on the outcome. There were no differences in the baseline parameters like, day 3 FSH and E2, antral follicle count, duration of the stimulation, total dose of gonadotropin, number of oocytes retrieved and 2 PN, and fertilization rates. In our study, all transfers are performed under ultrasound guidance and at a fixed distance of 1 cm from the fundus which is the favored location according to recent studies (13). The results of the present study show that difficult transfers were associated with a lower clinical pregnancy rate (CPR) compared to easy transfers and afterloading, but this did not reach statistical significance. Difficult transfers were associated with the lowest CPR, but the number of cases in this group was relatively smaller than the others.

Neithardt et al. compared direct ET with afterloaded ET and reported an improved implantation rate (20.5% vs. 24.7%) and CPR (34.9% vs. 52.4%) with afterloading, although the difference was not statistically significant. They also found significantly more transfer catheters with mucus in the direct transfer group. Mucus on the transfer catheter has been proposed to adversely affect implantation either by contamination of the cavity or by causing retention or displacement of the embryos. The authors explained this by avoidance of passage of embryos through the initial inner sheath placed in the cervical canal, which they

Table 1. Clinical and laboratory parameters of the groups

	Easy (n:232)	External guidance (n:45)	Difficult transfer with a stylet (n:36)	p
Age (years)	29.7±4.5	29.3±4.1	28.6±4.1	0.339
Infertility etiology				0.126
Male	107 (46.1%)	24 (53.3%)	25 (69.4%)	
Tubal	12 (5.2%)	2 (4.4%)	1 (2.8%)	
Unexplained	113 (48.7%)	19 (42.2%)	10 (27.8%)	
Baseline FSH (IU/L)	6.1 (0.5-12.1)	6.6 (3.6-10.7)	5.7 (2.5-9.2)	0.088
Baseline E2 (pg/mL)	55 (4.3-176)	58 (23.2-153)	56.5 (14-126)	0.590
Antral follicle count (n)	11 (2-16)	10 (3-14)	12.5 (1-16)	0.825
Duration of stimulation (days)	10 (6-23)	10 (6-14)	10 (6-17)	0.933
Gonadotrophin dose (IU)	1650 (475-7700)	1900 (600-5850)	1687.5 (750-4400)	0.498
Peak E2 (pg/mL)	2215 (192-6867)	2379 (547-5963)	2490 (910-4988)	0.706
Endometrial thickness (mm)	10 (5-18)	10 (5-15)	11 (6-19)	0.160
Oocyte number	9 (1-32)	8 (1-28)	10 (3-28)	0.440
2PN	4 (1-15)	5 (1-12)	4 (1-17)	0.308
Fertilization rate (%)	60 (0.5-100)	50 (12.5-100)	50 (10-100)	0.130
Clinical pregnancy rate (%)	92 (39.7%)	15 (33.3%)	9 (25.0%)	0.204

Values are expressed as mean±standard deviation, median (interquartile range), n (%), p<0.05 is significant,
FSH: follicle stimulating hormone; E2: estradiol; PN: pronucleus

believe decreased mucus contamination of the catheter (6). Our study is different from Neithardt's study since we excluded all ETs with blood or mucus on the catheter tip to evaluate the impact of the technique rather than other confounding factors; furthermore, we included only single ET cycles. Our findings are consistent with their results with regard to similar CPRs in external guidance and easy transfer with a soft catheter ETs.

In another study, a cohort of 784 consecutive cycles with four different types of catheters were compared: a) a rigid preloading-type catheter b) a rigid afterloading type with an obturator and a soft inner catheter c) a ball-pointed rigid afterloading catheter with a soft inner catheter d) an afterloading type catheter with an obturator and an inner ultrasoft catheter. All ETs were performed by a single operator. The ultrasoft catheter was found to produce the highest pregnancy and implantation rates compared to the more rigid Frydman catheter. Negotiation of the cervix, using the volsellum, and the presence of the blood on the catheter wall or on the cervix did not affect the results. Changing the catheter or blood on the catheter tip significantly diminished the pregnancy and implantation rates (12). Sallam et al. (12) reported a low CPR with a rigid catheter compared to external guidance. In our study, despite the decreased pregnancy rates in the rigid catheter group, there were no differences in the clinical pregnancy rates between the groups. This may be attributable to the small size of our rigid catheter group. Recently, Spitzer et al. (7), reported lower CPR and live birth delivery rate (LBDR) in the external guidance ET group than the soft catheter ET group and the group in which the cervix was probed with a stylet (7). However, the groups of patients were not homogenous in this study and D5 transfers were done in a great majority of patients, which is not the case in most clinical settings. Furthermore, LBDR is mainly related to embryonic characteristics rather than the ET technique. Spitzer et al. estimated that external guidance may be related to a greater risk of transferring mucus and blood into the fundus, which is in contrast to the findings of Neithardt et al. We excluded ETs with blood or mucus on the catheter tip to minimize the negative effect on implantation.

In conclusion, we recommend an afterloaded external guidance ET technique when direct ET with a soft catheter is not available, especially in training centers like our institution. It is a simple procedure with similar CPR as direct ET with a soft catheter. Randomized controlled trials with a large number of patients are required to identify the impact of different ET techniques on ART outcomes.

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

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

Peer-review: *Externally peer-reviewed.*

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Conventional box model training improves laparoscopic skills during salpingectomy on LapSim: a randomized trial

Geleneksel kutu tipi eğitim modeli ve laparoskopik becerilerin geliştirilmesi: Randomize çalışma

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Abstract

Objective: Box model trainers have been used for many years to facilitate the improvement of laparoscopic skills. However, there are limited data available on box trainers and their impact on skill acquisition, assessed by virtual reality systems.

Material and Methods: Twenty-two Postgraduate Year 1 gynecology residents with no laparoscopic experience were randomly divided into one group that received structured box model training and a control group. All residents performed a salpingectomy on LapSim before and after the training. Performances before and after the training were assessed using LapSim and were recorded using objective parameters, registered by a computer system (time, damage, and economy of motion scores).

Results: There were initially no differences between the two groups. The box trainer group showed significantly greater improvement in time (p=0.01) and economy of motion scores (p=0.001) compared with the control group post-training.

Conclusion: The present study confirmed the positive effect of low cost box model training on laparoscopic skill acquisition as assessed using LapSim. Novice surgeons should obtain practice on box trainers and teaching centers should make efforts to establish training laboratories. (J Turkish-German Gynecol Assoc 2013; 14: 157-62)

Key words: Laparoscopy, skill, box trainer, LapSim

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Özet

Amaç: Geleneksel kutu tipi laparoskopi eğitim modelleri yıllardır laparoskopik beceri gelişimini kolaylaştırmak amacıyla kullanılmaktadırlar. Fakat kutu tipi eğitim modelleri ve bunların laparoskopik beceri kazanılması üzerine etkilerinin sanal gerçeklik sistemler ile değerlendirilmesi konusunda sınırlı bilgi mevcuttur.

Gereç ve Yöntemler: Laparoskopik deneyimi olmayan 22 birinci yıl jinekoloji asistanı gruplardan birincisi kutu tipi eğitim modelinde yapılandırılmış eğitim alacaklar ve ikincisi kontrol grubu oluşturacak şekilde iki gruba randomize edilmiştir. Tüm asistanlar eğitim öncesinde ve sonrasında LapSim de salpenjektomi gerçekleştirmiştir. Eğitim öncesi ve sonrası performansları LapSim'in bilgisayar sistemi sayesinde elde edilen objektif parametreler kullanılarak (zaman skoru, hasar oranı, hareket ekonomisi skoru) değerlendirildi.

Bulgular: Başlangıçta iki grup arasında her hangi bir fark saptanmadı. Eğitim sonrasında, kutu modelde eğitim alan grup kontrol grubuna göre, zaman (p=0.01) ve hareket ekonomisi skorlarında (p=0.001) anlamlı olarak daha fazla gelişme göstermişlerdir.

Sonuç: Bu çalışma, düşük maliyetli kutu tipi laparoskopi eğitim modelinin laparoskopik beceri kazanımı üzerine olumlu etkisinin bulunduğu LapSim kullanılarak konfirme edilmiştir. Laparoskopi konusunda tecrübesiz cerrahlar kutu tipi eğitim modelinde egzersiz yapmalıdırlar. Ayrıca eğitim hastaneleri laparoskopi eğitim laboratuarlarına sahip olmak için gerekli çabayı göstermelidirler.

(J Turkish-German Gynecol Assoc 2013; 14: 157-62)

Anahtar kelimeler: Laparoskopi, beceri, kutu tipi eğitim modeli, LapSim

Geliş Tarihi: 9 Temmuz 2013 **Kabul Tarihi:** 25 Temmuz 2013

Introduction

Reduced mortality, faster postoperative recovery, shorter hospital stays, and better cosmetic results are the main advantages of laparoscopic surgery and thus it has become a standard approach for many conditions in most surgical fields (1, 2). On the other hand, it is obvious that laparoscopic surgery is associated with a longer operation time and a higher surgical complication rate during the learning curve of the laparoscopic technique, which requires novel and unique psychomotor skills, such as the transfer to 2-D from 3-D, long instruments that amplify tremor, reduced tactile feedback, and the fulcrum effect (3, 4). Additionally, laparoscopic skills are fundamentally different from those used for traditional open surgery, leading to a prolonged learning curve. Moreover, these skills cannot be acquired exclusively via the old apprenticeship model of observing and assisting (5).

Laparoscopic simulators, such as box model trainers and virtual reality simulators (VR) have been used for many years to facilitate the acquisition of skills needed for laparo-

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scopic surgery. The former, which incorporates conventional laparoscopic equipment, is a relatively inexpensive and highly versatile device that enables training on animal parts as well as synthetic inanimate models (6). The latter shows a great potential for both training and assessment purposes. VR replicates laparoscopic instruments as surgeons navigate through and interact with the environment using their natural senses and skills. Now, laparoscopic VR software can replicate procedure-specific tasks such as salpingectomy, tubatomy, myomectomy, and even hysterectomy. It can also be used more easily as an objective tool for assessing laparoscopic skills than the box trainer (5, 7).

The aim of the current study was to evaluate the effectiveness of training on the box trainer with regards to salpingectomy training using the LapSim (LapSim®, Surgical Science, Gothenburg, Sweden) VR simulator.

Material and Methods

The study was conducted as a prospective, randomized, controlled trial. Twenty-two Postgraduate Year 1 gynecology residents with no laparoscopic experience were randomly assigned to either a box trainer group or a control group using the sealed envelope technique. All residents completed a questionnaire regarding demographics, handedness, and any previous experiences with laparoscopic surgery, the box trainer, the LapSim simulator, or video games.

The aim of the study was explained to all the subjects, and informed consent was obtained prior to participation in the trial. The training was spread over a period of 5 weeks. During the first week, all residents were given didactics about the LapSim and each of them performed a salpingectomy on the ectopic pregnancy module of the LapSim as an initial test. Thereafter, during the subsequent four weeks (one hour weekly), the box group received a structured box model training curriculum and the control group did not receive any training. One week after the training period had finished, both groups performed another salpingectomy on the LapSim as a post-training test (Figure 1).

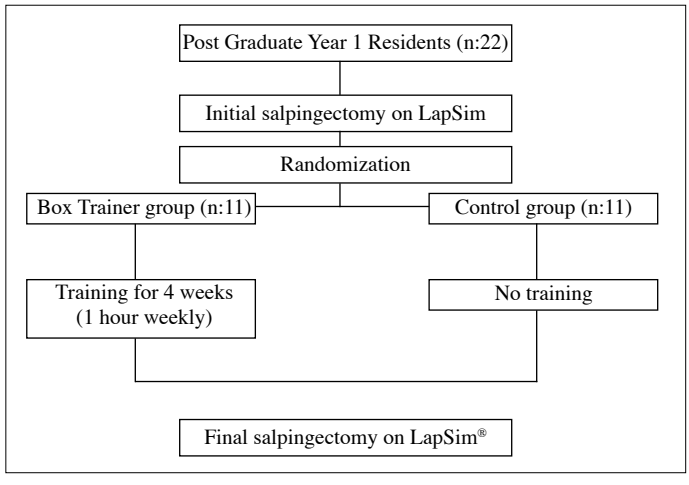

Figure 1. Experimental design

Each participant performed four sessions of a salpingectomy on the LapSim during both the initial and post-training tests. Since it was previously shown that there is a familiarization curve with the LapSim, the first three sessions were used for this purpose (8).

Performance before and after training was assessed and based on objective parameters registered by the computer system (time, damage, and economy of motion scores).

Equipment

LapSim® (Gothenburg, Sweden) is a PC-based VR system that consists of a 19 inch monitor and a laparoscopic interface module with two instruments and a foot-switch. The software is run on a dual-processor Pentium D 3 GHz computer with 1 GB RAM and GeForce 6800 graphics card using Windows XP Professional. The LapSim® has been extensively validated as a relevant tool for training of laparoscopic skills (Figure 2).

The ectopic pregnancy module of the LapSim provides a realistic image of the procedure with which subjects can interact to perform a salpingectomy (Figure 3). The difficulty level of the module can be altered from level 1 (easy) to level 3 (difficult); this was set to level 1 for the present study. The subject interacts with the simulator through a virtual laparoscopic interface. This is a frame holding two standard laparoscopic instruments in an appropriate position. The nature of the instrument is changed virtually and thus a laparoscopic grasper, bipolar grasper, diathermy scissors, suction and bag are available for use. A pedal, which

Figure 2. The LapSim

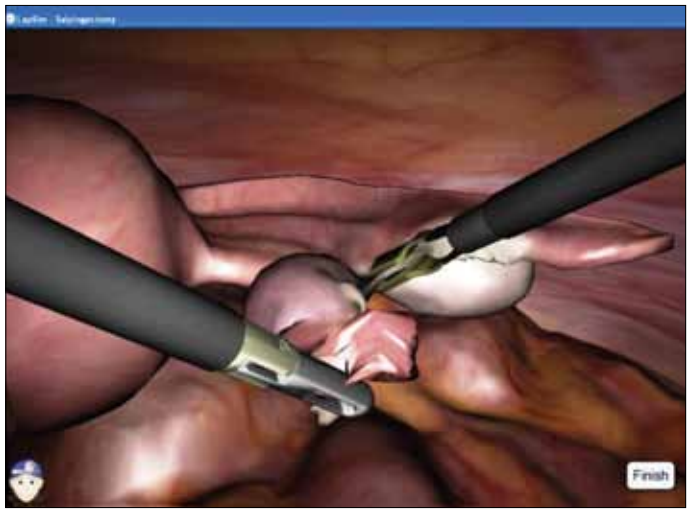

Figure 3. A demonstration of a simulated salpingectomy

Results for: Salpingectomy

Overall Score: 42%

Parameter	Value	Graph	Min	Max	Passed	Score
Total Time (s)	964.8		0	360	Failed	0%
Blood Loss (mL)	100.92		0	500	Passed	100%
Bleeding (mL/s)	0		0	1	Passed	100%
Pool volume (mL)	0.87		0	10	Passed	100%
Ovary Diathermy Damage (s)	0.54		0	10	Passed	100%
Tube Cut: Ulterus Distance (mm)	0		0	50	Passed	100%
Bleeding vessel cut (max 1)	1		0	1	Passed	0%
Evacuation from the body	0		1	1	Failed	0%
Left Instrument Path Length (m)	14.43		0	3	Failed	0%
Left Instrument Angular Path (dengrees)	2402.33		0	720	Failed	0%
Right Instrument Path Length (m)	17.36		0	5	Failed	0%
Right Instrument Angular Path (dengrees)	2642.33		0	1080	Failed	0%

Figure 4. Summary metrics of LapSim

is operated by the subject, doubles as a diathermy or suction device depending on which instrument has been selected.

The aim of the task is to perform a right-sided salpingectomy using the left hand grasper to identify the mesosalpinx and the right hand bipolar grasper to cauterize the mesosalpinx, followed by the right hand diathermy scissors. Once the salpingectomy has been fully completed, the tube should be placed in a virtual bag and any residual bleeding should be controlled. When the subject is satisfied that the task is completed, the simulation ends. Summary metrics include total time taken to complete each task (seconds), path length of each hand (meters), angular path length of each hand (degrees), total blood loss (milliliters), ovarian diathermy damage (seconds), residual bleeding rate (milliliters/seconds) and amount of un-removed dissected tissue (if any). These are subsequently recorded using the computer and can be downloaded into a spreadsheet format (Figure 4).

The box trainer was constructed using dark plastic in the shape of a rectangular prism (size 45×30×25 cm). Five holes were cut out for the camera and trocars. The right side of the box trainer was left open in order to put the training tools inside (Figure 5).

Figure 5. The Box Trainer

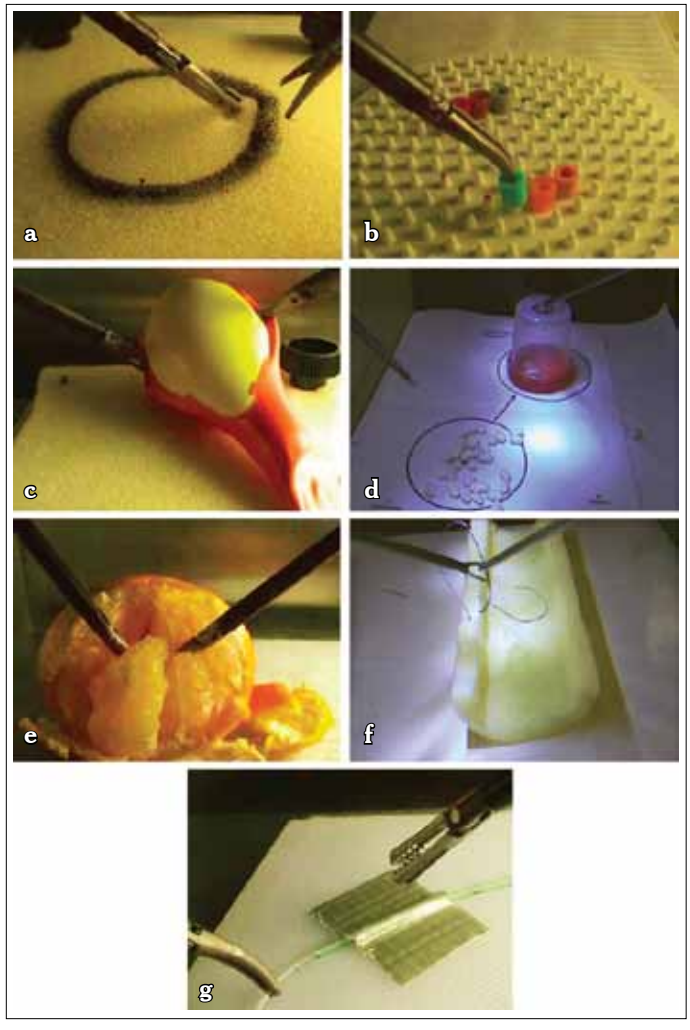

Figure 6. Box trainer exercises. Cutting out a drawn circle (a). Moving pegs on a board (b). Cutting out the inner balloon to simulate enucleating an ovarian cyst (c). Grasping and dropping beans into a small box (d). Peeling a mandarin (e). Suturing and knot tying (f). Tubulation (g)

Task description (Figure 6)

Task 1: Cutting out a drawn circle with a diameter of 4 cm from a thin sponge directly on the drawn line. The sponge material is fixed to the base of the box trainer.

Task 2: Moving pegs on a board. Ten small plastic pegs are moved to predefined spots.

Task 3: Cutting out the inner balloon of two balloons to simulate enucleating an ovarian cyst, without rupturing the inner balloon. The inner balloon is filled with ultrasound gel.
Task 4: Grasping and dropping beans into a small box.
Task 5: Peeling a mandarin.
Task 6: Suturing a sponge and tying a knot. The sponge is fixed onto a board.
Task 7: Introducing an epidural catheter into a piece of intravenous infusion tube. The piece of tube is fixed onto a board. The epidural catheter is initially placed beside the tube.

Evaluation of performance

Evaluation of performance was based on time, economy of motion score (left and right instrument path length and angular path) and damage score (bleeding, ovarian thermal damage).

Statistics

Data was analyzed using the SPSS 15.0 software package. The normality of the data was assessed using the Shapiro-Wilk test. Because the data were non-parametric, Mann-Whitney's U test was used to assess the differences between the two groups regarding all performance parameters. The Wilcoxon signed rank test for related data was used to assess differences in performance of all of the parameters. Significance was set at $p<0.05$. Post hoc power analysis was performed using NCSS-pass 2000 software package. Group sample sizes of 11 and 11 achieved 93%, 94% and 97% power to detect a difference between two groups for the time, instrument path length and instrument angular path scores, respectively, with a significance level (alpha) of 0.05 using a two-sided Mann-Whitney test.

Results

All 22 participants assigned to take part in the study completed it (Table 1). Analysis of the initial performance data showed no significant differences between the two groups in all parameters. In comparison with the control group, the box trainer group performed significantly better regarding the time and economy of motion scores in the post-training data, but there were no differences in damage scores (Table 2, 3).
Analysis of pre- and post-training performance data of the box trainer group showed a significant improvement in the time and economy of motion scores, but there were no differences in damage scores (Table 4).

Table 1. Demographics of the participants

Group Demographics	Box Trainer Group (n:11)	Control Group (n:11)
Age (year)	28.2±1.7	27.8±2.0
Female/male	6/5	6/5
Dominant hand (R:L)	10/1	10/1
Previous laparoscopic experience	None	None
Previous LapSim experience	None	None
Previous Box trainer experience	None	None
Video game play experience		
None	5	4
Occasionally	2	2
Formerly	1	3
Often	3	2

Table 2. Pre-training test performance. Better performance is represented by a lower score

	Box Trainer Group Median / IR	Control Group Median / IR	p
Time (sec)	321 (107)	325 (73)	0.818
Damage Score			
Ovarian diathermy damage (sec)	0.28 (0.41)	0.55 (0.47)	0.308
Bleeding (mL)	5.36 (12.24)	14.17 (12.37)	0.139
Economy of Motion			
Instrument path length (m)	7.35 (5.10)	8.52 (2.57)	0.412
Instrument angular path (degrees)	1264.34 (671.10)	1508.93 (600)	0.094
Mann Whitney U test, Numbers in the parenthesis represents the interquartile range (IR)			

Table 3. Post-training test performance. Better performance is represented by a lower score

	Box Trainer Group	Control Group	
	Median / IR	Median / IR	p
Time (sec)	196 (72)	280 (67)	0.01
Damage Score			
Ovarian diathermy damage (sec)	0.11 (0.45)	0.13 (1.41)	0.261
Bleeding (ml)	7.45 (6.81)	23.7 (31.66)	0.317
Economy of Motion			
Instrument path length (m)	5.03 (0.97)	10.07 (3.86)	0.001
Instrument angular path (degrees)	693.63 (163.14)	1838.81 (664.62)	0.001
Mann Whitney U test, Numbers in the parenthesis represents the interquartile range (IR)			

Table 4. Performance of the Box Trainer group pre- and post-training. Better performance is represented by a lower score

Box Trainer group			
	Pre-training	Post-training	
	Median / IR	Median / IR	p
Time (sec)	321 (107)	196 (72)	0.003
Damage score			
Ovarian diathermy damage (sec)	0.28 (0.41)	0.11 (0.45)	0.169
Bleeding (ml)	5.36 (12.24)	7.45 (6.81)	0.317
Economy of Motion			
Instrument path length (m)	7.35 (5.10)	5.03 (0.97)	0.003
Instrument angular path (degree)	1264.34 (671.60)	693.63 (163.14)	0.003
Wilcoxon's signed-rank test, Numbers in the parenthesis represents the interquartile range (IR)			

Discussion

The present study shows that the use of a box trainer can improve laparoscopic skill performance (measured by VR simulator). The improvement in economy of motion scores, which have been shown to have the greatest validity in the assessment of laparoscopic technical skills (9), was found to be the greatest. Box model trainers have been used to improve laparoscopic skills. There have been several studies in the literature which indicate that box trainers can improve laparoscopic skill (2, 10). For performance assessment, these studies have generally used an experienced surgeon who observed the procedure and/or special devices. This is time consuming and expensive. As can be seen, the main drawback of the box trainer is the lack of a feasible system for performance assessment. Despite this, box trainers carry important advantages include the use of real laparoscopic instruments, tactile feedback and the opportunity to work with animal parts (2).

The LapSim virtual simulator was used in the present study and has been found to improve relevant skills for surgical performance in real operations (1, 11, 12). One of the main advantages of LapSim is having a feasible performance assessment system thanks to its software. One study in which participants completed the basic training tests before and after training on a box trainer showed that structured laparoscopic skill training on a box trainer improves time, economy of motion and damage scores, as assessed using a simple test in the LapSim system (4). Mainly, damage scores for the simple test for LapSim are quite different to those for the salpingectomy module. Tissue damage, which is strongly associated with tactile feedback, is the damage score for the simple test. On the other hand, bleeding and ovarian diathermy damage are the damage scores for salpingectomy; these are mostly associated with procedural knowledge. It is also known that procedural knowledge does not change with training using either VR or the box trainer (1, 11, 13). Similarly, in our study, damage scores were not affected by training on the box trainer.

The small sample size may be one of the limitations for the present study, yet it can be seen in the available literature that most studies have similar sample sizes. Additionally, we used some common training exercises instead of approved or certificated exercises for the box trainer. Despite all our efforts, we could not find any certified exercises. Related to this issue, there is a lack of agreement regarding which exercises are most useful and effective for skill improvement (4). This will be taken into account in further studies.

In conclusion, training on a traditional low-cost box trainer can help improve basic laparoscopic skills. All surgeons who

want to improve their skills relevant to minimally invasive surgery should practice on trainers. Additionally, teaching centers should make more effort to have training laboratories which include box trainers and even VR simulators.

Ethics Committee Approval: *N/A.*

Informed Consent: *Informed consent was received from the participants of the study.*

Peer-review: *Externally peer-reviewed.*

Author contributions: *Concept – A.A., F.Ş.; Design – A.A., A.M.E.; Supervision – F.Ş., A.Ö.Y.; Resource – A.A., A.Ö.Y.; Materials – A.A., A.M.E.; Data Collection&/or Processing – A.A., A.M.E., Analysis&/or Interpretation – A.Ö.Y.; Literature Search – A.A.; Writing – A.A.; Critical Reviews – F.Ş.*

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

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The effects of prenatal sex steroid hormones on sexual differentiation of the brain

Prenatal seks steroit hormonlarının beyindeki cinsiyet farklılaşması üzerine etkileri

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Abstract

Most of the anatomical, physiological and neurochemical gender-related differences in the brain occur prenatally. The sexual differences in the brain are affected by sex steroid hormones, which play important roles in the differentiation of neuroendocrine system and behavior. Testosterone, estrogen and dihydrotestosterone are the main steroid hormones responsible for the organization and sexual differentiation of brain structures during early development. The structural and behavioral differences in the female and male brains are observed in many animal species; however, these differences are variable between species. Animal and human (in vivo imaging and postmortem) studies on sex differences in the brain have shown many differences in the local distribution of the cortex, the gray-white matter ratio, corpus callosum, anterior commissure, hypothalamus, bed nucleus of the stria terminalis, limbic system and neurotransmitter systems. This review aims to evaluate the anatomical, physiological and neurochemical differences in the female and male brains and to assess the effect of prenatal exposure to sex steroid hormones on the developing brain.
(J Turkish-German Gynecol Assoc 2013; 14: 163-7)

Key words: Brain gender, sex differences, sex steroid hormones, prenatal development

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Özet

Beyindeki cinsiyete bağlı anatomik, fizyolojik ve nörokimyasal farklılıkların birçoğu prenatal dönemde oluşmaktadır. Beyindeki seksüel farklılıklar nöroendokrin sistem ve davranışların farklılaşmasında önemli rol oynayan seks steroit hormonlarından etkilenmektedir. Testosteron, östrojen ve dihidrotestosteron erken gelişim döneminde beyindeki yapıların organizasyonundan ve seksüel farklılaşmasından sorumlu olan temel steroit hormonlardır. Dişi ve erkek beyinlerindeki bu yapısal ve davranışsal farklılıklar, birçok hayvan türünde gözlenmekle birlikte türler arasında çeşitlilik göstermektedir. Beyindeki cinsiyete bağlı farklılıklar ile ilgili hayvan ve insan (in vivo görüntüleme ve otopsi) çalışmaları korteksin bölgesel dağılımında, gri-beyaz madde oranında, korpus kallosumda, anterior komissürde, hipotalamusta, stria terminalisin bed nukleusunda, limbik sistemde ve nörotransmitter sistemlerde birçok fark ortaya koymuştur. Bu derlemede dişi ve erkek beyinlerindeki anatomik, fizyolojik ve nörokimyasal farklılıkların değerlendirilmesi ve seks steroit hormonların prenatal maruziyetinin gelişen beyin üzerine etkilerinin incelenmesi amaçlanmıştır.
(J Turkish-German Gynecol Assoc 2013; 14: 163-7)

Anahtar kelimeler: Beyin cinsiyeti, cinsiyet farklılıkları, seks steroit hormonlar, prenatal gelişim

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Sex steroid hormones and brain gender

The anatomical and physiological differences between the genders are referred as sexual dimorphism. The term dimorphism (from the Greek word meaning having two forms) indicates phenotypic differences between males and females of the same species. Sexual dimorphism is seen in the reproductive system and in the structure of the central nervous system and cognitive functions as well. Usually, sex steroid hormones are shown to be the main reason for the occurrence of female and male brain differences. Indeed, sexual differentiation is determined by chromosomal, genetic and hormonal factors (1, 2). Some studies have shown that sex differences in the central nervous system appear before the onset of the release gonadal hormones during embryonic development. Early genetic events, such as cell migration, can trigger sexual differentiation of brain, independent of hormonal action. After sex steroid hormones and their receptors become available, the influence of gonadal steroids on sexual differentiation become apparent (3). The maternal and fetal derived sex steroid hormones, especially testosterone, estrogen and dihydrotestosterone which play roles in sexual differentiation of neuroendocrine system and behavior, affect the fetal brain during gestation (1).

Production and the mechanism of action of sex steroid hormones

The typical male phenotype is determined during embryonic development by the sex-determining region Y (Sry) gene, which is located on the Y chromosome. Around the sixth week of gestation, this region of the chromosome activates testicular determining factor (TDF) that initiates testicular differentiation. In the testicles, Leydig cells begin to produce testosterone. Conversely, differentiation of the ovaries in the female fetus begins approximately at week

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8 of gestation (4). The fetal ovary seems to be inactive until late fetal development because it releases a small amount of estrogen prenatally. Furthermore, both female and male fetuses are exposed to high estrogen levels produced by the placenta. Although the gender differences seem to be important between weeks 8–24 of gestation, this is not the only period for differentiation. The maximum difference in serum testosterone concentration between genders is seen between 12 and 18 weeks. The fetuses are also exposed to small amounts of androgen from the fat tissue and adrenal gland of the fetus and mother (5, 6).

All sex steroid hormones are synthesized from cholesterol and carried to their target cells. Because of its small size and lipophilicity, circulating testosterone can cross the blood-brain barrier and pass through the cell membrane. Once testosterone enters the cytoplasm, it binds to its intracellular receptor (5, 7). Then, testosterone can be converted to dihydrotestosterone by 5α-reductase or to 17β-estradiol (a form of estrogen) by aromatase enzymes. The classic view suggests that the main mechanism for masculinization of the brain is via the neural aromatization of testosterone. Thus, interestingly, testosterone appears to be converted to an estrogen form and exerts its final effect via binding to estrogen receptors. However, dihydrotestosterone and other androgens also show some effects through androgen receptors. Therefore, the main factor is suggested to be the reciprocal interaction between estrogen receptors and androgen receptors in the male fetal brain (8).

The feminization process is different since the female fetal brain is exposed to high levels of estrogen produced by the placenta and mother. Alpha-fetoprotein, a plasma glycoprotein, binds estrogen and acts as a carrier. Thus alpha-fetoprotein seems to protect the fetal brain from the masculinizing effect of estrogen by preventing its entry into cells. However alpha-fetoprotein does not bind circulating testosterone. Taken together, it might be assumed that feminization may be a passive process, but controversy continues over this process (2, 9).

Some clinical studies have indicated that sex steroid hormones influence early human development. For example, individuals with complete androgen insensitivity syndrome (CAIS), who are 46, XY and lack functioning androgen receptors, have normal testes that produce normal male levels of testosterone. Because the target organs cannot respond to androgen, individuals with CAIS are born phenotypically female. As mentioned above, if neural aromatization of testosterone is the only mechanism for masculinization of the brain, then individuals with CAIS should show behavioral masculinization because their estrogen receptors are normal. However, individuals with CAIS display feminine behavior similar to that of normal girls (2). Another example is congenital adrenal hyperplasia (CAH), in which both sexes are exposed to excess adrenal androgen prenatally. Because of the excess androgens, females with CAH display more male-type behaviors including toy, playmate and activity preferences, even in childhood (6). Autism Spectrum Conditions (ASC), characterized by difficulties with social interaction and empathy, are more common in males. Many studies have indicated that elevated fetal testosterone is a potential risk factor for ASC (10).

Sex differences in the animal brain

Central nervous system exhibits structural and behavioral differences between genders due to exposure to sex steroid hormones during gestation. Most of the sexual behavioral differences are a part of reproductive behavior, whereas others pertain to cognitive functions; all of these behaviors vary among species. For example, Nottebohm and Arnold (1976) discovered that some brain regions are highly sexually dimorphic in songbirds. Singing is a reproductive behavior and songbirds (canaries, zebra finches, etc.) sing complex songs to attract mates. Interestingly, males but not females can produce intricate songs because of the nuclei in the vocal cord region in the brain that controls singing in songbirds. These nuclei are approximately three (canaries) to five (zebra finch) times larger in volume in males, who sing, compared to females, who do not. These nuclei are sexually dimorphic and sex differences in the volume of these nuclei arise from the action of sex steroid hormones. The sizes of these nuclei are larger in the females exposed to testosterone during development and females sing like males after testosterone treatment (4, 11). Similarly, in rodent and non-human primate studies, female animals exposed to testosterone during early development show increased male-typical behavior (12).

Gorski et al. (13) discovered a sexually dimorphic nucleus in the preoptic area of the male rat hypothalamus. The preoptic area, the most distinctive region for sexual morphometric differences, controls copulatory behavior in the male rat. The volume of the sexually dimorphic nucleus of the preoptic area (SDN-POA) is three or five times larger in males than in females. Normally, the SDN-POA is exposed to neuronal apoptosis in newborn rats. But, circulating testosterone, after being converted to estradiol, prevents SDN-POA neuronal apoptosis during the perinatal period. Thus, the volume of the SDN-POA remains larger in males than in females (14).

The anteroventral periventricular (AVPV) nucleus, which is also a sexually dimorphic area, is located in the rat hypothalamus. The volume of the AVPV is approximately two times larger in females than in males. The AVPV has been suggested to play a key role in regulating the phasic secretion of gonadotropin-releasing hormone in female rats. It contains many neurons expressing ovarian steroid hormone receptors (15, 16).

Sex differences in the human brain

Sexual dimorphism is also observed in the human brain. On average, the male brain is larger and heavier than the female brain. Autopsy and in vivo imaging studies have shown that the cerebrum of the men is 8-10% larger than in women. Regional and structural sex differences have been reported between relative cerebrum sizes. For example, relative to the size of the cerebrum, volumes of the frontal and medial paralimbic cortices are larger in women while men have a larger volume of the frontomedial cortex (17). The superior temporal cortex is also larger in women than men. Broca's area (Brodmann's areas 44 and 45) is located in the ventral and posterior portions of the frontal lobe; it processes information coming from

Wernicke's area (Brodmann's area 22) and converts it into a pattern for language production. In an autopsy study (10 men and 11 women free of neurological abnormalities), brains were measured using a stereological technique; the results showed that the average volume of Broca's area in women was approximately 20% larger than in men. This difference may be associated with the view that women have better language skills than men (18). Conversely, men have a greater sulcal volume and greater cerebrospinal fluid volume compared to women (19). Sexual differences are also observed in the blood circulation of brain. Global cerebral blood flow is higher in women than in men, both at rest and during cognitive activity (20). In addition, the gray/white matter ratio, corpus callosum, anterior commissure, hypothalamus, bed nucleus of the stria terminalis, limbic system and neurotransmitter systems also exhibit differences between genders.

The gray/white matter ratio

The gray matter of the brain consists of neuronal cell bodies and dendrites, while the white matter consists of myelinated axons. Women have greater cortical gray matter volume than men, whereas men have a greater percentage of white matter. In women, the gray/white matter ratio is higher, especially in the cingulate gyrus, insula, frontal, parietal, occipital and temporal lobes as compared with men (21). A study by brain magnetic resonance imaging (MRI) (40 men and 40 women) also showed similar findings for gray and white matter. Additionally, the study indicated that, in men, the left hemisphere has a greater percentage of gray matter than the right hemisphere, while the percentage of white matter shows no difference between the two hemispheres. Women show no asymmetries (19). Additionally, in women, the cortical gray matter volume reaches a peak one or two years earlier than in men (22).

The corpus callosum and anterior commissure

The corpus callosum consists of nerve fibers, and is located in the middle of the brain where it connects the left and right cerebral hemispheres. It is usually larger in women than in men (23). Women also have a larger anterior commissure, another structure that connects the left and right hemispheres and many brain regions (lateral amygdala, endopiriform cortex, nucleus accumbens etc.), as compared with men (24). Interestingly, an MRI study (12 homosexual men and 10 heterosexual men) showed that the corpus callosum with the isthmus was larger in homosexual than in heterosexual men (25). The massa intermedia, a structure that links the two thalami, is larger in women than in men and is absent more often in men (32%) than in women (22%) (26). Taken together, these studies suggest that interhemispheric connectivity may be better in women, although some studies report controversial findings (23).

The hypothalamus

The hypothalamus, relative to the size of the cerebrum, is larger in men than in women (17). Researchers have reported a region in the human hypothalamus which is analogous to the SDN-POA in the rat. Since the four nuclei are located in the anterior hypothalamus, they are called the interstitial nuclei of the anterior hypothalamus (INAH). The INAH are numbered 1 to 4 (INAH 1–4). Studies have shown that INAH-3 contains more neurons and is larger in men. Moreover, INAH 3 is thought to be analogous to the SDN-POA in the rat (27). In addition, Levay et al. (28) showed that INAH 3 is smaller in homosexual men than in heterosexual men.

The thalamus and suprachiasmatic nucleus

The thalamus contains a group of large nuclei located in the diencephalon that play a role in sensory perception, as well as in the regulation of limbic and motor functions. A positron emission tomography (PET) study in 120 healthy subjects (65 women and 55 men) showed that the thalamic nuclei are larger in women (29). Women also have a larger caudate nucleus compared to men; this structure is located within the basal ganglia (30). Another structure, the suprachiasmatic nucleus (SCN), lies just above the optic chiasm; it receives direct retinal input and generates circadian rhythms. Vasopressin (VP) and vasoactive intestinal polypeptide (VIP) are present in different subdivisions of the SCN. The VIP subnucleus of the SCN is larger in young adult men than in women (31). However, this sex difference may reverse (the female subnucleus becomes larger) after 40 years of age (32). The VP subnucleus of the SCN has a different shape in men compared to that in women. It has an elongated shape in women, and is more spherical in men (33). Interestingly, the VP subnucleus of the SCN is 1.7 times larger in homosexual men than in heterosexual men (34).

The bed nucleus of the stria terminalis

The bed nucleus of the stria terminalis (BNST) is a nucleus of the forebrain that receives projections from limbic system nuclei and sends projections to several hypothalamic and brainstem target areas. It may regulate behavioral responses to stress (35). The volume of the encapsulated region of the BNST is larger (97%) in male rats than in females (36). In humans, a similar sexual difference has also been reported in one study (26 age-matched men and women) showing that the volume of the posteromedial region of the BNST is 2.5 times larger in men than in women. Additionally, they suggested that BNST may play a role in aggressive and sexual behaviors (37). Another study showed that the volume of the central subdivision of the BNST is larger in men than in women. Interestingly, the authors found that the volume of the central subdivision of the BNST in six male-to-female transsexuals was similar to that in other women (38).

The limbic system

The limbic system is involved in many of our emotions (fear, anger and emotions related to sexual behavior), memory and learning. The most prominent components of the limbic system are the hippocampus and the amygdala. The hippocampus is larger in women than in men. This has been shown by two MRI studies (one with 10 men and 10 women, the other with 35 men and 34 women) (30, 29) and a PET study (55 men and 65 women) (29) in healthy people. The amygdala, in contrast to the hippocampus, is larger in men. For example, an MRI study (15 boys and 15 girls) reported that the amygdala was

larger in boys than in girls aged 7 to 11 years (39). Goldstein et al. (17) also found similar results in 48 healthy adults (21 men and 27 women) using MRI. The amygdala is very important for masculinized social behavior and is affected by testosterone. If a female is exposed to a high dose of testosterone during the neonatal period, she shows masculinized social behavior (40).

Neurotransmitter systems

The dopaminergic system is involved in the brain reward system, addiction and coordination of motor behavior. Some studies have shown that dopaminergic function is elevated in women due to higher striatal presynaptic dopamine synthesis and increased dopamine transporter availability (this regulates the uptake of dopamine into neurons) (21, 41, 42). Also, dopamine receptor density in the nucleus accumbens and the striatum is higher in male rats than in female rats during early development (43). A PET study (seven men and six women) showed that women have greater amphetamine-induced dopamine release in the right inferior frontal gyrus and right globus pallidus (44). The higher dopaminergic function in women may be protective against some diseases caused by disturbances in dopaminergic function, such as schizophrenia and alcoholism (21).

The serotonergic system controls complex sensory and motor patterns, and a disruption in serotonin (5-HT) synthesis can trigger schizophrenia, as well as mood, sleep and eating disorders. Sex differences in the serotonin system were first reported nearly 50 years ago in animal models. Rosecrans et al. (45) reported that female rats have higher central serotonin levels than male rats. In humans, some studies have shown similar differences between men and women; for example, whole blood serotonin levels are lower in men (46). In a PET study (eight healthy men and seven healthy women), Nishizawa et al. (47) reported that the mean rate of serotonin synthesis was 52% higher in men than in women. The 5-HT_{1A} autoreceptor, a subtype of serotonin receptor, is a pharmacological target for antidepressant drugs and plays a role in the modulation of anxiety and depression. A PET study in 25 healthy subjects (12 women and 13 men) showed that 5-HT_{1A} receptor numbers were higher in women than in men in certain brain regions such as the dorsal raphe, anterior cingulate cortex, amygdala, medial prefrontal cortex and orbital prefrontal cortex (48). These results may be related to the lower incidence of depression in men.

In addition, regarding the central cholinergic system, which is involved in cognitive function and memory, the expression of muscarinic cholinergic receptors in the cerebral cortex has been found to be higher in women (49). Furthermore, cortical levels of gamma-aminobutyric acid (GABA), which is the major inhibitory neurotransmitter associated with mood and memory, are higher in women than in men (50). The opioid neurotransmitter system controls pain, reward and addictive behaviors. A PET study reported that women have higher mu-opioid receptor binding than men in a number of cortical and subcortical regions (51).

Conclusion

Understanding sex-specific brain differences between men and women may be an important first step to explain the differences in sex-related behavior patterns. It seems likely that the prenatal hormone environment is responsible for the sexual dimorphism of the brain through effects on neural development.

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The metabolic effects of drugs used for the treatment of polycystic ovary syndrome

Polikistik over sendromu tedavisinde kullanılan ilaçların metabolik etkileri

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Abstract

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder among women of reproductive age. It is characterized by menstrual disorders, hyperandrogenism (clinical and/or biochemical) and ultrasonographic features. It is well known that PCOS has unfavourable effects on carbohydrate metabolism, the parameters of cardiovascular disease and lipid profile. Mode of treatment is mainly guided by the main complaint of the patient. A lot of medicines have been used for many years to treat these women. For that reason the recognition the effects of these drugs on the metabolic risk profile is important. The aim of this review was to evaluate the effects of these drugs on metabolic parameters in women with PCOS.

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Key words: Polycystic ovary syndrome, treatment, drug effects; metabolic parameter

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Özet

Polikistik over sendromu (PKOS), üreme çağındaki kadınlarda en sık görülen endokrin bozukluktur. Menstruel bozukluklar, hiperandrojenizm (klinik ve/veya biyokimyasal) ve ultrasonografik özellikleriyle karakterizedir. PKOS'nun karbonhidrat metabolizması, kardiyovasküler hastalık parametreleri ve lipid profili üzerine olan olumsuz etkileri iyi bilinmektedir. Tedavi şekli genelde hastanın asıl yakınmasına göre düzenlenir. Bu kadınların tedavisinde uzun yıllardan beri çeşitli ilaçlar kullanılmaktadır. Bu nedenle de tedavide kullanılan bu ilaçların, metabolik risk profili üzerine olan etkilerini bilmek önemli hale gelmektedir. Bu derlemenin amacı tedavide kullanılan bu ilaçların, PKOS'lu bayanların metabolik parametreleri üzerine olan etkilerini değerlendirmektir. (J Turkish-German Gynecol Assoc 2013; 14: 168-73)

Anahtar kelimeler: Polikistik over sendromu, tedavi, ilaç etkileri, metabolik parametre

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Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder among women of reproductive age, and is characterized by anovulation, hyperandrogenemia, obesity and insulin resistance (1-3).

PCOS was defined by Stein and Leventhal for the first time in 1935 as hyperandrogenemia, menstrual irregularity, large polycystic ovaries and obesity (4). The Rotterdam criteria, which we currently use frequently in PCOS diagnosis, were defined in 2003 (5). These criteria include; polycystic ovaries seen by ultrasound; ≥12 follicles in each ovary with a diameter of 2-9 mm and/or increase in the ovarian volume (>10 cm^3), chronic oligo-anovulation and hyperandrogenemia (determined clinically or in the laboratory). Other diseases should be excluded and at least two of these three criteria should be present to make a diagnosis. The PCOS criteria were rearranged by the Androgen Excess Society in 2009; these include androgen excess (clinical and/or biochemical hyperandrogenism), ovarian dysfunction (oligo-anovulation and/or polycystic ovarian morphology) and exclusion of other diseases with hyperandrogenism and exclusion of ovulatory diseases (6). The Androgen Excess Society recommends that all three criteria should be present for a diagnosis of PCOS.

The prevalence of the syndrome has been reported to be approximately 6-8% (7). The ultrasonographic prevalence of PCOS ranges between 14% and 23% (8, 9). There are no differences in PCOS prevalence in terms of ethnic background (10). The etiology of PCOS is not exactly known. Genetic and environmental factors have been blamed. The frequencies of hyperandrogenism and menstrual dysfunction are increased in the mothers and sisters of patients with PCOS. Serum androgen levels are increased in the fathers and brothers of patients with PCOS (11). Different studies in which potential genetic defects which might be involved in development of PCOS were examined showed that the syndrome is a complex polygenic defect (12). No specific environmental factors have been defined, but obesity is especially emphasized. The frequency of obesity in PCOS has been reported to be 40-60% (13). Obesity increases the prevalence of PCOS (14).

Three theories are emphasized in its pathophysiology. These include hypothalamic-pituitary dysfunction, ovarian hyper-

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androgenism and peripheral insulin resistance. Intraovarian androgen excess is responsible of both anovulation and the development of numerous follicle cysts. According to the abnormal pituitary function hypothesis, the increase in the frequency and amplitude of pulsatile secretion of LH increases androgen production in the ovaries. This results in discontinued follicle development (15). In addition, increased androgen blocks the inhibitory action of progesterone on the hypophysis. According to the hypothesis of ovarian hyperandrogenism, mainly primary functional ovarian hyperandrogenism is involved in PCOS (16). In vitro studies have shown an abnormal steroidogenic phenotype in isolated theca cell culture (17). According to the third hypothesis, insulin resistance and hyperinsulinemia are important factors in steroidogenic dysfunction in PCOS (18, 19). Insulin resistance and hyperinsulinemia stimulate androgen synthesis in the ovaries and lead to an increase in the levels of free testosterone by decreasing the synthesis of sex hormone binding globulin (SHBG) in the liver. Insulin resistance also contributes to the development of metabolic syndrome. Metabolic syndrome is present in 25% of adolescent PCOS cases and in 40% of adults aged 40 years with PCOS (20-23). One third of PCOS patients have an abnormal glucose tolerance test and 10% have type 2 diabetes mellitus (24).

PCOS generally presents with menstrual irregularity (oligoamenorrhea, dysfunctional uterine bleeding), hyperandrogenism findings (hirsutism, acne, oily skin, androgenic alopecia), infertility and obesity. Other clinical conditions related to PCOS include impaired glucose tolerance (IGT), type 2 diabetes mellitus (24), metabolic syndrome (20-23), non-alcoholic steatohepatitis (14), sleep apnea syndrome (25), malignancy (endometrium, ovary, breast cancer) (14, 26) and increased cardiovascular risk (27-29). An atherogenic lipid profile is observed as a result of increased LDL cholesterol, increased triglyceride levels and decreased HDL cholesterol levels in these patients (30-32).

Since the etiopathogenesis of PCOS is not clearly known, current treatment options are generally symptomatic. Two categories are emphasized in treatment. These include treatment of anovulatory infertility and treatment of the symptoms related to PCOS (menstrual dysfunction, hirsutism, infertility, etc.). The first step in the treatment of the symptoms related to PCOS includes lifestyle changes and weight loss, if the patient is obese. Medical treatment includes combined oral contraceptives (COC), spironolactone, finasteride, flutamide, metformin and combinations of these treatments.

COCs contain a progestin and ethinyl estradiol, which is a synthetic estrogen. Many of these progestins (levonorgestrel, norgestrel, desogestrel, gestodene, noretindrone) are derivatives of testosterone and show androgenic properties (33). Drospienone and cyproterone acetate (CPA), which are among the other progestins, are not structurally related to testosterone and show antiandrogenic activity. CPA blocks androgen receptors and inhibits 5-α-reductase activity (34). Thus, the serum androgen level is decreased. Drospirenone decreases blood pressure with its anti-mineralocorticoid action in addition to its anti-androgenic property. Conclusively, CPA and COCs which contain drospirenone suppress ovarian androgen production by inhibiting LH secretion, decrease serum free testosterone levels by increasing the synthesis of SHBG in the liver, block androgen receptors and thus regular menstruation, and prevent endometrial hyperplasia such that a decrease in the risk of endometrial cancer and regression of hirsutism are observed.

Spironolactone is an aldosterone antagonist, and its action is dependent on the dose. It is a competitive inhibitor of androgen receptors and also inhibits the activity of 5-α-reductase. Finasteride inhibits the activity of 5α-reductase and is less effective compared to the other anti-androgens (35). Flutamide is an androgen receptor blocker. Its efficiency is similar to spironolactone (36).

Metformin decreases ovarian androgen production by decreasing the serum insulin level and mitigating insulin resistance (37). Thus, the serum testosterone level decreases, the hirsutism score and menstrual dysfunction improve and infertility is reversed (38).

The negative effects of PCOS on carbohydrate metabolism, cardiovascular disease parameters and the lipid profile are well-known. Therefore, it becomes important to know the effects of the drugs used commonly in PCOS treatment on the metabolic risk profile.

Metabolic effects of drugs

COCs have been used in the treatment of PCOS for more than 30 years. There are various studies evaluating the effects of COCs on carbohydrate metabolism, the lipid profile and cardiovascular risk parameters.

Morin-Papunen et al. (39) compared the metabolic and endocrine effects of metformin and an oral contraceptive tablet (Diane 35) containing ethinyl estradiol (35 μg) and cyproterone acetate (CPA) (2 mg) in obese women with PCOS. Eighteen patients with a body mass index (BMI) above 27 were included in the study. Eight patients were given metformin and ten patients were given an oral contraceptive tablet for 6 months; the effects were evaluated at the beginning and at the end of the treatment period. The waist/hip ratio, as well as the serum free fatty acid, fasting insulin and fasting blood glucose levels decreased significantly in the group who received metformin ($p<0.05$), and impaired fasting glucose (IFG) returned to normal in one patient. The menstrual cycle improved, but the hirsutism score did not change in these patients. In the group who received Diane 35, the waist/hip ratio, serum free fatty acids, fasting insulin and fasting blood glucose did not change. One patient showed progression from IFG to diabetes mellitus and IGT developed in three patients. The menstrual cycle improved and hirsutism score decreased in the patients given Diane 35.

Mastorakos et al. (40) compared the effects of two different COCs on androgen and lipid parameters in patients with PCOS; 30 μg ethinyl estradiol + 0.15 mg desogestrel were given to group A (n=14) and 35 μg ethinyl estradiol + 2 mg cyproterone acetate were given to group B (n=14) for 12 months and the findings were compared at the beginning and at the end of the treatment period. In both groups, total and free testosterone levels decreased, the hirsutism score was reduced, total cholesterol (TC), LDL and HDL increased, the TC/HDL and LDL/HDL ratios did not change and a significant increase was found in TG levels in group B compared to group A.

In another study performed by Mastorakos et al. (41), the effects of two different COCs on carbohydrate metabolism were evaluated; 30 μg ethinyl estradiol + 0.15 mg desogestrel were given to group A (n=18) and 35 μg ethinyl estradiol + 2 mg cyproterone acetate were given to group B (n=18) for 12 months and the findings were compared at the beginning and at the end of the treatment period. In both groups, insulin resistance increased. The fasting blood glucose/insulin ratio decreased and first and second phase insulin secretion in OGTT was increased in group B. Conclusively, oral contraceptive drugs led to a change in insulin sensitivity. In addition, cyproterone acetate was related to an increase in insulin secretion and hyperinsulinemia.

Orbetsova et al. (42) evaluated anti-androgens and the effects of combinations of anti-androgens and insulin sensitizing agents on metabolic and hormonal parameters in women with PCOS. Forty-four patients were included in the study and divided into three groups. Diane 35 was given to the first group, Diane 35 + metformin were given to the second group and Diane 35 + rosiglitazone were given to the third group for 6 months. The body weight, body fat mass and abdominal fat distribution did not change in the first group. Despite mild hyperinsulinemic action, no change was found in carbohydrate tolerance. Negative effects were observed on atherogenic lipids. In the second group, the body fat mass and abdominal fat distribution decreased, blood glucose levels did not change, insulin levels decreased, diastolic blood pressure decreased and a positive effect was observed on HDL, while a neutral effect was observed on atherogenic lipids. In the third group, the body fat mass and abdominal fat distribution did not change, fasting blood glucose did not change, fasting insulin levels and the Homeostasis Model of Assessment-Insulin Resistance (HOMA-IR) decreased and a neutral effect was observed on atherogenic lipids.

In a meta-analysis comparing the efficiency of placebo with thiazolidinediones in PCOS (43), it was shown that thiazolidinediones decreased insulin and fasting blood glucose levels, but were not efficient in decreasing the Ferriman-Gallwey score and androgen levels and led to weight gain.

Gode et al. (44) evaluated the effects of ethinyl estradiol + cyproterone acetate on cardiovascular risk parameters. 40 patients were given a COC containing cyproterone acetate for 6 months. At the end of the treatment period, TC, LDL and TG increased compared to the baseline values ($p<0.05$). A significant increase was found in the carotid intima-media thickness (0.03±0.01 mm) ($p<0.05$). Brachial artery flow-mediated dilatation was observed to be decreased ($p<0.05$).

In a study in which the effects of medical treatment on endothelial function and insulin resistance were evaluated in patients with PCOS (45), one group was given metformin (n=36) and the other group (n=30) was given 35 μg ethinyl estradiol + 2 mg cyproterone acetate for 6 months. The effects were compared at the beginning and at the end of the treatment period. Glucose and insulin levels were evaluated using the oral glucose tolerance test. In the group who received metformin, the insulin, high sensitivity C-reactive protein (HS-CRP) and HDL levels decreased and brachial artery flow-mediated dilatation increased. In the group who received COC, the insulin, HS-CRP, glucose, SHBG and TG levels increased, while plasminogen activator inhibitor-1, LDL and testosterone levels decreased.

Ozkaya et al. (46) gave metformin twice a day to 19 women with PCOS for 3 months. The effects were evaluated at the beginning and at the end of the treatment period. Fasting insulin, free testosterone, dehydroepiandrosterone sulfate and visfatin levels decreased. HOMA-IR, BMI and waist circumference values also decreased. The changes in TC, HDL, LDL, TG and prolactin concentrations were not significant.

Kriplani et al. (47) investigated the effects of two different COCs on clinical and biochemical parameters in patients with PCOS. Group A was given (n=30) 30 μg ethinyl estradiol + 3 mg drospirenone and group B was given (n=30) 30 μg ethinyl estradiol + 0.15 mg desogestrel for 6 months and the effects were compared at the beginning and at the end of the treatment period. In group A, LDL decreased, HDL increased, fasting blood glucose, postprandial blood glucose and insulin level decreased, the hirsutism score improved and systolic-diastolic blood pressure decreased. In group B, fasting blood glucose, postprandial blood glucose and insulin level increased, the hirsutism score did not change, and systolic and diastolic blood pressure increased. Conclusively, it was interpreted that drospirenone was more efficient compared to the COC containing desogestrel because of its positive anti-androgenic effects on menstrual cycle regularity, blood pressure and lipid profile.

In a different study (48), 30 μg ethinyl estradiol + 3 mg drospirenone were given to 20 women with PCOS for 6 months. When the effects were evaluated at the beginning and at the end of the treatment period, it was found that testosterone levels decreased, SHBG levels increased and the hirsutism score improved, while no significant effects were observed on carbohydrate metabolism (FBG, fasting insulin, HOMA IR).

In a study where the effect of COC on body composition was evaluated in patients with PCOS (49), 30 μg ethinyl estradiol + 3 mg drospirenone were given to patients for 6 months. After 6 months of treatment, no changes were observed in body weight, BMI and waist/hip ratio, while a significant increase was found in the total fat percentage and central fat percentage. While the lipid parameters were increased (TC, LDL, TG, HDL), no changes were observed in insulin resistance and glucose metabolism.

Nakhjavani et al. (50) gave 100 mg spironolactone to 27 patients (20 PCOS + 7 idiopathic hirsutism) for 3 months and evaluated the lipid profile. Testosterone, dehydroepiandrosterone sulfate and prolactin levels decreased. TC, TG and FBG levels did not change. LDL, TC/HDL increased, while HDL decreased.

In a study which compared the efficiencies of spironolactone and metformin (51), group A (n=34) was given 50 mg/day spironolactone and group B (n=35) was given 1000 mg/day metformin for 6 months. In both groups, improvements were observed in the menstrual cycle, hirsutism score, glucose tolerance and HOMA-IR. The LH/FSH ratio and testosterone levels decreased. The blood pressure, BMI and waist/hip ratio did not

Table 1. Metabolic effects of the drugs used to treat PCOS

	Carbohydrate metabolism	Lipid profile	Hormonal profile	Hirsutism score	Menstrual dysfunction
Metformin	positive	positive	positive or neutral	positive or neutral	positive
Cyproterone acetate	negative	negative	positive	positive	positive
Drospirenone	positive or neutral	positive	positive	positive	positive
Desogestrel	negative	negative	positive or neutral	positive or neutral	positive
Spironolactone	positive or neutral	negative	positive	positive	positive

change. Conclusively, it was interpreted that both drugs were efficient in the treatment of PCOS, but spironolactone was superior to metformin in the treatment of hirsutism, menstrual imbalance and hormonal imbalance with a slight increase in side effects.

In a meta-analysis which evaluated the efficiency of exercise treatment in PCOS (52), the results of eight studies which included moderate physical activity and exercise periods ranging between 12 and 24 weeks were assessed. A 4.5-10% decrease in body weight, a 9-30% improvement in insulin resistance and improved ovulatory functions were observed with exercise. This was found to be independent of the type of exercise and the frequency and time of the sessions.

In summary, these studies generally show that cyproterone acetate has favorable effects on menstrual dysfunction, hirsutism score and hormonal profile and unfavorable effects on the lipid profile, carbohydrate metabolism and cardiovascular risk parameters. Drospirenone has favorable effects on menstrual dysfunction, hirsutism score, hormonal profile, carbohydrate metabolism, lipid profile and blood pressure. Spironolactone has favorable or neutral effects on carbohydrate metabolism, favorable effects on hirsutism score and hormonal profile and unfavorable effects on the lipid profile. Metformin has favorable effects on carbohydrate metabolism, menstrual dysfunction, lipid profile and favorable or neutral effects on the hormonal profile and hirsutism score (Table 1).

Conclusion

PCOS is a metabolic disease which has unfavorable effects on the lipid profile, carbohydrate metabolism and cardiovascular risk parameters. It has been shown that some drugs which are used in treatment of PCOS also have unfavorable effects on these parameters. Therefore, the metabolic effects of the drugs should be considered in treatment selection.

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Cutaneous metastasis in cancer of the uterine cervix: A case report and review of the literature

Uterin serviks kanserinde kutanöz metastaz: Bir olgu raporu ve literatürün gözden geçirilmesi

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Abstract

Carcinoma of the uterine cervix is a common neoplasm among Indian women; in fact, it is the commonest malignancy among rural Indian women. Uterine cervical cancer spreads mainly to the regional lymph nodes, with distant metastasis rarely occurring. Major sites of distant metastasis are lung, bone, and liver. Skin metastasis from carcinoma of the uterine cervix is a very rare event. The reported incidence ranges from 0.1 to 2%. Here we describe a 60-year-old woman with cervical cancer who developed metastatic lesions on the lower abdominal wall and also over the inner aspects of thigh.

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Key words: Skin metastasis, cancer, cervix, cutaneous metastasis in gynaecological cancer

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Özet

Uterin serviks kanseri Hintli kadınlarda yaygın görülen bir neoplazmdır; kırsal kesimdeki Hintli kadınlarda gerçekten en yaygın görülen malignitedir. Uterin serviks kanseri çoğunlukla bölgesel lenf bezlerine yayılmakta olup uzak metastazlar nadiren ortaya çıkmaktadır. Başlıca uzak metastaz bölgeleri akciğer, kemik ve karaciğerdir. Uterin serviks kanserinin cilt metastazı oldukça nadir bir durumdur. Bildirilen insidans %0.1 ila %2 arasında değişmektedir. Biz burada servikal kanseri olan, alt karın duvarında ve ayrıca uyluğun iç kısımlarında metastatik lezyonlar gelişen 60 yaşında bir kadın hastayı tanımlamaktayız.

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Anahtar kelimeler: Cilt metastazı, kanser, serviks, jinekolojik kanserde kutanöz metastaz

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Introduction

Cancer of the uterine cervix, in addition to local contiguous invasion, spreads mainly through local lymphatics. Local lymph nodes are commonly involved, meaning that distant metastasis is rare. Skin metastasis from cervical cancer is a very unusual event; particularly, skin metastasis as a primary or recurrent presentation (as in the below-described case) of cervical cancer is very unusual indeed. Although cervical cancer is one of the commonest malignant neoplasms among Indian women-in fact, it is the commonest malignancy among women in rural parts of India (1)-skin metastasis from cervical cancer is extremely rare.

Case Report

A 60-year-old female was admitted to our Medical College with multiple swellings on the lower abdomen and inguinal region. This was associated with vague abdominal discomfort. On general examination she was cachectic; abdominal examination revealed a suprapubic scar and a nodular swelling over it. There were three swellings on the left upper thigh and inguinal region (Figure 1). Per vaginal examination showed a healthy vault.

Previous records revealed that she had been diagnosed with non-keratinising moderately differentiated squamous cell carcinoma of the cervix. One year previously she was admitted with a cervical growth which proved to be malignant and was in FIGO Stage IIa. The initial tumour size was approximately 4cm at the largest diameter without any parametrial involvement. Routine work-up including chest X-ray and abdominal ultrasound did not reveal any para-aortic nodal or distant spread. She underwent Modified Radical Hysterectomy (Wertheim's Operation). Histopathological examination from the post-operative specimen showed involvement of the bilateral internal iliac nodes. However, she did not receive any adjuvant therapy, although she was advised to do so, and she did not return for follow-up.

On this occasion, biopsy was taken from the skin nodules over the left upper thigh, which showed cutaneous deposits of metastatic squamous cell carcinoma. Excision biopsy from the swollen inguinal nodes also revealed deposits of squa-

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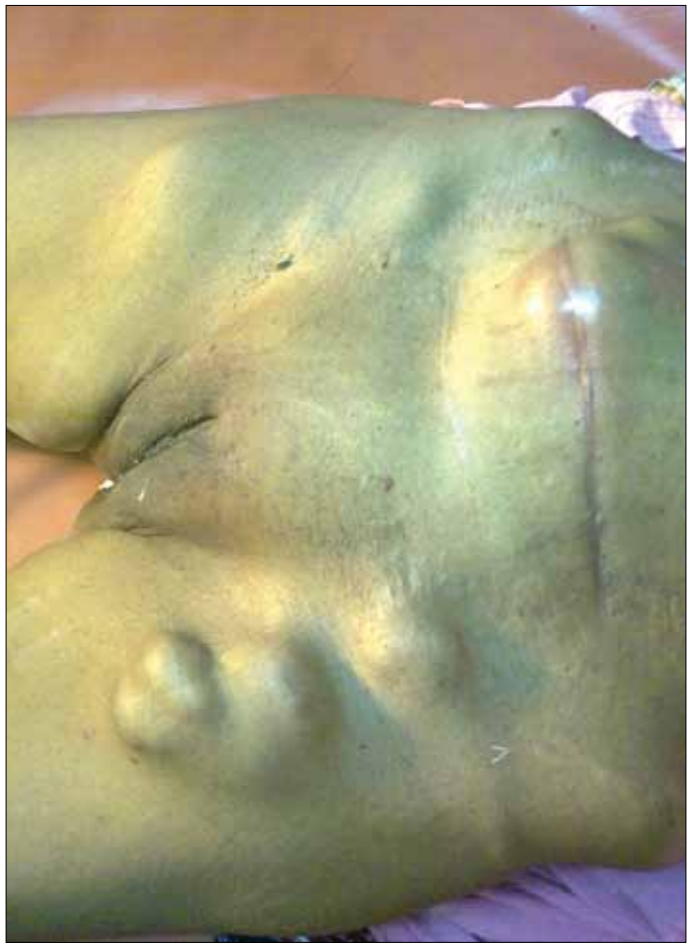

Figure 1. Skin nodules in upper thigh along with the inguinal & abdominal swellings

mous cell carcinoma in the lymph nodes. She was diagnosed as a case of metastatic uterine cervical carcinoma, i.e. cervical cancer spreading to the skin and inguinal lymph nodes. Clinical and colposcopic examination revealed that the vaginal vault was free from tumour. Radiological investigations did not show the metastatic involvement of any other organ.

The patient was treated with six cycles of chemotherapy with Cisplatin (80 mg/m^2) and Paclitaxel (225mg/m^2; infusion over 3 hours) on day 1, every 3 weeks. She received the treatment according to the schedule. At the end of the chemotherapy course there was a 50% reduction in the size of the lesion and the enlarged lymph nodes disappeared. She received palliative radiotherapy of 3000-CGy external beam irradiation in 10 fractions over 2 weeks according to a conventional fractionation schedule.

The lesions responded well to radiation therapy; however, four months later, she developed bilateral lung metastasis and died within three months. The period between the date of her presentation at our OPD with skin metastasis to the date of her death was seven month long.

Discussion

Metastatic carcinoma of the skin is an uncommon occurrence. Breast cancer is one of the most common primary tumours to metastasise to skin; gynaecological malignancies rarely give rise to metastatic deposits on the skin. Samaila et al. (2), from a search of twenty years institutional data from Nigeria, could find only four instances of cutaneous deposits from gynaecological malignancies, and in only one of these cases was the primary malignancy in the uterine cervix. Skin metastasis from uterine cervical carcinoma is a rare event. The reported incidence ranges from 0.1 to 2% (3).

To search PubMed for previously reported cases, we used the keywords “cancer cervix” and “skin metastasis”. All of the relevant literatures were thoroughly reviewed. Relevant cases are presented here in Table 1 (4-31). Studies dealing with cutaneous metastasis from all primary malignancies, among which some are from cancer of the cervix, are not included in Table 1 (3, 32, 33). Fifteen cervical cancer patients (out of 1190 patients) with skin metastasis from the study by Imchi et al. (34) are also not presented in the Table.

The most common sites of cutaneous metastases in cervical carcinoma are the abdominal wall and lower extremities (8). This is consistent with other carcinomas, in that metastatic spread to the skin is commonly located near the site of the primary tumour (33). The usual mode of spread has been suggested to be the lymphatic system (34). Imchi et al. (34), after reviewing 1190 patients with cancer of the cervix, including 15 of whom developed skin metastasis, observed that the incidence of skin metastasis was 0.8% in stage I, 1.2% in stage II, 1.2% in stage III, and 4.8% in stage IV. The incidence of skin metastasis seemed to be higher in patients with adenocarcinoma and undifferentiated carcinoma than in patients with squamous cell carcinoma. Macroscopically, three common patterns of skin metastases, such as nodules, plaques, and inflammatory telangiectasic lesions, have been recognised (33). Skin metastases from cervical carcinoma occur predominantly in cases of tumour recurrences, with metastases developing up to 10 years after the initial diagnosis and averaging less than 1 year (35). The prognosis associated with cutaneous metastasis of cervical carcinoma is poor. Usually, metastases to the skin occurring in patients with carcinoma of the cervix represent a pre-terminal event with a time from diagnosis to death of 3 months (3). Systemic treatment in patients with advanced disease is palliative. Cisplatin is the single most active agent for the treatment of cervical cancer; however, recent evidence suggests that combination chemotherapy with cisplatin and paclitaxel may improve progression-free survival over the use of cisplatin alone (36). Palliative radiation is useful for controlling symptoms (37). The most important prognostic factor, in cases like this, is the time interval between the initial diagnosis of the primary genital malignancy and the appearance of skin metastasis, whether metastasis is isolated or as a part of more widespread systemic recurrence. The earlier the metastasis occurs, the worse the prognosis for the patient is. The intent of treatment remains palliation, either by radiation, chemotherapy or surgery (alone or in combination). Most of the previously reported cases had undergone some kind of surgical intervention for their disease, either radical surgical intervention or lymphadenectomy (in some cases laparoscopic lymph node dissection), before the disease was treated by radiation

Table 1. Summary of results within the melatonin group and control groups

Case	Author	Sites of Metastasis	Previous Treatment Received	Histology
1	Daw & Riley (4)	Umbilicus	-	SCC
2	Tharakaram et al. (5)	Thigh	-	SCC
3	Chapman et al. (6)	Umbilicus	Initial Presentation	SCC
4.	Bohme et al. (7)	Two cases: One at Scalp, another in inguinal region	-	-
5.	Hayes et al. (8)	Upper back	-	SCC
6.	Lane et al. (9)	Laparoscopic port site	-	ASC
7.	Selo-Ojeme et al. (10)	Hysterectomy incision site	Radical Hysterectomy	AC
8.	Pertzborn et al. (11)	Hand	On Initial Presentation	-
9.	Maheswari et al. (12)	Scalp	Radiotherapy	SCC
10.	Tjalma et al. (13)	Laparoscopic port site	Radiotherapy	SCC
11.	Behtash et al. (14)	Abdominal wall (drain site)	Radical Surgery & Radiotherapy	SCC
12.	Liro et al. (15)	Abdominal wall	Radical Surgery & Radiotherapy	SCC
13.	Agarwal et al. (16)	Scalp	Radiotherapy	SCC
14.	Park et al. (17)	Scalp	-	SCC
15.	Srivastava et al. (18)	Incisional site	Radical Surgery & Radiotherapy	SCC
16.	Chung et al. (19)	Scalp presenting as alopecia neoplastica	Radical Surgery	SCC
17.	Sachdev & Jain (20)	Incisional site	Surgery	SCC
18.	Chen et al. (21)	Extremities, trunk, scalp	Chemo-radiotherapy	SCC
19.	Abhishek et al. (22)	Scalp		AC
20.	Behtash et al. (23)	Umbilicus	Chemo-radiotherapy	SCC
21	Ozdemir & Tuncbilek (24)	Nasal dorsum	-	SCC
22.	Deka et al. (Two Cases) (25)	Surgical scar	-	SCC
23.	Takagi et al. (26)	Scalp	Surgery & Chemoradiation	SCC
24.	Elamurugan et al. (27)	Palm (hand)	Radiotherapy	SCC
25.	Agrawal et al. (28)	Abdominal wall	Chemoradiotherapy	SCC
26.	Fogaca et al. (29)	All over	Surgery	Neuro-endocrine Cancer
27.	Lee et al. (30)	All over	-	
28.	Chung et al. (31)	-	-	

SCC: Squamous Cell Carcinoma; AC: Adenocarcinoma; ASC: Adenosquamous Carcinoma

therapy or chemoradiotherapy. Careful handling of tissue during operation, extension of radiation therapy portals to include the surgical scar and uncompromised primary treatment might prevent the development of skin metastases. Research targeted on the mechanism of local cancer spread and the interaction of cancer cells with the surgical wound environment may improve the knowledge regarding the pathogenesis of skin metastases and its clinical prognosis.

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 – B.B., S.M.; Design - B.B., S.M.; Supervision - B.B., S.M.; Resource - B.B., S.M.; Materials - B.B., S.M.; Data Collection&/or Processing - B.B., S.M.; Analysis&/or Interpretation - B.B., S.M.; Literature Search - B.B., S.M.; Writing - B.B., S.M.; Critical Reviews - B.B., S.M.*

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

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Role of 3D power Doppler sonography in early prenatal diagnosis of Galen vein aneurysm

3D power Doppler sonografinin Galen ven anevrizmasının erken prenatal tanısındaki rolü

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Abstract

Vein of Galen aneurysm malformation (VGAM) is a rare congenital vascular anomaly. Although the cause of VGAM remains to be elucidated, the current hypothesis is persistence of the embryonic vascular supply, which leads to progressive enlargement and formation of the aneurysmal component of a typical VGAM. Here, we present a 36-year-old woman at 23 weeks' gestation (gravida 3, para 2) who was evaluated using 3D power Doppler sonography for the prenatal diagnosis of a vein of Galen aneurysm. Investigation using 3D power Doppler sonography allowed for a non-invasive yet diffuse and detailed prenatal assessment of VGAM. Thus, we suggest that prenatal sonography with 3D power Doppler may be an option in cases of VGAM. (J Turkish-German Gynecol Assoc 2013; 14: 178-81)

Key words: Galen vein aneurysm, power Doppler, 3D sonography

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Özet

Galen ven anevrizma malformasyonu (VGAM) nadir görülen bir konjenital vasküler anomalidir. VGAM nedeni tam olarak aydınlatılmış olmamasına rağmen, güncel hipotez, tipik anevrizmanın progresif genişlemesine yol açan embriyonik vasküler desteğin persiste etmesidir. Bu çalışmamızda 36 yaşında ve 3D power Doppler sonografi kullanarak Galen ven anevrizması değerlendirilen 23 haftalık gebeliği (gravida 2, para 2) bulunan bir olguyu sunduk. VGAM'nin prenatal değerlendirmesinde non-invaziv yoldan geniş ve ayrıntılı bilgi sağlamak için 3D power Doppler sonografi kullanımı uygun görülmektedir. Bu nedenle VGAM bulunan vakalarda 3D power Doppler sonografiyi opsiyonel bir seçenek olarak önermekteyiz. (J Turkish-German Gynecol Assoc 2013; 14: 178-81)

Anahtar kelimeler: Galen ven anevrizması, power Doppler, 3D sonografi

Geliş Tarihi: 21 Kasım 2012 **Kabul Tarihi:** 12 Aralık 2012

Çevrimiçi Yayın Tarihi: 10 Temmuz 2013

Introduction

Vein of Galen aneurysm malformation (VGAM) is a rare congenital vascular anomaly that generally manifests in the neonatal period with symptoms of congestive heart failure. Hydrocephalus-related obstructive effects, seizures, encephalomalacia, and subcortical calcification may be present during the infantile period (1-4). In paediatric and adult patients, VGAM is rare and presents with headache and symptoms of subarachnoid haemorrhage (5, 6). VGAM has been reported to comprise 30% of paediatric vascular malformations (7).

Although the cause of VGAM remains to be elucidated, the current hypothesis regarding the possible mechanism is persistence of the embryonic vascular supply known as the choroidal arteries and the anterior segment of the median prosencephalic vein of Markowski, which normally regresses. This persistence leads to progressive enlargement and formation of the aneurysmal component of a typical VGAM (8).

Currently, the widespread utilisation of foetal sonography has resulted in the frequent diagnosis of this rare vascular malformation. The most common diagnostic criterion on real-time imaging is the detection of an intracranial cystic structure. Doppler sonography with colour flow imaging makes it easier to identify the blood flow within the cyst. Recent investigations of intracranial vascular lesions by using the 3D power Doppler mode reveals some benefits compared to conventional colour Doppler sonography, such as obviating aliasing distortions, angle dependence, overwhelming noise, and more detailed anatomical correlation. Cranial examination may also reveal mild to moderate hydrocephalus at advanced gestational ages. Jugular vascular ectasia and cardiomegaly with congestive heart failure and its peripheral findings, such as ascites, may be seen in the affected foetus or neonate due to high-velocity flow in this arteriovenous (AV) fistula and are accepted as the main factors determining prognosis (9).

In this case report, we present the prenatal diagnosis of a VGAM and evaluate it using the 3D power Doppler mode.

Case Report

A 36-year-old woman at 23 weeks' gestation (gravida 3, para 2) was referred to our prenatal diagnosis unit for further

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evaluation of corpus callosum agenesis. Her obstetric and medical history was unremarkable except for the presence of Rh incompatibility. Indirect Coombs test was negative in the second trimester evaluation. The triple test during the 16th week revealed a 1/81 risk for Down syndrome. Although both parents were informed of the test results, they did not pursue karyotyping.

Using ultrasonography, we diagnosed a 43×32 mm supratentorial cystic structure that was located posterior to the third ventricle communicating with the widened straight sinus (Figure 1). Colour flow examination revealed turbulent flow through the lesion (Figure 2). Doppler velocity waveforms showed high blood flow through the lesion that consisted of increased peak systolic velocity and decreased resistance, which resembled an AV shunt. Investigation using 3D power Doppler revealed a much more detailed appearance of the widened vascular sinus and its communications (Figure 3). Similar vascular dilatation and turbulent flow were observed in the cervical region on colour Doppler sonography. Mild ventriculomegaly of the lateral ventricles (11 mm) was noted during the initial examination.

The ventricular dilatation increased progressively until it reached 23 mm at term.

Biometric parameters were all compatible through the first trimester sonographic examination. Mild hydramnios and significant cardiomegaly were noted. Although the patient's screening test for gestational diabetes was positive, the diagnostic test was negative and the Hb_{A1C} level was within the normal range.

The cardiac structures were normal on echocardiographic examination; however, compartments such as the aorta, pulmonary artery, and both ventricles were dilated and the heart-to-thorax ratio was increased. Periodic echocardiographic examination revealed no regurgitation at the mitral or tricuspid valve. Neither ascites nor pericardial effusion, both of which are indicators of cardiac dysfunction, was detected at any time. Parents were informed of the diagnosis of Galen vein aneurysm and neonatal complications. They opted for conservative therapy until term and surgery in the neonatal period.

A female foetus weighing 3.250 g was delivered at the 39th gestational week by caesarean section. Her Apgar scores were 7 and 7. Oxygen saturation was 70%, so the patient was intubated. Physical examination revealed dilation of both carotid arteries, hypotonic posture, and weak neonatal reflexes. She was referred to paediatric cardiology. Echocardiography was performed, and marked dilatation at the carotid arteries and its branches from the level of the arcus aorta was observed. The patient's heart circulation was hyperdynamic because of an AV fistula. Cranial magnetic resonance imaging (MRI) and magnetic resonance angiography (MRA) were performed. MRI revealed hydrocephaly and a thin parenchyma. The AV fistula was detected on MRA and the presentation was more serious than expected. Embolisation could not be performed, and the patient died on the third day after birth.

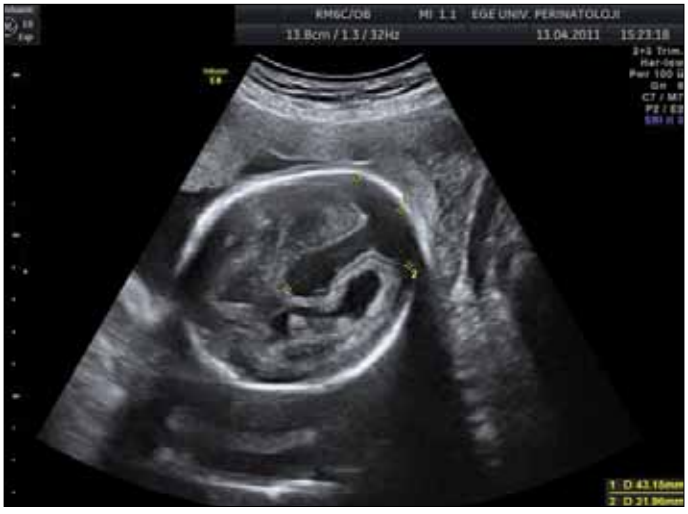

Figure 1. Cystic structure of VGA in gray scale sonography

Discussion

The current paper aimed to investigate the usefulness of 3D power Doppler sonography in a foetus diagnosed with VGAM during the second trimester. VGAM is a complex AV malformation that is usually characterized by multiple communications between the Galen vein system and the cerebral arteries (choroidal branches of posterior cerebral artery and the transmes-

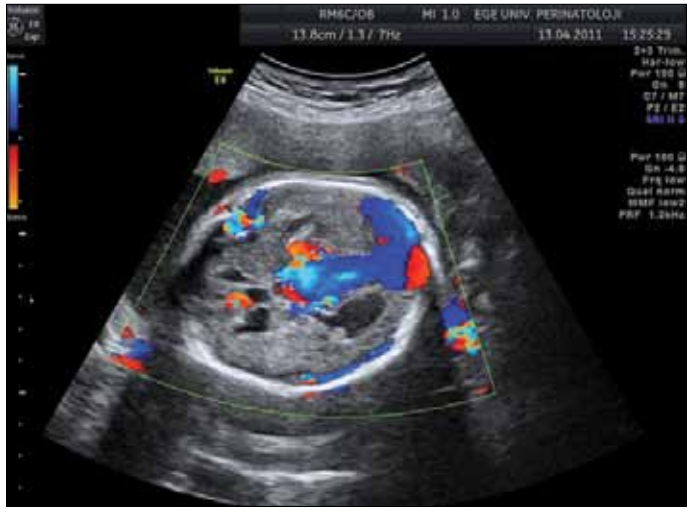

Figure 2. Turbulant blood flow in VGA through color Doppler mode

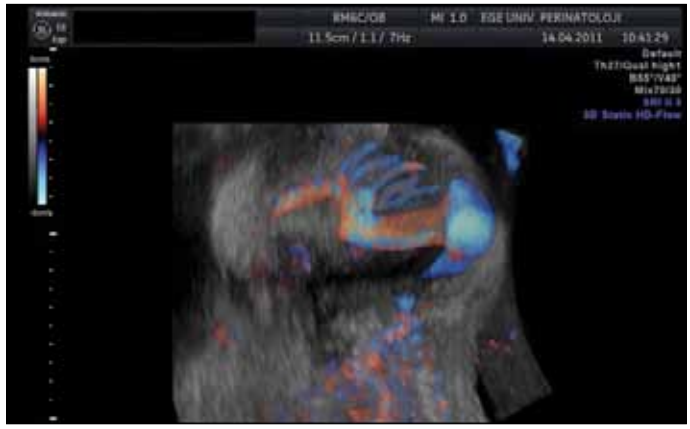

Figure 3. 3D power Doppler investigation reveals more detailed anatomic plans without disadvantages of other sonographic modes

encephalic arteries) (7). This vascular malformation may cause multi-system disturbances via hyperdynamic vascular flow or a mass effect on the intracranial structures. High output cardiac failure is the endpoint of cardiac influence. Cardiomegaly may be the initiator, and non-immune hydrops may lead to cardiac failure. In our case, the foetus was followed to term by a paediatric cardiologist to diagnose presumptive cardiac dysfunction, but no sign of cardiac pathology other than the cardiomegaly was observed.

The intracranial effects of this vascular lesion are generally directed by the mass effect of the aneurysm to the adjacent tissues such as the ventricular system. This may cause progressive dilatation of the ventricle, which was eventually diagnosed as hydrocephaly. Both hydrocephaly and diversion of the vascular flow from the cerebral cortex to the aneurysm (steal phenomenon) may cause porencephaly and leukomalacia (6). In our case, the lateral ventricle diameter increased from 11 mm to 23 mm from the initial examination to term.

In VGAM, the jugular veins may be tortuous and distended, as seen in our case. Sepulveda reported that ventriculomegaly, cardiomegaly, and jugular vein dilations were the most common findings of this pathology (10).

The diagnosis of VGAM relies on imaging studies such as ultrasonography, angiography, computed tomography, and MRI. Ultrasonography is the most common imaging method of intrauterine diagnosis. Since grey-scale sonography can only detect cystic dilatations and other peripheral manifestations, colour Doppler sonography may help to further diagnose these cystic malformations as vascular lesions. Finally, 3D power Doppler sonography shows both the nature and the anatomic structure of the lesion from different perspectives during or after examination (without the disadvantages of colour mode sonography and MRI) (11). The use of MRI during the prenatal or postnatal periods reveals detailed lesion conformation (11). Similarly, the use of angiography in the postnatal period may be helpful with diagnosis and treatment prediction.

Cardiac failure and associated brain pathologies are indicators of poor prognosis. Dilated drainage tract, multiple feeding vessels, dilated jugular veins, retrograde aortic diastolic flow, and cardiomegaly are the other factors that may influence prognosis. Hydropic changes and anatomical changes in the brain of the foetus are the worst prognostic findings. Recent studies have aimed to predict prognosis using scoring systems (12). In our case, some of the cranial reflections of poor prognosis were determined but cardiac status was stable throughout the gestational period. However, stable cardiac status does not guarantee postnatal survey. In the prenatal period, the low vascular resistance of the placenta conserves the formation of congestive heart failure by competing with the low-resistance aneurysm, but this compensated state does not predict a benign neonatal course. Neonatal congestive heart failure may exist after delivery when the placenta is removed (13, 14). The current case was similar. Although cardiomegaly was the single sign of cardiac pathology, the patient exhibited signs of cardiac failure and required intubation just after delivery.

Although treatment options have not yet been established, neurosurgical alternatives such as excision, ligation, and embolisation may be applied. Cardiac decompensation and gross brain involvement are the main indications of emergent treatment. In stable cases, treatment options are determined using a multidisciplinary approach (at 4–5 months of age). On the other hand, some cases of spontaneous regression via thrombosis have also been reported (15). In our case, both cardiac and cranial lesions were determined to have caused the mortality. In conclusion, 3D power Doppler is a non-invasive way to provide a diffuse and detailed prenatal assessment of VGAM. Although we have discovered many unknown features of VGAM, knowledge of other possible mechanisms to predict the survival of the patients with this condition is lacking.

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

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

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Two patients with marginal symptoms showing hyperthecosis at the edge of malignancy: Presentation of two cases

İki farklı semptom ve malignensi riski ile yansıyan hipertekozis: iki vaka sunumu

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Abstract

It is important to define the aetiology of increased levels of androgens in women. Ovarian stromal hyperplasia (OSH) and ovarian hyperthecosis (OHT) are non-neoplastic pathologies. They show the excess of androgen production and have a wide clinical range like hirsutism, virilisation, abnormal menses, obesity, hypertension and insulin resistance. Ovarian stromal hyperplasia and hyperthecosis are commonly seen in postmenopausal women and generally involve both ovaries. Laboratory testing is the gateway; testosterone and dehydroepiandrosterone sulphate (DHEA-S) are the first hormones that should be measured. OSH and OHT could also be a reason for endometrial malignancy by unopposed oestrogenic status. Hyperthecosis must be differentiated from several other diseases, especially malignant conditions, and the treatment for postmenopausal women should be bilateral oophorectomy. (J Turkish-German Gynecol Assoc 2013; 14: 182-5)

Key words: Hyperthecosis, hirsutism, menopause, malignancy

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Özet

Bayanlarda artmış androjen seviyelerinin etyolojisini açıklamak önemlidir. Overyan stromal hiperplazi (OSH) ve overyan hipertekozis (OHT) neoplastik olmayan patolojilerdir ve artmış androjen üretimini gösterirler. Hirsutizm, virilizasyon, menstrüel düzensizlik, obezite, hipertansiyon ve insülin rezistansı geniş klinik spektrumun parçalarıdır. Overyan stromal hiperplazi ve hipertekozis genellikle postmenopozal bayanlarda görülür ve sıklıkla her iki overi de içerir. Laboratuvar testleri tanıda anahattır; testosteron ve dehidroepiandrosteron sülfat (DHEA-S) bakılacak ilk hormonal testlerdir. OSH ve OHT, endometrial malignensi açısından karşılanmamış östrojen üretimi nedeniyle risk oluşturur. Hipertekozis diğer birçok hastalıktan ve özellikle malign durumlardan ayrılmalıdır ve postmenopozal bayanlarda bilateral ooferektomi tedavi için önemlidir. (J Turkish-German Gynecol Assoc 2013; 14: 182-5)

Anahtar kelimeler: Hipertekozis, hirsutizm, menopoz, malignensi

Geliş Tarihi: 13 Aralık 2012 **Kabul Tarihi:** 02 Ocak 2013

Çevrimiçi Yayın Tarihi: 10 Temmuz 2013

Introduction

It is important to define the aetiology of increased levels of androgens in women because of the possibility of malignancy originating from the ovaries or adrenal gland. Ovarian stromal hyperplasia (OSH) and ovarian hyperthecosis (OHT) are both similar and non-neoplastic pathologies. Stromal hyperplasia is the nodular or diffuse proliferation of the ovarian stroma, whereas ovarian hyperthecosis is stromal proliferation accompanied by luteinised stromal cells. They both show an excess of androgen production and have a wide clinical range, including hirsutism, virilisation, abnormal menses, obesity, hypertension and insulin resistance (1). This androgenic process leads to an elevation of testosterone. Additionally, by peripheral aromatisation of androgenic hormones, an increase in oestrogenic hormones occurs (2).

OSH and OHT with increased hormonal states were considered to be an aetiological database for endometrial cancer in a few case reports (3). The prevalence of this disease is unknown as most of the information about the disease has been derived from case reports. Here, we describe two cases of ovarian stromal hyperthecosis with different symptoms.

Case Reports

Case 1

A 59-year-old multiparous woman presented with a one year history of progressive hirsutism, acne and hair loss.

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She had her menarche when she was 14 years old and had normal periods since then until menopause (at the age of 51). She was hypertensive and also had an insulin resistance syndrome with hyperthyroidism. Initial investigations revealed significantly elevated androgen levels. The serum concentration of testosterone was 195 ng/dL, dehydroepiandrosterone sulphate (DHEA-S) was 88.9 μg/dL and cortisol was 30 μg/dL. Other laboratory parameters were normal. Sonography showed bilaterally enlarged ovaries with no other signs. Total abdominal hysterectomy and bilateral salpingooophorectomy was performed with frozen section analysis because of the risk of malignancy and the final pathology result revealed stromal hyperthecosis for bilateral ovaries (Figure 1a, 1b and 1c show the pathology images of hyperthecosis for this patient). After surgery, the symptoms of the patient reduced over time, and improvements in laboratory results were obtained.

Case 2

A 55-year-old multiparous woman presented with postmenopausal bleeding. She did not have any other symptoms; no hirsutism, no acne or hair loss. Her physical examination was normal and she had been in menopause from the age of 50 years. She had abdominal obesity, hypertension and hypothyroidism. Her laboratory findings showed levels of testosterone, oestradiol and Follicle stimulating hormone (FSH): 105 ng/dL, 27 pg/mL and 75 mIU/mL, respectively. The sonograph showed an endometrial thickness of 10mm with ovaries that were slightly echogenic, but there was no solid cystic lesion visible on the ovaries. An endometrial sampling operation was per-

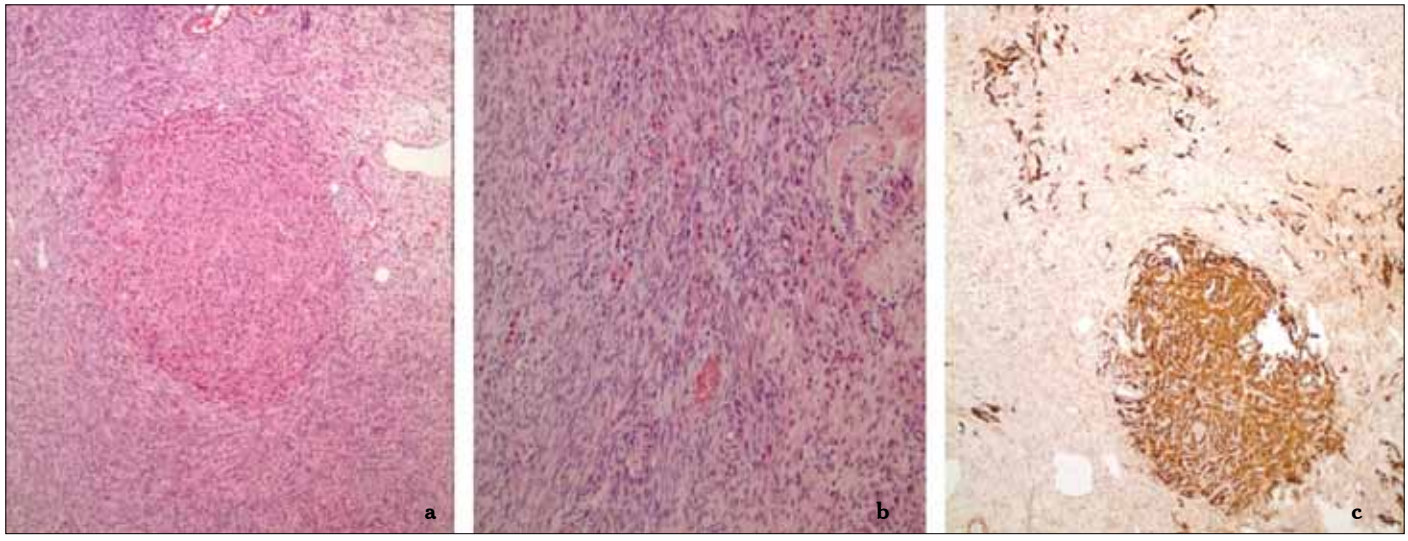

Figure 1. A nodule made up of eosinophilic, luteinised theca cells was seen in the ovarian stroma (Figure 1a). As well as forming nodules, these cells were diffusely dispersed into the stroma (Figure 1b). The same nodule and the other theca cells dispersed in the stroma displayed strong calretinin positivity immunohistochemically (Figure 1c) (x10, calretinin staining)

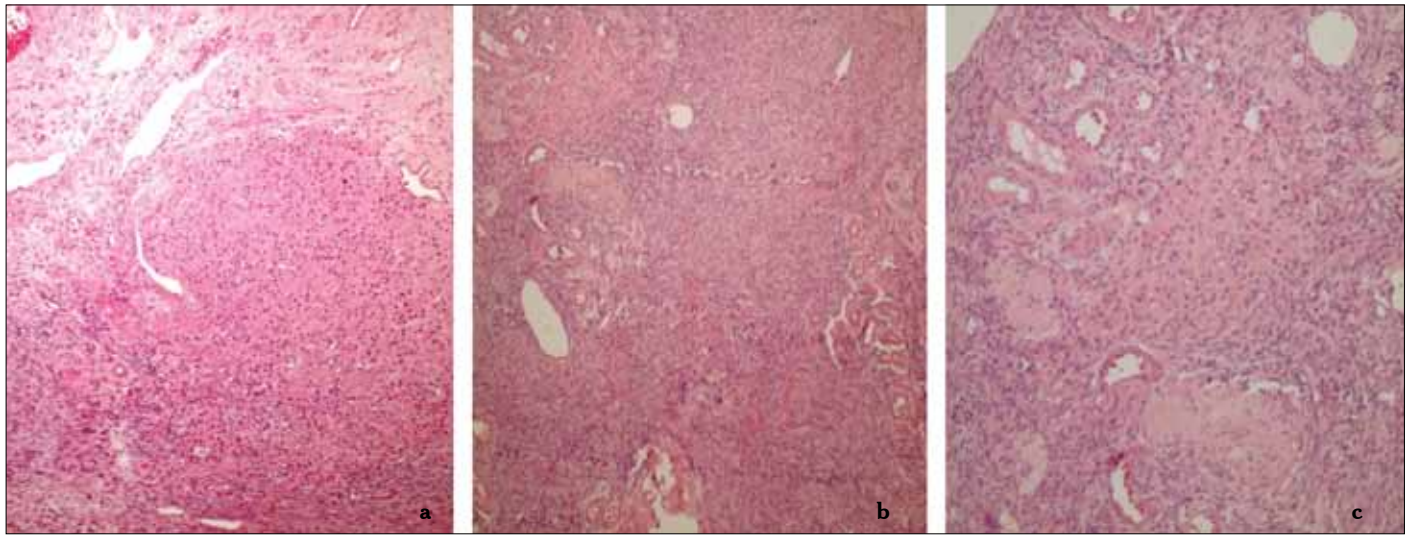

Figure 2. The ovarian stroma displayed vaguely nodular (Figure 2a) or diffusely dispersed (Figures 2b, 2c) cells with eosinophilic cytoplasm and indistinct cell borders (H&E)

formed and the pathology result revealed endometrial intraepithelial neoplasia (EIN). Total abdominal hysterectomy and bilateral salpingooophorectomy was performed with frozen section analysis and the final pathology result revealed stromal hyperthecosis for bilateral ovaries (Figure 2a, 2b and 2c show the pathology images of hyperthecosis for case 2).

Discussion

Ovarian hyperthecosis and polycystic ovary syndrome (PCOS) have similar presentations, making it difficult to distinguish between these two diseases. Symptoms of hyperandrogenism for ovarian hyperthecosis continue beyond the menopause, and the major cause of hyperandrogenemia cases in menopause is ovarian hyperthecosis. Women of this pathology present with severe hirsutism and other clinical evidence of virilisation that are not seen in PCOS, such as cliteromegaly, temporal balding, voice changes and diffuse acne (4).

In 1982, Hugheston suggested that hyperthecosis is a severe form of PCOS (5). Hyperthecosis is rarely seen in young women and is generally encountered in menopausal women (6). A complete medical history and full physical examination for virilisation, acne, abdominal obesity, hair loss, menstrual regularity, timing of menopause, and the duration of symptoms are all needed, as all are important hallmarks of diagnosis. The first patient had severe hirsutism, acne and hair loss, whereas the second patient did not have any of these symptoms.

Laboratory testing is the gateway for diagnosis; testosterone and dehydroepiandrosterone sulphate (DHEA-S) are the first hormones to be measured (7). Testosterone levels may be as high as in androgen secreting tumours (200ng/dL). Generally, testosterone levels are not as high as expected, but are nearly always above the postmenopausal range and are higher than the levels observed in PCOS (6). If the serum total testosterone level is >150 ng/dL, imaging of the adrenal glands and ovaries must be performed, because high levels of testosterone is the single most important laboratory finding (8). In both of the cases described here, the testosterone levels were elevated, so sonographic evaluation was performed for the identification of the underlying cause.

Ovarian stromal hyperplasia and hyperthecosis are commonly seen in postmenopausal women and generally involve both ovaries (9). To evaluate the ovaries, a pelvic ultrasound or magnetic resonance imaging may be predictive and helpful for defining pathologies and excluding malignant conditions in women with elevated testosterone levels. A computed tomography or magnetic resonance imaging of the adrenals is important for women with high levels of DHEA-S (10). In contrast to the knowledge that isolated testosterone elevation is related to ovarian pathology, rare cases of testosterone-secreting adrenal tumours have also been described in the literature (11). DHEA-S levels were normal for the two patients reported here.

In severe hyperthecosis, ultrasonography shows bilateral expansion in the ovarian stroma; ovaries appear more solid, and a few cysts may also be seen (9). Ovarian size is also frequently increased in postmenopausal women with hyperthecosis (12). Insulin resistance is present in almost all cases and the risk of type 2 diabetes and cardiovascular diseases increase. Additional findings like obesity and skin tags may also be seen (4). The first patient had bilateral enlarged ovaries, insulin resistance, and obesity, as described in the literature. The second patient had no symptoms other than postmenopausal bleeding. Her sonographic evaluation showed only echogenic ovaries.

Increases in oestrogen production by androgens secreted on the ovaries result in an increased risk of endometrial hyperplasia and endometrial carcinoma, especially in postmenopausal women (13). Our second patient had an endometrial intraepithelial neoplasia resulting from endometrial biopsy. Hyperthecosis must be differentiated from several other diseases, especially malignant conditions. Treatment should be bilateral oophorectomy for postmenopausal women (14). However, if the patient has multiple comorbidities, then long-term GnRH agonist treatment may be an option in cases where malignancy is excluded (15). For premenopausal women, treatment should be the same as for PCOS in cases where malignancy is excluded (7).

In conclusion, effective and timely treatment, especially bilateral oophorectomy in postmenopausal women, can reverse the cardio-metabolic consequences of hyperandrogenemia, and can reveal symptoms like acne and hair loss; also the risk of endometrial cancer is reduced.

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

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

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Malignant melanoma arising in an in vitro *fertilisation pregnancy: A case report*

In vitro *fertilizasyon gebeliğinde malign melanom: Olgu sunumu*

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Abstract

Malignant melanoma diagnosed during pregnancy results in confusion about staging and management. In this case report, a 39-year-old pregnant woman, who had undergone conception via *in vitro* fertilisation, was diagnosed with malignant melanoma of a growing lesion on her back in the 20th week of gestation. She delivered her baby by caesarean section in the 38th week. Metastasis was not found by chest X-ray, ultrasonography and positron emission tomography after delivery. She has been disease free for 6 months postpartum. Surgical resection of malignant melanoma and postponing of the sentinel lymph node biopsy has been proposed. Risk of adverse perinatal outcomes has not been increased; but the prognosis of malignant melanoma is known to be poorer when diagnosed during pregnancy. As a conclusion, any pigmentary change in the nevi should be assessed carefully during pregnancy. (J Turkish-German Gynecol Assoc 2013; 14: 186-7)

Key words: Malignant melanoma, pregnancy, *in vitro* fertilisation

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Özet

Gebelikte tanı alan malign melanom olgularında yönetim oldukça sıkıntılıdır. Hastalığın eksizyon sonrasında, evrelendirmesi ve tedavisi konusunda da fikirbirliği bulunmamaktadır. Bu olgu sunumunda, *in vitro* fertilizasyon (IVF) ile gebe kalan, gebeliğinin 20. haftasında sırtında ortaya çıkan lezyon biyopsisi malign melanom olarak gelen 39 yaşında bir hasta sunulmaktadır. Otuzsekizinci haftada sezeryanla sağlıklı bebek doğuran hastanın doğum sonrası tetkiklerinde metastaz saptanmamıştır. Postpartum 6 aylık süreçte nüks görülmemiştir. Cerrahi eksizyon ile malign melanom tanısı konduğunda evreleme için gerekli olan sentinel lenf nodu biyopsisinin doğum sonrasına ertelenmesi önerilmektedir. Perinatal risk artmamakla birlikte gebelikte tanı konan malign melanom olgularında prognozun daha kötü olduğu belirtilmektedir. Gebelikte, nevuslardaki herhangi bir değişiklik dikkatli değerlendirmelidir.

(J Turkish-German Gynecol Assoc 2013; 14: 186-7)

Anahtar kelimeler: Malign melanom, gebelik, *in vitro* fertilizasyon

Geliş Tarihi: 12 Kasım 2012 **Kabul Tarihi:** 20 Ocak 2013

Çevrimiçi Yayın Tarihi: 10 Temmuz 2013

Introduction

Malignancy in pregnancy causes confusion about diagnosis, management and pregnancy prognosis. The most common malignancy in pregnancy is breast cancer and malignant melanoma is quite common in advanced age. Although the actual incidence of malignant melanoma in pregnancy is not known, a figure of 2.8 per 1000 pregnancies has been reported (1). The risk benefit ratio of the diagnostic workup, surgery, radiotherapy and chemotherapy should be carefully assessed in pregnancy. There is no consensus about staging and treatment options after excisional procedure. Moreover, the long-term effect of pregnancy on disease progression is unclear. Here, malignant melanoma diagnosed in the 20th gestational week in a 39-year-old woman who conceived via *in vitro* fertilisation (IVF) is presented.

Case Report

A 39-year-old woman, suffering from primary infertility for 5 years was admitted to our department. The patient had conceived in her 6th *in vitro* fertilisation cycle. The pregnancy was uneventful until the 20th week of gestation when the patient noticed a growing 0.8x0.5 cm livid lesion with irregular margins on her back. Excisional biopsy revealed malignant melanoma (nodular type, Clark level III) filling up the superficial dermis, with 2.775 mm thickness (Breslow). Neither ulceration in the epidermis nor lymphovascular and neural invasion was present. She refused to undergo sentinel lymph node biopsy or other radiographic evaluations for staging. Radiotherapy was planned but the patient refused all treatment options. At the 38th week of gestation, she delivered a healthy 3300 g female by caesarean section. The placenta was grossly normal and pathology revealed no metastasis. Neither metastasis nor recurrence was found by chest X-ray, ultrasonography and positron emission tomography after delivery. She has been disease free for 6 months postpartum.

Discussion

Melanocytic nevi may develop, enlarge or darken during pregnancy due to the melanogenic effects of high oestrogen levels

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and melanocyte-stimulating hormone (2). Recently, studies have failed to show oestrogen receptors on human melanoma cells (3). Because melanoma accounts for 8% of malignancies during pregnancy, any pigmentary change in the nevi should be assessed and suspicious nevus should be excised properly. Surgery is the treatment of choice in patients with early melanoma. Resection of the primary tumour and postponing of the sentinel lymph node biopsy until after birth has been proposed. The only feasible procedure was surgery in our case because of trunk localisation.

Perinatal outcome in malignant melanoma is another issue. No substantially increased risk of adverse outcome, such as preterm birth, low birth weight and congenital abnormalities, has been reported; however, a possible increased risk of stillbirth in subsequent pregnancies is an exception in melanoma diagnosed within 2 years of delivery (4). Although placental or foetal metastasis is extraordinarily rare, melanoma is the most common malignancy that metastasises to the placenta (30%) and foetus (58%). Therefore, evaluation of the placenta is suggested. Our patient had a healthy baby at term with no metastasis to the placenta.

For adjuvant therapy, different treatment modalities have been widely investigated. Stage III (locoregional metastasis) and stage II (Breslow thickness >1.5 mm) patients were included in adjuvant melanoma trials discussing interferon, interleukin, chemotherapy, vaccines, colony stimulating factors and combination therapies (5). In this case, because of the long duration between excision and delivery, interferon was not used.

Malignant melanoma diagnosed in pregnancy had always been a matter of concern for poor prognosis. Lesions arising in pregnancy tend to be at higher stage and disease free survival is shorter in pregnant patients (6). However, the risk of death from melanoma does not increase when compared to non-pregnant women (7). No difference was found in the 10-year disease-free and overall survival rates between the pregnant and non-pregnant groups. Survival depends on tumour thickness and the presence of ulceration (8). Previous pregnancy can exert some favourable influence on prognosis; women with at least one pregnancy had a 94% probability of surviving melanoma compared to nulliparous women, of whom only 83% survived (9). Higher parity and young age at first pregnancy was found to be associated with reduced risk (10). In our case, advanced age and infertility can be poor prognostic factors. The high levels of sex steroids during IVF trials might have triggered the malignant process. Although meta analysis showed no association between melanoma prognosis and the use of oral contraceptives and hormone replacement therapy (10), further data are needed to clarify if there is any promoting or initiating effect of sex steroids on melanoma.

In conclusion, no evidence supporting pregnancy as an initiating factor for primary melanoma development exists. Pregnancy does not appear to effect survival in localised melanoma diagnosed before, during or after pregnancy. There is still controversy about the safety of diagnostic and staging procedures such as sentinel lymph node biopsy. Management depends on the stage of the disease and patient preferences. The most important prognostic factor for overall survival is the thickness of the lesion, regardless of pregnancy.

Ethics Committee Approval: *N/A*

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

Peer-review: *Externally peer-reviewed.*

Author contributions: *Concept –M.K.,İ.K., R.P.; Design –M.K, G.S.C, M.B.Y.; Supervision –R.P., G.S.C.*

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What is your diagnosis?

A 32-year-old patient who was G9 P6 Y6 A2 was admitted to our clinic for detailed examination at the 21st week of gestation. Her medical history revealed that there was no antenatal follow-up. Abdominal sonographic examination showed one live fetus at 20 weeks of development. Detailed ultrasonographic examination revealed a 2x2 cm cystic enlargement localized in the fetal posterior fontanelle (Figure 1). Magnetic resonance imaging (MRI) was performed after the ultrasonography (Figure 2). What is your diagnosis?

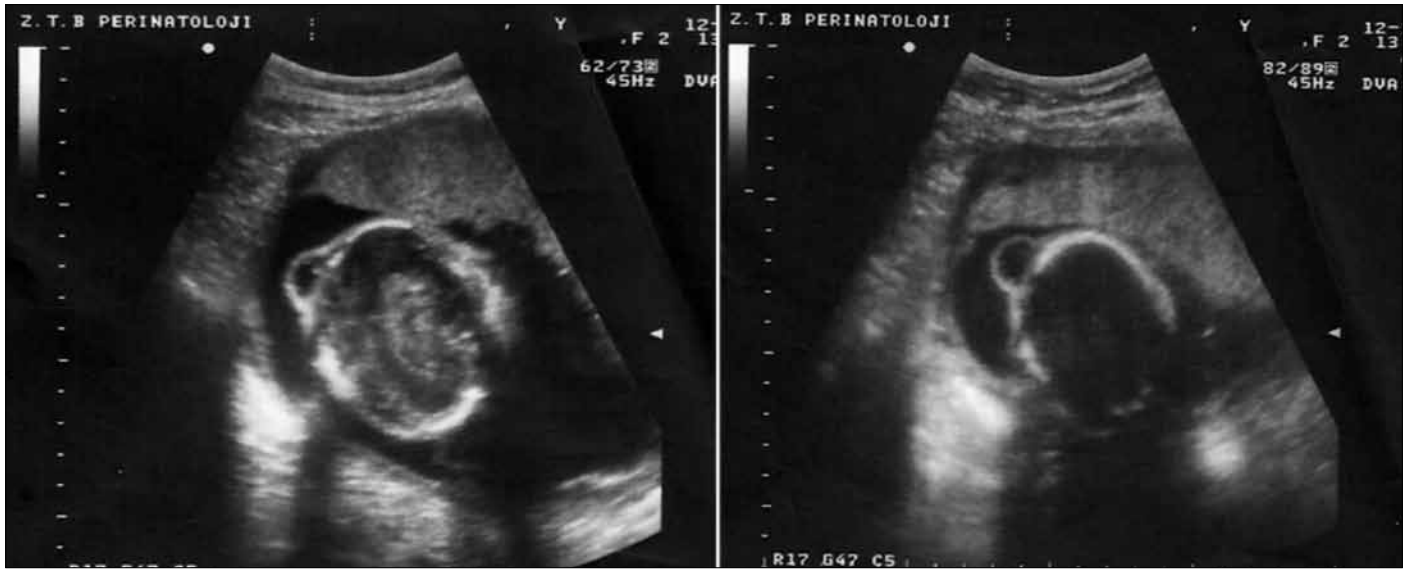

Figure 1. Fetal cranial ultrasonogram

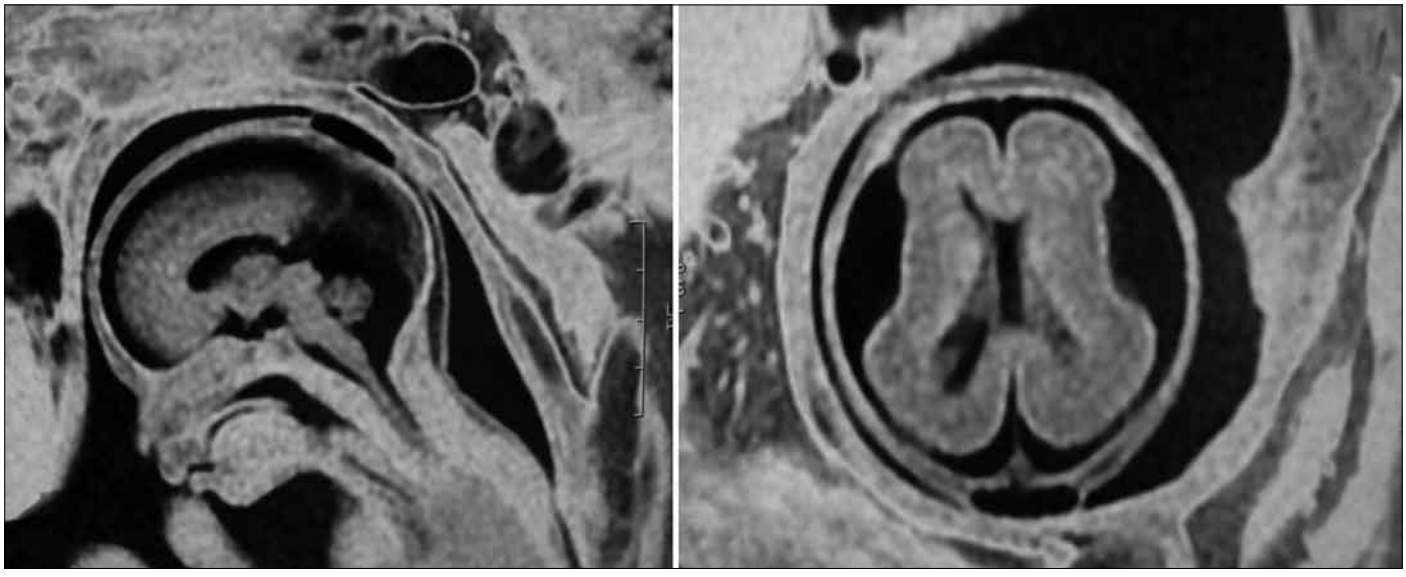

Figure 2. Fetal cranial MRI

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Answer

This case shows the importance of fetal MRI in prenatal diagnosis. With only sonographic examination, the most probable diagnosis was encephalocele. But, with the addition of MRI, the diagnosis of this case changed. The lesion was localized under the skin and there was no relationship with intracranial structures. A cystic lesion was found in the parieto-occipital region with a size of 2x1x5x1 cm had a hypodense appearance on T1 and a hyperdense appearance on T2 (Figure 2). Intracranial structures were normal on MRI. With all these findings, the lesion was determined to be a subcutaneous epidermal cyst. The family was informed and it was decided to continue the pregnancy. However, the patient was lost to follow-up.

The use of fetal MRI was first reported in 1983 (1). Since then, with rapidly increasing technological developments and accumulated knowledge and experience, MRI is predominantly used for central nervous, respiratory and gastrointestinal anomalies because of the limitations of ultrasonography. MRI is not recommended in the first trimester due to a lack of sufficient information about its safety (2).

Encephalocele is an extremely rare neural tube defect. The incidence of encephalocele is 0.8-5/10,000 live births (3). Although the mechanism of encephalocele is not completely known, an anterior neural tube closure defect is the most likely mechanism (3). As a result of this fetal defect, changes occur to the calvarium and dura mater, along with herniation of the leptomeninges to the extracranial region. This sac sometimes consists of only cerebrospinal fluid and sometimes cerebrospinal fluid and brain tissue. It can appear in the occipital, frontal and basal regions. The localization of herniation is associated with a racial distribution. The occipital localization of encephalocele is the most recognizable type during the antenatal period. Because of the localization and appearance of the cyst, our initial diagnosis was encephalocele. However, MRI was also used for a differential diagnosis and with this method we could provide sufficient information to the family. Obesity and oligohydramniosis have negative effects on ultrasonographic images, but these conditions have no negative effects on MRI. On the other hand, MRI has some limitations, like excessive fetal movement and fetal position changes. Today, images are obtained using an ultra-fast image capture technique (4). Before the procedure, an empty stomach and an empty bladder are recommended. Imaging is often performed in the supine position, but in later weeks of pregnancy, imaging is performed in the left lateral position because of supine hypotension.

Our case demonstrates once again that for soft tissue imaging, MRI is specific and sensitive. Our case also shows that T2 imaging is best for soft tissue while T1 imaging is more sensitive for hemorrhage, adipose tissue and diaphragmatic hernia.

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CONGRESS CALENDAR

INTERNATIONAL MEETINGS

5-8 September 2013	**SGI Summit Turkey** İstanbul, Turkey www.sgiturkey2013.org
18-21 September 2013	**10th Congress of the European Society of Gynecology (ESG)** Brussel, Belgium http://www.seg2013.com/
6-9 October 2013	**23rd World Congress on Ultrasound in Obstetrics and Gynecology** Sidney, Australia http://www.isuog.org/WorldCongress/2013
12-17 October 2013	**69th ASRM Annual Meeting to be held Conjoint with IFFS** Boston, MA, USA http://www.asrm.org/IFFS-ASRM2013
16-19 October 2013	**22nd Annual International Congress of the European Society of Gynaecological** Berlin, Germany http://www.esge.org/berlin-2013-esge/homepage
19-22 October 2013	**18th ESGO International Meeting** Athene, Greece http://www.esgo.org/esgo18/
24-27 October 2013	**18th World Congress on Controversies in Obstetrics, Gynecology & Infertility (COGI)** Vienne, Austuria http://www.seg2013.com/
25-27 October 2013	**The 8th ESIDOG European Conference** London, England http://www.esidog.com/5.html
7-9 November 2013	**The 3rd World Congress on the International Society for Fertility Preservation (ISFP)** Valencia, Spain http://www.comtecmed.com/ISFP/2013/Default.aspx
10-14 November 2013	**42nd AAGL Global Congress on Minimally Invasive Gynecology** Washington D.C., USA http://www.aagl.org/annual-meeting/
5-6 December 2013	**5th Annual Meeting on Laparoscopic, Robotic & Vaginal Hysterectomy with Comprehensive Hands-on Workshop on Laparoscopic Suturing & Knot-Tying with Simulation** New York, USA http://www.aagl.org/event/36/

NATIONAL MEETINGS

26-29 September 2013	**4th Congress of the Society of Reproductive Medicine** Antalya, Turkey http://www.utd2013.org
26-29 September 2013	**5th National Symposium of Osteoporosis** Muğla, Turkey http://www.osteoporoz2013.org/
8-10 November 2013	**Maternal Fetal Medicine and Perinatology Association of Turkey Ultrasonography Course** İstanbul, Turkey http://www.tmftpultrason2013.org/
20-23 November 2013	**3rd National – 2nd Congress of Midwifery** Antalya, Turkey http://www.ebko2013.org/
23-26 November 2013	**6th National Urogynecology Congress** İstanbul-Turkey http://urojinekoloji2013.org/
5-6 December 2013	**Derin İnfiltratif Endometriozis Sempozyumu** İstanbul, Turkey www.endometriozis2014.org
18-19 January 2014	**8th National Congress of Menopause Osteoporosis** İstanbul, Turkey http://turkiyemenopozosteoporoz.org/
30 April-3 May 2014	**TAJEV 2014** Titanic Deluxe Belek Hotel, Antalya, Turkey www.tajev2014.org - www.tajev.org

JTGGA CME/CPD CREDITING

Questions on the article within the scope of CME/CPD

1. Which of the followings is not a metabolic risk factor for polycystic ovary syndrome?
   a) Impaired glucose tolerance
   b) Type 2 diabetes mellitus
   c) Non-alcoholic steatohepatitis
   d) High LDL cholesterol level
   e) Low HDL cholesterol level

2. Which of the following factor effect the prevalence of polycystic ovary syndrome?
   a) Aging
   b) Obesity
   c) Smoking
   d) Alcohol
   e) Stress

3. Which of the following is not an effect of cyproterone acetate?
   a) Improvement in hirsutism score
   b) Improvement in menstruel dysfunction
   c) Worsening of carbohydrate metabolism
   d) Decrease in testosterone level
   e) Decrease in LDL level

4. Which of the following is an effect of spironolactone?
   a) Improvement in lipid profile
   b) Worsening in menstruel dysfunction
   c) Worsening of carbohydrate metabolism
   d) Decrease in testosterone level
   e) Weight gain

5. Which of the following is not an effect of drospirenone?
   a) Increase in HDL level
   b) Improvement in menstruel dysfunction
   c) Worsening of carbohydrate metabolism
   d) Decrease in testosterone level
   e) Drop in blood pressure

6. Which of the following is not an effect of metformine?
   a) Weighting loss
   b) Improvement in menstruel dysfunction
   c) Improvement in carbohydrate metabolism
   d) Increase in high sensitivity –CRP level
   e) Improvement in hirsutism score

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

1st Question				
A	B	C	D	E

2nd Question				
A	B	C	D	E

3rd Question				
A	B	C	D	E

4th Question				
A	B	C	D	E

5th Question				
A	B	C	D	E

6th Question				
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