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Editorial Office | Address: Abdi İpekçi Cad. 2/7 34367 Nişantaşı, İstanbul-Turkey
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Aims and Scope

Journal of the Turkish-German Gynecological Association is an official journal of the Turkish-German Gynecological Education and Research Foundation, Turkish-German Gynecological Association and the Turkish Society of Reproductive Medicine and is published quarterly on March, June, September and December.

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

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

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Editorial

Dear Colleagues,

It is my great pleasure to be introducing the final issue of our journal in 2012. Many interesting articles are submitted in this issue from Turkey and other countries. We are proud that the number of citations from our journal is increasing day by day.

Our journal-JTGGA is indexed by many internationally accepted databases such as SIIC, Tübitak/Ulakbim Turkish Medical Index, Turkish Citation Index, EBSCO host, SCOPUS, Excerpta Medica (EMBASE), DOAJ database, Gale/Cengage Learning, ProQuest, CINAHL and Index Copernicus. Our aim is to be indexed by as many indexes as possible. It will be our great pleasure to receive your qualified research studies to be published in a peer-reviewed and internationally indexed journal which will be very important in the many exams you will face. You can also contact our editorial team directly if you would like to be a reviewer and be actively involved in the evaluation process of our journal.

I am glad to inform you that Assoc. Prof. Dr. Yaprak Engin Üstün from Ankara Dr. Zekai Tahir Burak Women Health Training and Research Hospital has been appointed as an editor for our journal. I would like to thank her very much again for all her continuous efforts for our journal and wish her success to be everlasting. I would also like to thank all editorial board members and referees involved in the publication process of our journal for their admirable work through the year.

Our next congress - 10th Turkish German Gynecology Congress is planned to held in the spring of 2014. We have made a strategic decision and decided to postpone our congress for a year because of the heavy congress traffic and current situation of the industry. In this respect, we will also find more time to prepare a satisfactory congress not only scientifically but also socially. The tenth congress does also have an additional meaning to be the 10th anniversary of the traditional Turkish German Gynecology Congresses. I will inform you more about our congress preparation in the following issues.

I hope the New Year will be a fruitful era for all our community and bring us together in the light of science. Wish you all again success in your studies and looking forward to meeting you in the first issue of the next year.

Best regards,

Prof. Dr. Cihat Ünlü
Editor in Chief of the JTGGA
President of TAJEV

Platelet profile in patients with gestational diabetes: a retrospective study

Gestasyonel diabetik hastalarda trombosit profili: retrospektif değerlendirme

Muhammet Erdal Sak[1], Hatice Ender Soydinç[1], Ali Özler[1], Mehmet Sıddık Evsen[1], Abdülkadir Turgut[1], Sibel Sak[2], Talip Gül[1]

[1]Dicle University, School of Medicine, Department of Obstetrics And Gynecology, Diyarbakır, Turkey

[2]Diyarbakır Maternity And Children Hospital, Department of Obstetrics And Gynecology, Diyarbakır, Turkey

Abstract

Objective: To assess and compare alterations in the morphology and function of platelets occurring in gestational diabetes and healthy pregnancies.

Material and Methods: A retrospective study was performed of 77 pregnant women: 42 cases with gestational diabetes and 35 healthy controls. The two groups were compared in terms of demographics and platelet parameters derived from complete blood counts.

Results: The mean platelet volume ($p=0.001$) and HbA1c ($p<0.001$) were significantly increased in the patients with gestational diabetes. The mean platelet volume was well correlated with the platelet distribution width (rs=0.404, $p<0.001$) and the platelet count (rs=0.355, $p=0.002$)

Conclusion: The mean platelet volume and other platelet parameters may significantly aid the identification of diabetic pregnants at risk for vascular complications. The role and possible clinical relevance of these changes during diabetic pregnancy need to be investigated in further studies. (J Turkish-German Gynecol Assoc 2012; 13: 223-6)

Key words: Pregnancy, diabetes, gestational diabetes, platelet, mean platelet volume

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Özet

Amaç: Gestasyonel diabet ve sağlıklı gebelerde trombosit morfolojisi ve fonksiyonlarını değerlendirmek ve karşılaştırmak amaçlanmıştır.

Gereç ve Yöntemler: Kırk iki gestasyonel diabet, 35 sağlıklı kontrol olarak toplam 77 gebe hasta retrospektif olarak değerlendirildi. İki grup demografik veriler ve tam kan sayımından elde edilen trombosit parametreleri açısından karşılaştırıldı.

Bulgular: Gestasyonel diabet olgularında; ortalama trombosit hacmi ($p=0.001$) ve HbA1c ($p<0.001$) anlamlı olarak artmış saptandı. Ortalama trombosit hacmi , trombosit dağılım genişliği (rs=0.404, $p<0.001$) ve trombosit sayımı (rs=0.355, $p=0.002$) ile ilişkili bulundu.

Sonuç: Ortalama trombosit hacmi ve diğer trombosit parametreleri vasküler komplikasyonlar açısından risk altında olan diyabetik gebelerin belirlenmesinde önemli ölçüde yardımcı olabilir. Gestasyonel diyabetik gebelerdeki bu değişikliklerin rolü ve olası klinik ilişkisi için ileri çalışmalar yapılmalıdır.

(J Turkish-German Gynecol Assoc 2012; 13: 223-6)

Anahtar kelimeler: Gebelik, diabet, gestasyonel diabet, trombosit, ortalama trombosit hacmi

Geliş Tarihi: 26 Temmuz 2012 **Kabul Tarihi:** 04 Ekim 2012

Introduction

Altered platelet morphology and function have been reported in patients with diabetes mellitus (1). These changes may be associated with increased risk of vascular disease and venous thromboembolism (1-3). Although normal pregnancy may result in the activation of primary hemostasis and coagulation, these issues have not been widely investigated in gestational diabetes (GD).

Patients with diabetes mellitus show altered platelet function, including decreased nitric oxide synthase activity and increased peroxynitrite production (4-6). Platelet volumes are direct indicators of increased platelet synthesis (4). In normal pregnancies, a small increase in platelet aggregation occurs. This increase is compensated for by increased platelet synthesis and, consequently, in an increased mean platelet volume (MPV) (3, 7). The MPV is an indicator of platelet activation. In a normal pregnancy, changes in platelet volumes may be more sensitive than platelet numbers as a measure of altered platelet function (8). It is also increased in acute myocardial infarction, acute ischemic stroke, pre-eclampsia and renal artery stenosis (9). Importantly, an elevated MPV predicts a poor outcome following myocardial infarction, restenosis following coronary angioplasty, and the development of pre-eclampsia (5, 8, 9).

The present study was designed to compare and assess the demographic and laboratory findings in healthy pregnant women and GD patients. As far as we know, such a comparative study has not been reported previously in the literature.

Material and Methods

A retrospective study was performed in the obstetrics and gynecology department of a tertiary care center on the medical records of 77 pregnants (42 GD cases, 35 controls) diag-

Address for Correspondence: *Muhammet Erdal Sak, Dicle University School of Medicine, Department of Gynecology and Obstetrics, 21280, Diyarbakır, Turkey*
Phone: +90 533 511 22 72 e.mail: drmesak@yahoo.com

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nosed between February 2010 and February 2012. After obtaining the approval of the Institutional Review Board, patients' files were reviewed to collect relevant demographic, clinical, and laboratory data. Women with systemic diseases (hypertension, collagen tissue disease, heart disease, renal disease, hepatic disease) or a poor obstetric history requiring medication during gestation (recurrent pregnancy loss, previous occurrence of pre-eclampsia, preterm labor, intrauterine growth retardation, or intrauterine demise) were excluded from the study.

All women with GD had normal medical histories before pregnancy. The patients were screened with a 50-gram (g), 1-hour (h) glucose tolerance test between 24 and 28 weeks of pregnancy. In accordance with the recommendations of the American Diabetes Association, patients with a plasma glucose threshold value of 140 mg/dl one hour after glucose load intake under nonfasting conditions were included in the study. When the plasma glucose level reached ≥140 mg/dl following the 50-g oral glucose loading test, which was administered at 24-28 gestational weeks, a 100-g, 3-h oral glucose tolerance test was administered. The patients who had high values from both tests were considered to have GD. The patients were instructed to monitor their blood glucose levels. The patients were given information about nutrition and advised to perform physical activity for 30 min/day. Patients who had not achieved target levels were treated with human regular and neutral protamine hagedorn (NPH) insulin preparations used in multiple injection regimens. The body mass index and HbA1c of the patients were measured at the time of diagnosis. A fasting peripheral venous blood sample was obtained from all the participants in their last trimester (32-36 weeks). To avoid the platelet swelling induced by ethylene diamine tetra acetate (EDTA), blood samples were analyzed within half an hour of collection. An automated blood counter (CELL-DYN 3500, Abbott Diagnostics, Santa Clara, CA, USA) was used to measure complete blood count (CBC) parameters. Positivity for ketone bodies in the urine was investigated using the dipstick method (Siemens Multistix 10SG, Germany).

Statistical analysis was carried out using the SPSS 11.0 computer program (SPSS Inc, Chicago, IL, USA). Nonparametric tests were chosen for comparison due to the relatively small sample size. The Mann-Whitney test, student's *t* test, and Spearman correlation analysis were utilized when appropriate. $p<0.05$ was regarded as significant.

Results

Analysis of the data showed that the body mass index and the number of parities were increased in parallel with the advancement of age (rs=0.344, p=0.002; and rs=0.515, p<0.001 respectively). The MPV was well correlated with the platelet distribution width (rs=0.404, p<0.001) and the platelet count (rs=0.355, p =0.002).

Comparison of the two groups revealed that the MPV (p=0.001) and the HbA1c (p<0.001) were increased in GD (Table 1, Figure 1).

Discussion

Altered platelet morphology and function have been reported in patients with diabetes (6, 8, 9). Patients with diabetes have increased platelet activation compared to nondiabetic subjects (8-10). Platelet hyperactivity was accompanied by increased synthesis of thromboxane and/or decreased prostacycline production. MPV is a marker of platelet function and activation (11). Larger platelets are both more reactive and aggregable. They contain denser granules, secrete more serotonin and b-thromboglobulin, and produce more thromboxane A2 than smaller platelets. This points to a relationship between platelet function and micro- and macrovascular complications of diabetes mellitus (DM) (8-12).

Recently, an increase in MPV in the late phase of myocardial infarction has been shown to be an independent predictor for

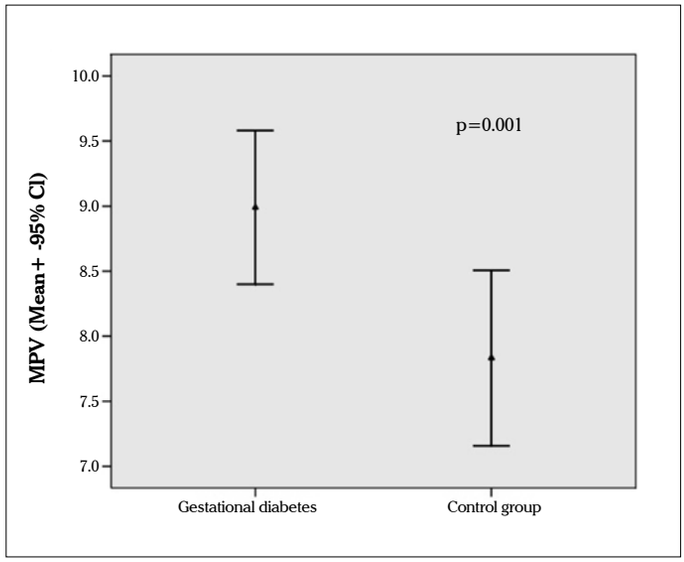

Figure 1. Comparison of MPV values in gestational diabetes and controls

Table 1. Demographic, clinical, and laboratory properties of the study and control groups (mean±standard deviation)

	Diabetes	Control	p Value
Age	31.2±4.1	30.7±3.8	0.224
Parity	1.8±1.6	1.6±1.5	0.634
BMI	28.6±2.4	27.5±3.6	0.048*
Leukocyte count	9.9±2.2	10.2±2.8	0.701
HbA1c	5.7±1.0	4.7±0.6	<0.001*
Hct	34.7±3.2	33.5±2.8	0.024*
Platelet count	250.4±64.4	256.8±63.8	0.567
MPV	8.9±1.9	7.8±2.0	0.001*
PDW	18.2±1.2	18.1±1.4	0.566

(BMI: body-mass index, HbA1c: glycosylated hemoglobin, Hct: hematocrit, MPV: mean platelet volume, PDW: platelet distribution width)
* denotes statistical significance

recurrent myocardial infarction (5, 7). Platelet hyperactivity in DM may be a contributor to severe and profound vasculopathies associated with this disorder (6, 8). Increased platelet aggregation has been demonstrated in DM, and this may potentially have a role in the development of vascular complications (2, 3, 5). Activated platelets respond to activated leukocytes and endothelial cells via adhesion molecules linking inflammation and thrombosis (4). Platelets of recent-onset Type 1 diabetic patients have been shown to be activated independently of metabolic control (5).

Platelet volume is a marker of platelet activation and function and is measured using the MPV (10). MPV values can be an effective marker for blood glucose (10-12). MPV values were found to be higher. However, after the blood glucose was reduced, there was a significant decrease in these MPV values (8, 10). MPV values have been found to be higher in diabetic patients when compared with normal controls (11). Patients with retinopathy and microalbuminuria had higher MPV values than patients without diabetic complications (9). In previous studies, MPV was observed to be higher in nonpregnant diabetics when compared with the normal population (8-12). Furthermore, in patients with impaired fasting glucose, which is thought to be indicative of prediabetes, a high MPV has been noted (12). In comparison to normal sized platelets, trombocytes with high MPV values are more reactive (9, 10). This situation may lead to vasoconstriction and vein occlusion and a decrease in the concentration of prostacylin, resulting in vasoconstriction at the vascular vein level (2, 9). It has been argued that an increase in the MPV sets the stage for micro- and macrovascular complications in diabetic patients (9-12).

Increased MPV values have also been reported in various cardiovascular diseases (7-9). MPV values can be an effective marker of blood glucose levels (11-12). Some studies have found that increased aggregation and multiplication functions occur in diabetic patients' megakaryocyte stem cells (7, 8). The glycoprotein IB molecule, a marker of megakaryocyte stem cells, is found more frequently in the cell membrane of platelets with high MPV values in diabetic patients (10). Other studies have argued that the number of peripheral platelets may depend on variables such as the platelet production rate and the mean platelet survival (9-11).

In our study, we found that HbA1c levels were increased in GD. This finding was expected. The identification of a larger MPV in GD patients suggests that the MPV may be used as a marker for follow-up of diabetic patients. Its potential needs to be confirmed in further prospective, randomized, controlled studies. Recently, Bozkurt et al. (10) claimed that GD patients had higher MPV values than normal control subjects and that patients with high MPV values had low platelet counts. It has been reported that platelet survival is shorter in diabetic patients (9, 12). This may be explained by variables such as platelet production and mean platelet survival. The platelet distribution width displays a good correlation with the MPV. We did not detect a significant difference between the platelet distribution width values between the two groups.

Gestational DM is a systemic disease that affects both the mother and fetus (10, 11). These patients are more likely to develop Type 2 DM; hence, they must be monitored closely. As an increased MPV may reflect increased platelet activation, further studies on platelet parameters and functions might be helpful in decreasing the mortality and the morbidity associated with GD.

We are aware of the limitations of this study due to the retrospective design and limited number of study participants, which may fail to demonstrate small differences between the groups. Gestational DM may not always constitute a good model for extrapolation of results to Type 2 diabetes. However, we suggest that modifications in glycemia undetectable by standard clinical laboratory methods can be reflected via alterations in platelet features. We also compared the influences of short-term (gestational) diabetes on platelet parameters of CBC.

DM is associated with serious potential systemic and metabolic risks during pregnancy. Diabetic pregnants need to be closely observed during their antenatal checkups. Close observation is essential to prevent complications of diabetic illnesses associated with hyperglycemia, which has a negative influence on all maternal systems and on fetal homeostasis. Further research may indicate higher MPV values in pregnants with poor diabetic control. As studies related to platelet functions in diabetic pregnants increase, we strongly believe that improvements will occur in prenatal and postnatal observation and treatment, which will subsequently result in a decrease in fetomaternal complications.

In conclusion, the present work shows that measurement of the MPV and other platelet-related parameters is a simple procedure, available in most hospital laboratories. These parameters may significantly aid the identification of diabetic pregnants at risk for vascular complications. The role of changes in these parameters in the hemostatic system during diabetic pregnancy and the possible clinical relevance concerning the risk for thrombosis call for further studies.

Conflict of interest

No conflict of interest was declared by the authors.

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C-reactive protein and lipoprotein-a as markers of coronary heart disease in polycystic ovary syndrome

Polikistik over sendromlu hastalarda koroner kalp hastalıklarının belirteci olarak c-reaktif protein ve lipoprotein-a bakılması

Nilgün Güdücü, Herman İşçi, Alin Başgül Yiğiter, İlkkan Dünder

Department of Obstetrics and Gynecology, İstanbul Bilim University Avrupa Florence Nightingale Hospital, İstanbul, Turkey

Abstract

Objective: The aim of this study was to investigate the risk factors of coronary heart disease, CRP and Lipoprotein-a in polycystic ovary syndrome patients.

Material and Methods: Prospectively collected data of polycystic ovary syndrome patients (n=62) and control group (n=40) were compared.

Results: PCOS patients had higher HOMA-IR, CRP, DHEAS, free testosterone, FAI, LH and prolactin levels when compared to the control group. Lipoprotein-a levels did not differ between the groups. The obese PCOS group had statistically significantly higher fasting blood glucose, total cholesterol, triglyceride, free testosterone, insulin, CRP and HOMA-IR and statistically significantly lower HDL and SHBG when compared to normal weight PCOS persons. Fasting blood glucose, total cholesterol, LDL, SHBG, CRP, Lipoprotein-a, FSH, LH, TSH, DHEAS and prolactin levels did not differ between the normal weight and obese control groups.

Conclusion: CRP levels increase in polycystic ovary syndrome patients and can be used as a marker of coronary heart disease. Future studies can be directed at treatments to decrease CRP levels, including antiinflammatory treatments.

(J Turkish-German Gynecol Assoc 2012; 13: 227-32)

Key words: Polycystic ovary syndrome, obesity, CRP, lipoprotein-a, coronary heart disease

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Özet

Amaç: Bu çalışmanın amacı polikistik over sendromlu hastalarda koroner kalp hastalıkları risk faktörlerini, CRP ve Lipoprotein-a araştırmaktı.

Gereç ve Yöntemler: Prospektif olarak polikistik over sendromlu (n=62) ve kontrol grubunun (n=40) kan örnekleri çalışıldı.

Bulgular: Polikistik over sendromlu hastalarda HOMA-IR, CRP, DHEAS, serbest testosteron, FAI, LH and prolaktin seviyeleri yüksek, FSH ve SHBG seviyeleri ise düşük bulundu. Lipoprotein-a gruplarda farklı değildi. Obez polikistik over sendromlu hastalarda açlık kan şekeri, total kolesterol, trigliserit, serbest testosteron, insulin, CRP ve HOMA-IR daha yüksek, HDL ve SHBG daha düşük bulundu. Açlık kan şekeri, total kolesterol, LDL, CRP, SHBG, Lipoprotein-a, FSH, LH, TSH, DHEAS ve prolaktin seviyeleri obez ve normal kilolu kontrol gruplarında benzerdi.

Sonuç: CRP polikistik over sendromlu hastalarda yükselir ve koroner kalp hastalıklarının belirteci olarak kullanılabilir. Gelecekte CRP'nin düşürülmesine yönelik, antienflamatuar tedavileri de içeren araştırmalar planlanabilir. (J Turkish-German Gynecol Assoc 2012; 13: 227-32)

Anahtar kelimeler: Polikistik over sendromu, obesite, lipoprotein-a, koroner kalp hastalığı, CRP

Geliş Tarihi: 09 Mayıs 2012 **Kabul Tarihi:** 12 Kasım 2012

Introduction

Polycystic ovary syndrome (PCOS) is a heterogenous disease characterized by hyperandrogenism, chronic oligo-anovulation, infertility and insulin resistance (IR) (1). This common endocrinopathy, encountered in 5-10% of women of reproductive age (2), necessitates exclusion of other etiologies of hirsutism and anovulation. The wide-spectrum of the disease and the changing nature of the clinical presentation with weight fluctuations may be challenging. Most women with PCOS present with features of metabolic syndrome (MS), including abdominal obesity, IR, hypertension and dyslipidemia (3). PCOS patients were reported to have an increased prevalence of MS, about 2-3 times higher than age-matched controls (3, 4). Metabolic syndrome is a known risk factor for coronary heart disease (CHD), and PCOS patients are a group of young women with a high risk of early-onset CHD (5). In a recent study women with PCOS were reported to have an increased incidence of cardiovascular events and a lower event free survival (6). Lipoprotein-a (Lp-a) is a genetically determined LDL-like atherogenic lipoprotein (7). The aim of this study was to investigate the previously suggested biochemical markers of CHD in women with PCOS, namely C-reactive protein (CRP) and Lp-a (8, 9).

Material and Methods

This is a prospective cross-sectional study carried out in İstanbul Bilim University School of Medicine, Department of Obstetrics and Gynecology Clinics between April 2010

Address for Correspondence: *Nilgün Güdücü, Hüseyin Cahit Yalçın Sk. No: 1 Fulya, İstanbul, Turkey*
Phone: +90 533 640 40 10 e.mail: nilgun.kutay@gmail.com

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and December 2011. The study was in agreement with the Declaration of Helsinki, 1975 and all of the involved patients gave their informed consent. Some of the data used in this study was used in another study which has been reported previously.

The study group consisted of 62 women with PCOS and 40 healthy, normally menstruating women without any concomitant disease. All PCOS patients were diagnosed as PCOS according to the 2003 Rotterdam ESHRE/ASRM PCOS Consensus Workshop Group criteria (10) if at least two of the following criteria were present: oligo/amenorrhea, clinical or biochemical hyperandrogenism and PCO on ultrasonography. Clinical hyperandrogenism was defined as the presence of a Ferriman-Gallwey score>8. PCO was defined as the presence of an ovary with 12 or more follicles measuring 2-9mm in diameter. All subjects in the control group had a normal pelvic ultrasound, regular periods and no clinical or biochemical hyperandrogenism. Patients with systemic diseases such as diabetes mellitus, cardiovascular diseases, hypertension, thyroid diseases, chronic renal failure, malignancy, Cushing syndrome, congenital adrenal hyperplasia, hyperprolactinemia and gastrointestinal malabsorptive diseases were excluded. None of the patients were on any medications for at least 3 months before the study including oral contraceptives, glucocorticoids, lipid-lowering, antiobesity, antidiabetes, antiandrogenic, antihypertensive or ovulation-inducing agents.

All the patients underwent a physical examination and appropriate laboratory tests were performed. BMI was calculated as body weight in kilograms divided by height in square metres (kg/m^2). Patients were also separated into two groups according to their body mass index (BMI). Twenty-one patients with PCOS were obese (BMI≥ 25) and 41 were non-obese (BMI< 25). In the control group, 11 patients were obese (BMI≥ 25) and 29 were non-obese (BMI <25). Weight, height and waist and hip circumferences were measured. Waist circumference (WC) was obtained as the smallest circumference at the level of the umbilicus. Hip circumference (HC) was obtained as the widest circumference at the level of the buttocks. Serum samples were obtained from all women in the early follicular phase after an overnight fast, during the 3^{rd}-4^{th} days of the cycle. Levels of fasting plasma glucose, insulin, total cholesterol, high-density lipoprotein (HDL), low density lipoprotein (LDL), triglycerides (TG), LH, FSH, prolactin, TSH, dehydroepiandosterone sulfate (DHEAS), free testosterone, cortisol, freeT4, 17-OH proges-

Table 1. Anthropometric, biochemical and hormonal characteristics of the study groups

	PCOS n=62	Control n=40	
	Mean±SD (Median)	Mean±SD (Median)	p
Age (years)	24.77±4.85	28.13±5.66	0.002**
Height (cm)	163.37±6.48	162.98±6.76	0.768
Weight (kg)	64.60±14.88	61.82±14.69	0.357
Waist/Hip ratio	0.81±0.09	0.79±0.06	0.197
BMI (kg/m^2)	24.15±5.35	23.35±5.33	0.465
Fasting glucose(mg/dL)	91.95±6.99	90.35±7.22	0.267
Insulin (uU/mL)	11.41±6.89	8.73±3.85	0.014*
Total Cholesterol (mg/dL)	162.39±36.32	162.41±28.92	0.998
Triglyceride (mg/dL)	69.23±30.30	65.67±25.23	0.540
HDL (mg/dL)	56.60±15.97	54.42±13.67	0.479
LDL (mg/dL)	93.62±27.24	99.70±27.24	0.275
+DHEAS (ug/dL)	273.98±109.45 (280.55)	235.63±103.69 (219.00)	0.036*
+F. Testosterone (ng/dL)	0.73±0.54 (0.63)	0.38±0.23 (0.30)	0.001**
FSH (mIU/mL)	5.46±1.39	6.87±2.08	0.001**
LH (mIU/mL)	8.72±4.17	6.15±2.50	0.001**
Prolactin(ng/mL)	20.87±9.21	16.53±6.16	0.006**
TSH (uIU/mL)	2.43±1.221	2.13±1.07	0.198
HOMA-IR	2.58±1.71	2.01±1.07	0.042*
+SHBG (nmol/mL)	44.44±25.5 (35.59)	50.39±22.98 (42.42)	0.029*
FAI	4.31±2.78	2.36±1.52	0.017*
+Lipoprotein-a (mg/dL)	24.69±20.05 (18)	22.11±28.77 (9.7)	0.097
+CRP (mg/dL)	0.27±0.28 (0.16)	0.19±0.25 (0.10)	0.046*

Student t Test, +Mann Whitney U Test, *p<0.05, **p<0.01

terone, sex-hormone binding globulin (SHBG), CRP (cobas integra 400, Roche) and Lp-a (cobas integra 800, Roche) were measured. Insulin resistance was calculated by the homeostasis model assessment (HOMA) index with the formula: HOMA-IR=fasting plasma immunoreactive insulin (μU/mL)x fasting serum glucose (mg/dL)/405, after excluding those with a serum glucose>200mg/dL, using insulin and thiazolidine.

Statistical analyses were performed using the Number Cruncher Statistical System (NCSS) 2007& Power Analysis and Sample Size (PASS) 2008 Statistical Software (Utah). Data showing normal distribution of parameters were compared with the Student's t-test, data showing non-normal distribution of parameters were compared with Mann Whitney U test, relation of CRP and Lp-A with other parameters was compared with Spearman's correlation analysis. At a confidence interval of 95%, p-values<0.05 were considered statistically significant.

Results

The anthropometric, biochemical and hormonal data of the groups were shown in Table 1. BMI, waist to hip ratio (WHR), fasting plasma glucose, total cholesterol, TG, HDL, LDL, lipoprotein-a (Lp-a), insulin and TSH levels were similar in the PCOS and control groups. PCOS patients had higher HOMA-IR, CRP, DHEAS, free testosterone, FAI, LH and prolactin levels when compared to the control group and lower FSH and SHBG levels. Lp-a levels did not differ between the groups.

Lp-a correlated negatively with DHEAS and positively with LDL in the control group, but did not correlate with any parameter in women with PCOS (Table 2). CRP correlated positively with weight, BMI, insulin, LDL and HOMA-IR in women with PCOS, with weight, WHR, BMI, insulin, TG and HOMA-IR in the control group and correlated negatively with HDL in the control group (Table 2).

The obese PCOS group had statistically significantly higher fasting blood glucose, total cholesterol, triglyceride, free testosterone, insulin, CRP and HOMA-IR and statistically significantly lower HDL and SHBG when compared to the normal weight PCOS controls (Table 3). Lp-a, FSH, LH, TSH, DHEAS and prolactin levels did not differ between the PCOS groups (Table 3). Obese controls had statistically significantly higher triglyceride, insulin, free testosterone and HOMA-IR and statistically significantly lower HDL when compared to normal weight controls (Table 4). Fasting plasma glucose, total cholesterol, LDL, SHBG, CRP, Lp-a, FSH, LH, TSH, DHEAS and prolactin levels did not differ between the normal weight and obese control groups (Table 4).

Table 2. Correlations according to Lipoprotein-a and CRP between the groups

	Lipoprotein-a				CRP			
	PCOS		Control		PCOS		Control	
	r	p	r	p	r	p	r	p
Age	-0.111	0.400	0.159	0.335	-0.056	0.672	0.186	0.250
Weight	0.127	0.337	0.229	0.162	0.411	0.001**	0.457	0.003**
Waist/Hip ratio	0.049	0.711	0.231	0.158	0.032	0.808	0.314	0.049*
Height	0.155	0.241	-0.049	0.769	-0.053	0.689	0.041	0.800
BMI	0.094	0.480	0.252	0.121	0.389	0.002**	0.503	0.001**
Fasting glucose	-0.026	0.847	0.051	0.760	0.189	0.147	0.202	0.212
Insulin	0.143	0.285	0.209	0.202	0.421	0.001**	0.453	0.003**
T. Cholesterol	0.115	0.474	0.095	0.625	0.267	0.092	0.041	0.833
Triglyceride	-0.008	0.950	0.148	0.367	0.113	0.390	0.404	0.010*
HDL	0.156	0.239	-0.223	0.173	-0.090	0.495	-0.325	0.041*
LDL	-0.017	0.899	0.333	0.038*	0.295	0.022*	-0.025	0.877
DHEAS	-0.022	0.868	-0.407	0.010*	0.172	0.189	0.073	0.657
F. Testosterone	-0.252	0.056	-0.063	0.706	0.151	0.253	0.256	0.116
FSH	0.155	0.245	0.157	0.340	0.086	0.518	-0.016	0.920
LH	-0.052	0.697	0.078	0.639	-0.017	0.900	0.004	0.980
Prolactin	-0.064	0.638	-0.242	0.138	-0.058	0.667	-0.202	0.211
TSH	-0.233	0.078	0.031	0.849	-0.123	0.354	0.051	0.756
HOMA-IR	0.169	0.200	0.232	0.156	0.372	0.003**	0.467	0.002**
SHBG	0.086	0.697	0.191	0.273	-0.234	0.271	-0.150	0.384
FAI	-0.122	0.571	-0.252	0.384	0.170	0.416	0.059	0.834

r: Spearman's correlations, *p<0.05, **p<0.01

Table 3. Comparison of parameters according to BMI in PCOS patients

n=62	BMI<25	BMI>25	p
	Mean±SD (median)	Mean±SD (median)	
Fasting glucose (mg/dL)	90.39±5.98	95±7.92	0.013*
Insulin (uU/mL)	9.14±5.26	16.07±7.61	0.000**
T. Cholesterol (mg/dL)	153.75±27.09	181±46.87	0.023*
Triglyceride (mg/dL)	62.61±22.03	82.80±39.88	0.045*
HDL (mg/dL)	59.49±16.63	50.70±12.98	0.043*
FSH (mIU/mL)	5.49±1.57	5.43±0.98	0.859
LDL (mg/dL)	87.32±3.41	106.55±32.82	0.008**
+DHEAS (ug/dL)	266.60±114.04 (282.10)	288.40±101 (278)	0.337
+F. Testosterone (ng/dL)	0.59±0.45 (0.50)	1.03±0.59 (0.87)	0.001**
LH (mIU/mL)	9.30±4.48	7.62±3.35	0.138
Prolactin (ng/mL)	22.23±10	18.33±7.06	0.119
TSH (uIU/mL)	2.27±1.04	2.73±1.46	0.211
HOMA-IR	1.95±1.02	3.80±2.11	0.002**
+SHBG (nmol/mL)	57.90±24.50 (47.30)	24.24±6.71 (23.61)	0.001**
FAI	3.04±1.66	6.35±3.073	0.002**
+Lipoprotein-a (mg/dL)	24.64±20.55 (18.50)	24.80±19.49 (17.60)	0.542
+CRP (mg/dL)	0.20±0.24 (0.11)	0.42±0.31 (0.42)	0.001**
Student t Test, +Mann Whitney U Test, *p<0.05, **p<0.01			

Table 4. Comparison of parameters according to BMI in the control group

n=40	BMI<25	BMI>25	p
	Mean±SD	Mean±SD	
Fasting glucose (mg/dL)	89.90±5.90	91.55±10.19	0.526
Insulin (uU/mL)	7.8±3.18	11.13±4.56	0.013*
T. Cholesterol (mg/dL)	161.64±28.62	164.86±32.06	0.803
Triglyceride (mg/dL)	59.00±19.67	83.27±30.50	0.005**
HDL (mg/dL)	57.80±10.73	45.55±16.93	0.009**
LDL (mg/dL)	96.93±27.34	107±26.82	0.303
+DHEAS (ug/dL)	230.75±103.15 (218)	248.50±109 (220)	0.671
+F. Testosterone (ng/dL)	0.34±0.22 (0.30)	0.51±0.24 (0.53)	0.035*
FSH (mIU/mL)	6.73±2.19	7.24±1.81	0.494
LH (mIU/mL)	6.33±2.68	5.67±2.00	0.464
Prolactin (ng/mL)	16.58±5.21	16.40±6.33	0.933
TSH (uIU/mL)	1.93±0.74	2.65±1.57	0.166
HOMA-IR	1.76±0.80	2.66±1.41	0.016*
+SHBG (nmol/mL)	53.63±25.71 (43.31)	40.68±4.64 (41.81)	0.083
FAI	2.17±1.95	2.66±054	0.565
+Lipoprotein-a (mg/dL)	20.47±32.31 (8.9)	26.28±17.32 (21)	0.058
+CRP (mg/dL)	0.14±0.15 (0.10)	0.33±0.39 (0.13)	0.098
Student t Test, +Mann Whitney U Test, *p<0.05, **p<0.01			

Discussion

Although there is no standard evaluation for cardivascular risk assessment, CRP is a known predictor of future CHD risk (11) and Lp-a was reported to be associated with coronary heart disease (9). High sensitivity CRP is a marker of inflammation, an acute phase reactant that is synthesized in the liver as a response to tumor necrosis factor-a (TNF-α) and interleukin-6 (11). CRP was also shown to play a role in cardiovascular events by complement activation, adhesion molecule expression and promotion of LDL uptake by macrophages (12). In this study, we detected increased CRP levels in PCOS patients, as reported previously (13,14). In our study, PCOS patients had statistically significantly increased CRP levels and obese PCOS patients had higher CRP levels than non-obese PCOS. We did not find such a relationship in the control group. Both non-obese and obese PCOS were reported to have higher CRP concentrations when compared to the control groups with similar BMI (8, 15). Gen et al. (16) reported similar CRP levels in non-obese PCOS and healthy women. These findings suggest an increased body weight as the major determinant of metabolic abnormalities related to CHD in PCOS women, as reported previously (16, 17) and CRP as the marker of increased CHD risk.

A significant correlation was previously reported between CRP and IR in PCOS patients (13-15). In our study, CRP correlated with IR both in PCOS patients and control group. In PCOS patients correlation of CRP with IR was independent of WHR, but in the control group it was related to the presence of abdominal obesity. These findings suggest an increased risk of CHD in PCOS patients independent of abdominal obesity.

Hyperandrogenic women not fulfilling PCOS criteria were reported to have CRP levels similar to the control group (18). There was no correlation between CRP levels and androgens in our study, as reported previously (13).

We hypothetized that by measuring Lp-a levels, cardiovascular risk factors not related to insulin resistance could be determined and treatment strategies could be developed. We found similar Lp-a levels in PCOS patients and healthy controls without concomitant disease. Moreover Lp-a plasma levels showed no variation when groups were compared according to the BMI and had no correlation with other metabolic parameters. Patients with MS, a syndrome with features similar to PCOS, were reported to have higher Lp-a levels when compared to the control group (19). Previously, PCOS patients were shown to have increased concentrations of Lp-a (9). In our study there was no difference in Lp-a levels between PCOS patients and controls. Elevated plasma levels of Lp-a have been suggested to increase the risk of CHD in a way independent of insulin resistance (20). Other studies suggested modifications in Lp-a by glycation, this modification was reported to increase the risk of CHD both in type 1 and type 2 diabetic women (9, 21, 22). Obese PCOS were reported to have higher Lp-a levels when compared to non-obese PCOS, while other studies found elevated Lp-a both in non-obese and obese PCOS (23, 24). In our study, there was no correlation between Lp-a levels and IR markers. In our control group, Lp-a levels correlated positively with LDL and negatively with DHEAS levels. These findings obscure the suggestion of Lp-a as a marker of CHD in PCOS patients. As Lp-a is genetically and racially determined (7), these findings may be unique to our population.

Insulin resistance is a common feature of PCOS, it affects about half the women with PCOS, whether obese or non-obese (25). PCOS patients were reported to have a form of IR intrinsic to PCOS (26). Forty percent of obese PCOS patients developed diabetes or impaired glucose tolerance until the age of 26 years (27). Obesity acts as an additive factor, increasing IR (25). Our PCOS patients had higher IR when compared to the control group.

Conclusion

CRP levels were increased in PCOS patients and the increase was higher in obese PCOS patients. Weight loss may decrease CHD risk in PCOS patients and CRP can be used as a marker of CHD. The role of antiinflammatory drugs in decreasing CRP and CHD of PCOS patients can be studied in the future clinical trials.

Conflict of interest

No conflict of interest was declared by the authors.

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Investigation of oxidative balance in patients with dysmenorrhea by multiple serum markers

Dismenoresi olan hastalarda çeşitli serum belirleyicileri ile oksidatif dengenin araştırılması

Nilgün Turhan[1], Havva Çelik[1], Candan İltemir Duvan[1], Yüksel Onaran[1], Murat Aydın[2], Ferah Armutcu[2]

[1]Department of Gynecology and Obstetrics, Faculty of Medicine, Fatih University, Ankara, Turkey

[2]Department of Biochemistry, Faculty of Medicine, Fatih University, Ankara, Turkey

Abstract

Objective: To investigate the level of oxidative stress in patients with dysmenorrhea by multiple serum markers including malondialdehyde (MDA), nitrotyrosine (3-NT), deoxyguanosine (8-OHdG) and superoxide dismutase (SOD).

Material and Methods: Fifty-eight women, aged between 20 and 34, who had had regular menses for at least six previous cycles, were involved. The women were divided into two groups. The study group consisted of 33 patients with primary dysmenorrhea, and the control group consisted of 25 healthy women.

Results: Demographic characteristics of patients were similar between the two groups. The serum MDA levels were 1.32±0.46 and 0.91±0.26 nmol/mL for the dysmenorrhea and control groups, respectively ($p<0.001$). The differences in plasma levels of 3-NT, SOD and serum 8-OhdG were similar in both groups ($p>0.05$). Also, no correlation was found between the severity of dysmenorrhea and the levels of oxidative markers.

Conclusion: Oxidative stress is slightly aggravated in patients with dysmenorrhea. (J Turkish-German Gynecol Assoc 2012; 13: 233-6)

Key words: Primary dysmenorrhea, malondialdehyde, nitrotyrosine, deoxyguanosine, superoxide dismutase

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Özet

Amaç: Dismenoresi olan hastalarda malondialdehit (MDA), nitrotirozin (3-NT), deoksigunaozin (8-OhdG) ve süperoksit dismutaz (SOD) gibi belirleyiciler ile oksidatif stres düzeyinin araştırılması.

Gereç ve Yöntemler: Yaşları 20 ile 34 arasında değişen, en az 6 aydır düzenli adet gören 58 kadın çalışmaya dâhil edildi. İki gruba ayrıldılar. Primer dismenoreli 33 hasta çalışma grubunu, sağlıklı 25 hasta ise kontrol grubunu oluşturdu.

Bulgular: Hastaların demografik karakteristik özellikleri her iki grupta da birbirine benzerdi. MDA düzeyleri, dismenore ve kontrol grupları için sırasıyla; 1.32±0.46 ve 0.91±0.26 nmol/mL idi ($p<0.001$). 3-NT, SOD plazma ve 8-OhdG serum düzeyleri her iki grupta da biribirine benzerdi ($p>0.05$). Dismenorenin şiddeti ile oksidatif belirleyicilerin düzeyleri arasında herhangi bir bağlantı bulunamadı.

Sonuçlar: Oksidant/Antioksidant dengesi, dismenoreli hastalarda hafifçe reaktif oksijen radikalleri lehine değişikliğe uğramıştır.

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Anahtar kelimeler: Primer dismenore, malondialdehit, nitrotirozin, deoksiguanozin, süperoksit dismutaz

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Introduction

Primary dysmenorrhoea is defined as pelvic pain around the time of menstruation in the absence of an identifiable pathologic lesion (1). Dysmenorrhoea occurs due to myometrial contraction induced by prostaglandins originating from the secretory endometrium. The secretory endometrium contains sunstantial stores of arachidonic acid, which is converted to prostaglandin F2α (PGF2α), prostaglandin E2 (PGE2), and leukotrienes during menses. Other symptoms associated with dysmenorrhoea including headache, nausea and vomiting, backache and diarrhea are related to the elevation of circulatory prostaglandins (PGF2α, PGE2) and their metabolites. The posterior pituitary peptides, vasopressin and oxytocin, have also been implicated in the aetiology.

In a study, both vasopressin and PGF2 alpha are higher and markedly fluctuating vasopressin levels were found in the women with dysmenorrhea (2, 3). Furthermore the differences in oxytocin, vasopressin, FSH and 17beta-E2 concentrations found in women with dysmenorrhea plasma suggest an involvement of these hormones in mechanisms of primary dysmenorrhea (4).

Most of the release of prostaglandins during menstruation occurs within the first 48 hrs, which coincides with the greatest intensity of the symptoms (5). It is well known that the presence of elevated concentrations of free radicals and/

Address for Correspondence: *Candan İltemir Duvan, Hoşdere Cad. No: 145-147, Y. Ayrancı, 06540 Ankara, Turkey*
Phone: +90 533 630 88 10 e.mail: candanduvan@yahoo.com

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or lowered antioxidant potential leads to oxidative stress (OS). Recently, oxidative stress has been implicated in more than 100 diseases (6, 7). Dysmenorrhea also has been reported to lead to an increase in lipid peroxidation, an index of oxidative stress (8). Previous studies about OS and dysmenorrhea investigated only malondialdehyde (MDA) levels as a marker of oxidative status (9, 10). However, measuring MDA levels is limited in detecting oxidative stress in some cases, and might not be sufficient to show real oxidative stress in dysmenorrhoea (11, 12). Reactive oxygen species (ROS) produced either endogenously or exogenously can attack lipid, protein and nucleic acid simultaneously in living cells (13). In order to understand the extent of oxidative stress in dysmenorrheal women, in addition to MDA we examined nitrotyrosine (3-NT), which is a marker of the oxidation chain of protein and deoxyguanosine (8 OHdG) level, which is also a sensitive marker of oxidative DNA damage in cells (14, 15). To our knowledge, there are no other studies in the literature investigating the protein and DNA oxidation pathway in women with dysmenorrhea. The development of oxidative stress in dysmenorrhea may be related to its severity. Furthermore, redox levels may modulate the severity and the dynamics of dysmenorrhea. To the best of our knowledge there are no data revealing the relation of redox levels and severity of dysmenorrhea. In the present study, we evaluated oxidative stress and relation between redox levels and severity of dysmenorrhea.

Material and Methods

Fifty-eight eligible women were enrolled in this study, between June 2007 and July 2008 in the Department of Obstetrics and Gynecology, Fatih University. The study was approved by the Institutional Review Board of Fatih University and written informed consents were obtained from each of the women before the start of the study. The inclusion criteria for women were as follows: Women with a history of primary dysmenorrhoea, nulliparous, aged between 21 and 32, body mass index (BMI) < 23kg/m^2, who use an acceptable method of barrier contraception, but who do not use an intrauterine contraceptive device or an oral contraceptive. Patients with pelvic pathology or a history of alcohol and tobacco use were excluded. Care was taken to make sure that each participant had not taken any analgesic within 24 hrs prior to the study and to be in their most painful phase of menstrual cycle.

The following criteria are used to define dysmenorrhoea:

- Onset of pain within 6-12 hrs after menstruation.
- Lower abdominal or pelvic pain associated with onset of menses and lasting 8-72 hrs.
- Lower back pain during menses.
- Medial or anterior thigh pain.
- Menstrual pain with associated features such as headache, diarrhea, nausea and vomiting (1).

In addition, the severity of dysmenorrhea in all patients was scored on a 5-point scale ranging from 0 to 4 (i.e. 0=no pain, 1=mild pain requiring no medication, 2=moderate pain responding to mild pain relievers, 3=severe pain necessitating potent pain relievers, and 4=incapacitating pain unresponsive to potent pain relievers (16).

Patients were allocated into two groups. The study group consisted of 33 patients (Group 1) with primary dysmenorrhea, and the control group (Group 2) consisted of 25 healthy women with matching demographics. At the first screening visit, women had a complete history taken and pelvic examination to rule out uterine irregularity, cul de sac tenderness, or nodularity, which may suggest endometriosis, pelvic inflammatory disease, or a pelvic mass. Pelvic ultrasonography was also performed to evaluate the presence of leiomyomata or ovarian cysts consistent with endometriosis. All patients had a pregnancy test, a Pap smear, and whole blood cell counts and blood biochemical parameters including glucose, liver and kidney functions. Whole blood samples (~5 mL) were drawn from a peripheral vein in the morning hrs (8:00-10:00) after an overnight fast. Serum samples for clotting were kept in flat tubes with gel at room temperature for 30 minutes, then they were centrifuged at 2700 g for 10 minutes. After centrifuging the blood samples at 1500 g at +4°C for 20min, plasma samples were kept at -80°C until use.in K3 EDTA tube.

Measurement of plasma malondialdehyde levels

MDA level of the plasma was measured by the TBA method (17). The resulting pink stained TBA was determined in a spectrophotometer at 532 nm. The calibration curve was performed using 1,1,3,3-tetramethoxypropane subjected to the same treatment as that of the samples. Intra- and inter-assay coefficients for TBA assay were 4.5% n=8 and 4.7% n=10 respectively. Results were expressed as nanomoles per milliliter (nmol/mL).

Measurement of plasma level of 3- Nitrotyrosine

Plasma Nitrotyrosine levels were measured using an ELISA kit according to the manufacturer's protocol (Hycult Biotechnology Elisa Kit, Holland). Measurable concentration range of Hycult Biotechnology Elisa Kit 2-1.500 nM. Intra-assay coefficients of variation were 6.1%.

Measurement of serum level of 8-OH Deoxyguanosine

Serum values of 8-OH Deoxyguanosine were determined with test kits by the enzyme linked immunabsorbant assay (ELISA) method (Assay Designs DNA Damage ELISA Kit 8- hydroxy-2'-deoxyguanosine, USA) The sensitivity of Assay Designs's DNA Damage ELISA kit were determined to 0.59 ng/mL. Inter and intra-assay coefficients of variation were 4.1% and 5.2% respectively.

Measurement of plasma level of Superoxide dismutase (SOD)

SOD activity was determined according to the method of Sun et al. (18). The principle of the method is based on the inhibition of nitro blue tetrazolium (NBT) reduction by the xanthinexanthine oxidase system as a superoxide generator. Activity was assessed in the ethanol phase of the lysate after 1.0 mL ethanol/chloroform mixture (5/3, v/v) was added to the same volume of sample and centrifuged. One unit of SOD was defined as the amount of enzyme causing 50% inhibition of the NBT reduction rate. SOD activity was expressed as units per milliliter (U/mL).

Statistical Analysis

Data analysis was performed by using Statistical Package for Social Sciences (SPSS) version 11.5 software (SPSS Inc.,

Chicago, IL, United States). Shapiro-Wilk test was used to test the normality of distribution for continuous variables. Data were expressed as mean±standard deviation or median (minimum-maximum), where applicable. Nominal data were shown as the number of case and (%). While the mean age, height, weight, 8-OhdG and MDA were compared by Student's t test, the Mann Whitney U test was applied for the evaluation of menarche age, nitrotyrosine and SOD levels. A p value less than 0.05 was considered statistically significant.

Results

The demographic data for both groups are summarized in Table 1. In both groups, there were no significant differences in terms of body mass index, age, age at menarche. The mean plasma levels of SOD (14.98±2.08, 14.92±1.49 U/mL; P=0.48) were similar in both groups (P>0.05). The mean serum levels of corrected [8-OhdG] (25.93±3.89, 27.85±3.24 ng/mL, respectively; P=0.05) and nitrotyrosine (97.94±41.16, 85.04±18.8 nM/mL, respectively; P=0.48) were similar between the study and control groups (P>0.05). However, mean plasma levels of MDA were significantly higher in the study group compared to the control groups (1.32±0.46; 0.91±0.26, P<0.001, respectively) (Table 2). The dysmenorrhea group was divided into Grade II (moderate) group and Grade III (severe) group according to MDS classification. Biochemical findings were compared between the groups of Grade II and Grade III. MDA level in Grade II group was 1.12±0.38 nmol/mL whereas in Grade III group was 1.42±0.47 nmol/mL (p>0.05). 8-OHdG level in Grade II dysmenorrheal group was 25.19±2.99 ng/mL and was 26.30±4.28 ng/mL in Grade III. Nitrotyrosine was demonstrated as (81.4 (51.6- 240.8 nM/mL), SOD was (14.5 (12.9-16.8 U/mL) in Grade II and Nitrotyrosine was 82.4 (49.6–166.7 nM/mL), SOD was 14.7 (10.6-23.6 nM/mL) in Grade III, respectively. There were no significant differences between these findings either (Table 3).

Discussion

Plasma levels of MDA were higher in the subjects with primary dysmenorrhea compared to those in the control group. Currently, it is reported that reactive oxygen species (ROS) have been implicated in the pathogenesis of a variety of injury models. It is possible that dysmenorrhea is one of these conditions (8). Several studies investigated the role of free radicals in dysmenorrhea. However, no researches were able to clarify the balance of ROS and antioxidant systems in dysmenorrhea. Previously, Yeh et al. (10) showed that plasma MDA and interleukin-6 levels were significantly higher in subjects with dysmenorrhea compared to those in healthy subjects. Similarly, plasma levels of MDA in the subjects with primary dysmenorrhea were found to be higher compared to those in the control group in the present study. Dikensoy et al. (9) also reported that the plasma levels of MDA increased in subjects with primary dysmenorrhea. In their study, in addition to plasma MDA levels, serum nitric oxide (NO) and adrenomedullin (AM) levels also increased. However, no acceptable (limited number of study investigated antioxidant markers) antioxidant markers were studied in any of the above studies. In study of Dikensoy et al. (9) they used only AM, which is not a specific antioxidant; it has multipotent properties, including vasodilator function. Furthermore, they reported that AM levels were increased by compensatory mechanisms in patients with dysmenorrhea. However, oxidative stress occurs when an increase in the amount of reactive oxygen species or depletion in the levels of antioxidants occurs. As SOD levels, which is a specific antioxidant marker, were unchanged between two groups, our study

Table 1. Demographic data of the subjects

	Group I (n=25)	Group II (n=33)	p value
Age (years)	25.0±2.9	24.2±3.1	0.340[a]
BMI (kg/m^2)	22.4±3.5	21.9±2.7	0.547[a]
Age at menarche (year)	12.7±1.0	12.3±0.7	0.096[b]
Pain (n%)			-
Moderate	-	11(33.3%)	
Severe	-	22 (66.7%)	

BMI: Body mass index, Values expressed as mean±SD, [a]Student's t test, [b]Mann Whitney U test

Table 2. Malondialdehyde, Corrected [8-OhdG], Nitrotyrosine and SOD levels of Study and Control groups

	Group I (n=25)	Group II (n=33)	p value
Corrected [8-OhdG] ng/mL	27.8±3.25	25.9±3.89	0.051[a]
Nitrotyrosine nM/mL	81.4 (53.6-117.1)	81.4 (49.6-240.8)	0.489[b]
SOD U/mL	14.7 (14.0-21.8)	14.7 (10.6-23.6)	0.956[b]
MDA nmol/mL	0.9±0.26	1.3±0.46	<0.001[a]

SOD: Superoxide Dismutase, MDA: Malondialdehyde, Values expressed as mean±SD, [a]Student's t test, [b]Mann Whitney U test

Table 3. Biochemical findings of Grade II and Grade III in patients with dysmenorrhea

	Moderate (n=11)	Severe (n=22)	p
Corrected [8-OhdG] ng/mL	25.2±2.99	26.3±4.29	0.447[a]
Nitrotyrosine nM/mL	81.4 (51.6-240.8)	82.4 (49.6-166.7)	0.778[b]
SOD U/mL	14.5 (12.9-16.8)	14.7 (10.6-23.6)	0.440[b]
MDA nmol/mL	1.1±0.38	1.4±0.47	0.077[a]

SOD: Superoxide Dismutase, MDA: Malondialdehyde, Values expressed as mean±SD, [a]Student's t test, [b]Mann Whitney U test

supported the opinion that increased oxidative stress in dysmenorrhea depended only on excess of free oxygen radicals. Additionally, in the present study, we did not detect any assocation between the severity of dysmenorrhea and these markers. Dysmenorrhea is caused by frequent and prolonged PG-induced uterine contractions that decrease blood flow to the myometrium, resulting in ischemia. Substantial evidence suggests that hypoxia-ischemia activates phospholipase A2, a lipolytic enzyme that hydrolyses the acylglycerolipids and generates free fatty acids, especially arachidonic acid. Hence, arachidonic acid accumulates during the hypoxic-ischemic period. Upon perfusion, when oxygen is available, arachidonic acid is metabolized mainly by three different groups of enzymes-cyclooxygenase, lipooxygenase, and cytochrome P450-resulting in eicosanoid formation and the generation of activated oxygen species (19). Studies on oxidative stress and dysmenorrhea measured only MDA levels as an oxidative stress marker. However, it is well known that oxidative stress may disturb the destruction of all major classes of biological macromolecules, including nucleic acids, proteins, carbohydrates, and lipids (20). Hence, to make any conclusion about the level of oxidative stress in patients with dysmenorrhea, all of these oxidative stress indicators should be measured. In the present study, in addition to the MDA level, we also investigated plasma levels of 3-NT, which are markers of oxidative damage of protein and 8 OhdG, which also shows the oxidation of DNA. To our knowledge, there are no other studies in the literature investigating the detail of oxidation including protein and DNA oxidation in dysmenorrhea. In addition, no study has investigated antioxidant status by real antioxidant markers in patients with dysmenorrhea.

In conclusion, according to our results, as we only detected lipid peroxidation while protein and DNA oxidation is normal, we can suggest that increased oxidative stress may ocur in dysmenorrhea, but this oxidative stress is not really prevalent in dysmenorrhea. Before any other suggestions, further clinical researches with larger numbers of patients are required to clarify the relation of oxidant/antioxidant balance in dysmenorrhea.

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

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The effect of long term nicotine exposure on nicotine addiction and fetal growth

Uzun süreli nikotine maruziyetin nikotin bağımlılığına ve fetüsün büyümesine etkisi

Soycan Mızrak[1], Volkan Turan[2], Mustafa Coşan Terek[2], Gülinnaz Ercan[1]

[1]Department of Medical Biochemistry, Faculty of Medicine, Ege University, İzmir, Turkey

[2]Department of Gynecology and Obstetrics, Faculty of Medicine, Ege University, İzmir, Turkey

Abstract

Objective: To investigate the effect of nicotine exposure starting before coitus and continuing during pregnancy and lactation period on delivery rate, fetal growth and nicotine addiction in rats.

Material and Methods: Ten female Swiss Albino rats were divided into 2 groups as the nicotine group (NG) (n=5), and the control group (n=5), conceived by adding 2 male rats to each group. While the control group was given only normal drinking water, 0.4 mg/kg body weight (BW)/day nicotine was given to the NG in drinking water. After delivery, the BWs of pups were recorded weekly for 6 weeks and their drinking water preferences were assessed. Meanwhile, pups of the NG continued to receive 0.4 mg/kg/day nicotine for 12 months while the controls continued with normal drinking water.

Results: At the end of the 6th week, it was determined that 30 (69%) rats out of 43 in the NG and only 7 rats (20%) out of 35 in the control group preferred the nicotine added drinking water ($p<0.05$). No significant difference was observed between control and NGs in postnatal birth weights and BWs recorded for 6 weeks. On the contrary, a significant decrease ($p< 0.05$) was observed in the BWs of NG at the end of 12 months nicotine exposure.

Conclusion: Use of maternal nicotine in pregnancy and lactation periods, even at a low dose, may be effective in nicotine addiction development although it may not affect delivery rate, and BWs of pups after delivery and during six weeks follow up in the lactation period.

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Key words: Nicotine, pregnancy, nicotine addiction, fetal growth, delivery rate

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Özet

Amaç: Sıçanlarda koitusdan önce başlayıp gebelik boyunca ve laktasyon döneminde devam eden nikotin maruziyetinin doğum oranı, fetal büyüme ve nikotin bağımlılığına olan etkilerinin araştırılması

Gereç ve Yöntemler: On dişi Swiss Albino sıçan kontrol grubu (n=5) ve nikotin grubu (n=5) olarak iki gruba ayrıldı ve aralarına 2 erkek sıçan eklenerek gebe kalmaları sağlandı. Kontrol grubuna normal içme suyu verilirken, nikotin grubuna içme suyu içerisinde 0.4 mg/kg vücut ağırlığı/gün nikotin eklendi. Doğumdan sonra yavru ratların vücut ağırlıkları, 6 hafta boyunca haftalık kaydedildi ve su tercihleri değerlendirildi. Bu arada nikotin grubunun yavru sıçanları 0.4 mg/kg/gün nikotin 12 ay boyunca devam ederken, kontroller normal içme suyuna devam etti.

Bulgular: Altı hafta sonunda nikotin grubundaki 43 sıçanın 30'u (%69) ve kontrol grubundaki 35 sıçanın 7'si (%20) nikotin eklenmiş suyu tercih etti ($p<0.05$). İki grup arasında doğumda ve 6 hafta boyunca kaydedilen vücut ağırlıkları arasında herhangi bir farklılık saptanmazken, 12 aylık nikotin maruziyeti sonunda nikotin grubunun vücut ağırlığı istatistiki olarak anlamlı düşük bulunmuştur ($p<0.05$).

Sonuçlar: Nikotin, gebelik ve laktasyon dönemlerinde düşük dozda dahi kullanımı nikotin bağımlılığının gelişmesine etkili iken; doğum oranı ve yavru ratların doğumda ve 6 haftalık takibinde vücut ağırlıkları üzerine bir etkisi izlenmemiştir.

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Anahtar kelimeler: Nikotin, gebelik, fetal büyüme, doğum oranı, nikotin bağımlılığı

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Introduction

Cigarette smoking is defined as a bio-socio-psychological state of intoxication by the World Health Organization (WHO). Cigarette smoking is a quite common habit worldwide and 90% of smokers start this habit before the age of 20. Interestingly, since the number of female smokers is increasing daily, this leads to more frequent encounters with pregnant smokers (1). During the last two decades, smoking among pregnant women in the developed countries decreased by about 60-75% (2). 20-25% of pregnant women in South America and 30-36% of pregnant women in Spain smoke and approximately 41% of them attempt to give up this habit every year. However, only 10% of them succeed in quitting smoking. Nicotine replacement therapies (NRT) have been developed for nicotine addiction so as to boost this achievement (3).

There are more than 4000 chemical compounds in the cigarette. Some of them are carcinogenic substances and the most hazardous ones are arsenic, benzene, cadmium, hydrogen cyanide, toluene, ammonia and propylene glycol (4). Although it is not known for certain which of these chemicals

Address for Correspondence: *Gülinnaz Ercan, Department of Medical Biochemistry, Faculty of Medicine, Ege University, 35100 Bornova, İzmir, Turkey*
Phone: +90 505 911 37 36 e.mail: gulinnazalper@yahoo.com

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are harmful for the fetus, it is believed that nicotine especially may affect the pregnancy outcomes negatively (5).
Cotinine, the metabolite of nicotine, passes the placental barrier, which has been proven by showing it both in the amniotic fluid and fetal cord blood (6). Nicotine has been proposed to have negative effects on the fetus but it has not been explained exactly by which mechanism this situation may occur. Cheryl A. et al. (7) suggested that the direct toxic effect of nicotine on the fetus may be attributed to a decreased oxygen amount as a result of vasoconstriction in uterine arteries. The most-studied complication of smoking during pregnancy has been low birth weight (< 2500) (8,9). It was demonstrated that, in female smokers, the risk of having babies with low birth weight increases by 1.5-3.5 fold and that this risk increase is correlated with the increase in cigarette consumption ratio (10).
The nicotine dose in one cigarette is not life-threatening, but it has an addictive effect, which makes smokers keep on smoking and inhaling the other chemicals in tobacco, causing the health risks (11). In cigarette smoking, nicotine is quickly absorbed into the blood circulation, reaching the brain in 10 seconds, much quicker than other tobacco products. This is one of the major reasons why cigarette smoking has a high potential of having an addictive behaviour (12). Nicotinic acetylcholine receptors (nAChRs) are concentrated particularly in the areas of cognitive function such as the prefrontal cortex, basal ganglia, nucleus ceruleus and in the mesolimbic area. Nicotine activates the nAChRs and activation of these reseptors not only leads to release of acetylcholine, dopamine and glutamate but also modulates the other neurotransmitters such as noradrenaline and serotonin (13).
In this study, our aim is to investigate the effect of nicotine exposure starting before coitus and continuing during pregnancy and lactation period on fetal growth and nicotine addiction and to assess the effect of long term nicotine exposure on the physiological development of the pups by measuring the BWs of rats at the end of 12 months of nicotine exposure.

Material and Methods

In this study, approved by the Animal Ethics Committee of Ege University School of Medicine, Bornova, İzmir, Turkey,10 female and 4 male Swiss-Albino rats (200±50 g) were used. Rats were housed in a temperature-controlled room with a 12-hour light/dark cycle. The animals were maintained on standard laboratory animal chow and given water ad libitum. They were maintained in accordance with the guidelines for animal welfare. Female Swiss Albino rats were divided into 2 groups as the nicotine group (n=5), and the control group (n=5). They were conceived by adding 2 male rats to each group. While the control group was given normal drinking water, 0.4 mg/kg body weight (BW)/day nicotine (nicotine hydrogen tartrate, SIGMA 2.22 mg/kg/BW) was prepared freshly every day and added to the nicotine group's drinking water. After birth, firstly, the BWs and malformations were recorded and the offspring were left in the same cage with their mothers and breast-fed. During this lactation period, daily water consumption and body weights were recorded every week.
Pups, which had been kept beside their mothers as they breast-fed in the first six weeks following the birth were separated from their mothers at the end of 6th week and put individually in different cages in order to detect nicotine preference. Their preference for normal or nicotine added drinking water was determined through the method of "two bottle free choice". Nicotine was offered under the homecage, two-bottle choice regimen between nicotine added water and normal water with unlimited access for 24 h/day. The bottles were refilled every day with a fresh solution and their left–right positions interchanged daily to avoid development of position preference. Saccharin (Huxol sweetener, 2.4 g saccharin/200 mL) was dissolved in nicotine added water so as to mask the bitter taste of the water caused by nicotine, and make it easier for the rats to drink it. The normal water was also sweetened by adding saccharin during the test in order not to influence their water preferences. Daily nicotine was added and normal water consumed in each cage was measured and the water which the pups preferred was noted. This test was performed every day for one week. Then, low dose nicotine administration to pups born in the nicotine group was continued as 0.4 mg/kg/day for 12 months and their BWs were compared to controls at the end of this period.

Statistics

The statistics program of SPSS 13.0 was used in the statistical assessment of findings. Non-parametric tests (Mann Whitney U and Chi Square) were used to compare body weights and nicotine addiction. A $p<0.05$ value was accepted as significant.

Results

Forty-three pups were born in the nicotine group while 35 pups were born in the control group. No congenital malformation was observed in any of these rats.
No significant difference was observed between the pups in control and nicotine groups when the rats were compared in terms of their first measured post-natal birth weights and their weekly BWs recorded weekly for a period of 6 weeks (Table 1, Figure 1).
Pups of both the nicotine and control groups were kept beside their mothers as they continued breastfeeding in the first six weeks following the birth. At the end of the 6th week, they were put individually in separate cages in order to assess nicotine preference and addiction, and their preference for normal or nicotine added drinking water was determined with graduated bowls. Each day for one week, the type of water the pups preferred was recorded. It was determined that 30 (69%) pups out of 43 in the nicotine given group and only 7 pups (20%) out of 35 in the control group preferred the nicotine added water, while the others preferred the nicotine-free water ($p< 0.05$) (Table 2).
At the end of the 12th month, when the final BWs of the control and nicotine groups were measured, it was determined that the final BW of the nicotine group was significantly lower than the final BW of the control group ($p<0.05$) (Figure 2).

Table 1. The follow up of pups' body weights for six weeks

Groups (n=78)	1st day (g)	1st week (g)	2nd week (g)	3rd week (g)	4th week (g)	5th week (g)	6th week (g)
Control Group (n=35)	5.6	10.2	18.7	26.4	37.1	55.9	69.1
Nicotine Group (n=43)	5.7	10.9	19.8	30.4	44.1	61.9	70.0
No significant difference was observed between the pups in control and nicotine groups for a period of six weeks (p>0.05 each week)							

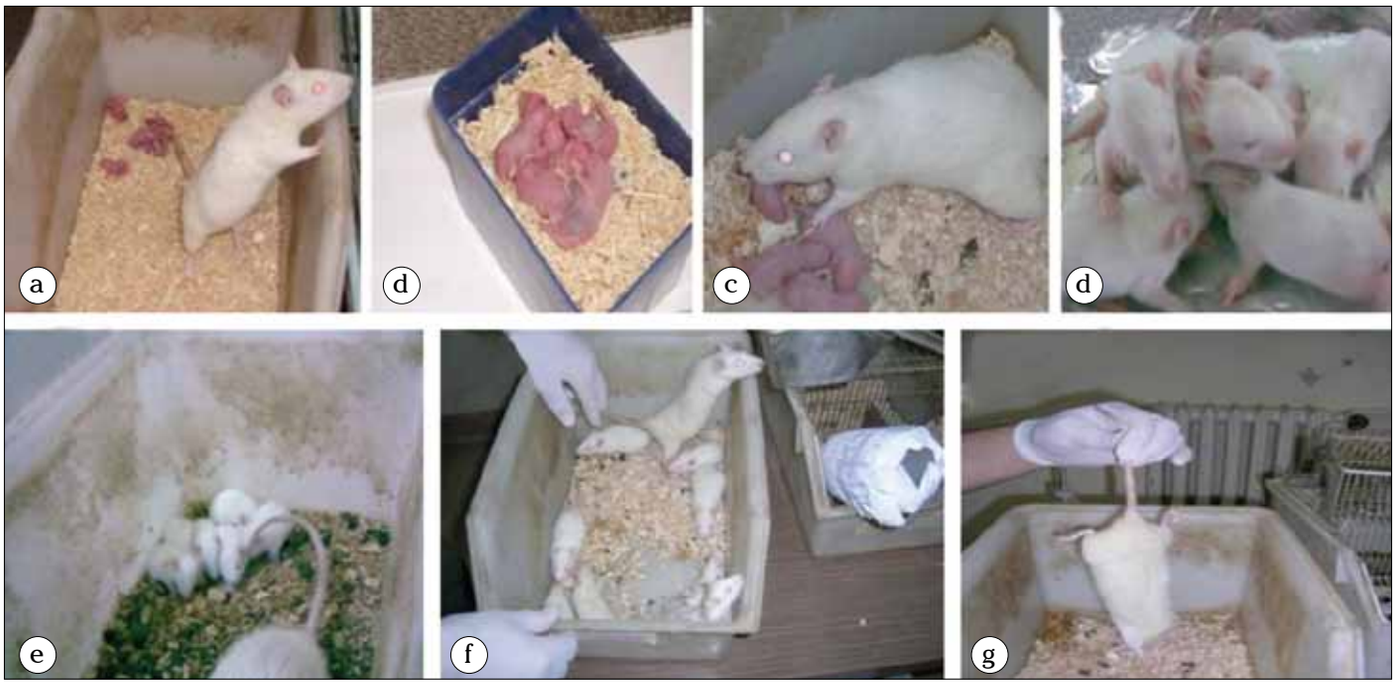

Figure 1. The follow up period of rats for six weeks a) after delivery, b) one week old, c) two weeks old, d) three weeks old, e) four weeks old, f) five weeks old, g) at the end of sixth week

Table 2. Water prefence ratio of rats in two bottle free choice test. Nicotine added water preference ratio was significantly higher in the nicotine group compared to the control group (p< 0.05)

Groups (n=78)	Normal drinking water n (%)	Nicotine-added water n (%)
Nicotine group (n=43)	13 (31%)	30 (69%)
Control group (n=35)	28 (80%)	7 (20%)

Discussion

It was reported that an average of 1.0 mg nicotine is obtained from smoking a cigarette and this intake varies between 0.37 mg and 1.56 mg according to individual differences in its metabolism (14). Nicotine intake via a parenteral method equals many times more nicotine intake when compared to per oral method. For this reason, it would be more accurate to assess the real effects of nicotine by a method mimicking its natural usage such as smoking and nicotine replacement treatment. This is the first study in the literature investigating

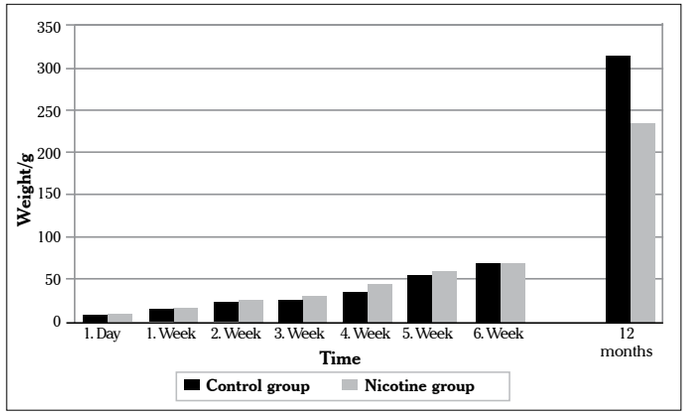

Figure 2. The body weights of the study groups during 12 months follow up

12 months mimicking long-term chronic nicotine exposure. Furthermore, even the studies that are published as chronic nicotine administration lasted 3 months at most (15-17). However, since smoking causes harm to the organism after chronic usage, determining the long term effects of nicotine is required. In the present study, nicotine exposure which started before

coitus and continued during pregnancy and lactation periods was evaluated. In addition, giving low dose nicotine in drinking water to pups of the nicotine group continued for 12 months after a two-bottle free-choice test in order to assess their physiological development by measuring BWs and compared with the BWs of pups in the control group which continued with normal drinking water.

Huang et al. (15) used the oral gastric intubation model and nicotine (6 mg/kg/day) was given in milk-formula for seven postpartum days. At the end of the study, the nicotine group was found to have lower BWs compared to controls. This report can be accepted as an experimental model mimicking chronic nicotine consumption. In addition to this the nicotine dose given to pups with BWs nearly 10-15 g by milk formula corresponds to an amount of at least 10 times higher plasma concentrations of the nicotine dose. For this reason, even this short time of nicotine exposure had an irreversible detrimental effect on pups and their suggestions should not be considered as reliable. In the present study, after conducting the weekly body weight follow-ups of pups, no statistically significant difference was found in final body weights between the nicotine and control groups at birth and during six week follow up. This shows that exposure to low dose nicotine during pregnancy and lactation has no negative effect on the physiological development of new-born pups until the end of the 6th post-natal week. These findings are compatible with other studies that claim that nicotine has no effect on fetal growth (18-20).

The results of the present study are consistent with the studies reporting that short-term nicotine administration has an anorexic effect on animals during the developmental period, especially the puberty period (21, 22). Weight loss induced by nicotine was found to be associated with fat tissue decrease and changes in fat composition (23). It can also be explained by a decrease in food intake desire and increase in energy spending (24, 25). Nicotine controls the food intake and energy spending directly or indirectly via activating the nicotinic acetylcoline receptors (nAchR) and presynaptic receptors in the hypothalamus regulating nutrition and energy metabolism (26). Flyn et al. (27) reported that after oral nicotine administration in different concentrations, “behavior of preference” occurred on the 8th day in adolescent Sprague-Dawley rats, while in another animal study it was stated that no particular nicotine preference occurred on the 12th day of the experiment (28). In the present study, this test was applied after the pups stopped breast-feeding at the end of the 6th postnatal week, and the nicotine preferences of pups were found to differentiate following day 2. It was found that the nicotine added water preference ratio was significantly higher in the nicotine group compared to the control group. The results of this study are very important in terms of showing that using nicotine during pregnancy and lactation period may be effective in developing nicotine addiction in new-borns.

Many studies reported that nicotine has been proved to be a potent pro-oxidant to the spermatozoa population and is able to alter the fertility potential by inducing membrane damage and changing both the sperm morphology and motility in man and by decreasing granulosa cell proliferation and ovarian vascularization but on the contrary increasing ovarian cell apoptosis in females (29, 30). However; in the present study, the rats drinking nicotine-added water became pregnant in a shorter time than controls and delivered more pups having similar body weights in comparison to the pups of the controls . This may be associated with euphoriant effects of nicotine which led to an increase in physical motility and sexual behaviour of rats according to our unpublished observations (31). Also, giving direct nicotine to the body parenterally leads to much greater nicotine intake for the body compared to administering the same dose of nicotine orally or via inhalation. When the studies reporting typical smokers who systemically absorb about 0.3 mg nicotine/kg BW per day based on the average daily nicotine consumption of 17 cigarettes (nearly one package) in the U.S.A, studies giving nicotine directly to the systemic circulation by injection is like giving a toxic dose of nicotine to an individual, as it corresponds to smoking more than 10 packages of cigarette per day, which is impossible in daily life (32, 33). Parenteral high doses of nicotine may affect sperm and ovarian follicle function but the low dose nicotine in our study probably has no adverse effect on fertility, since the daily low dose nicotine consumption did not affect delivery rates and number of pups at each delivery of the pregnant rats under nicotine exposure in comparison to controls.

Many guides that deal with NRT administration in pregnancy are contradictory. In the 2002 edition of Current Guidelines for Smoking Cessation in Pregnancy which deals with quitting smoking in pregnancy, it is reported that all nicotine gums and bands are contraindicated during pregnancy and it is stressed through various animal experiments that they may harm the fetus (34). On the other hand, the American Agency for Health Care Policy and Research supports the use of NRT since quitting nicotine abruptly during smoking cessation may lead to withdrawal symptoms and severe consequences in the fetus even though the efficiency of NRT in pregnant women is not known for certain (35).

In conclusion, contrary to common belief, the results of this study show that low dose nicotine does not cause the intrauterine growth retardation in the fetus frequently seen in smokers. Its chronic usage in the later period may influence growth and development negatively. Using maternal nicotine during pregnancy and lactation periods, even in small doses, may be effective in developing addiction in the new-born fetus. For this reason, nicotine replacement therapy for pregnant women intendinf to quit smoking, but can’t accomplish this with physiological and behavioral methods, may be recommended after taking into account the advantages and disadvantages of nicotine intake if they are not able to stop smoking. Therefore; pregnant women using NRT instead of smoking are protected from exposure to the other harmful materials in cigarette.

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Conflict of interest

No conflict of interest was declared by the authors.

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Fetal pulmonary injury following single high-dose intra-amniotic betamethasone treatment in preterm goat kids

Preterm keçi yavrusunda tek ve yüksek doz intra-amniyotik betametazon tedavisi sonrasında fetal pulmoner zedelenme

Meltem Antalyalı[1], Özlem Özmen[2], Mehmet Halıgür[2], Mekin Sezik[1]

[1]Department of Gynecology and Obstetrics, Faculty of Medicine, Süleyman Demirel University, Isparta, Turkey

[2]Department of Pathology, Faculty of Veterinary Medicine, Mehmet Akif Ersoy University, Burdur, Turkey

Abstract

Objective: Fetal lung maturation is an extremely important process that is necessary for the survival of the neonates. Conventionally, corticosteroids are administered maternally for inducing fetal lung maturation in preterm fetuses. Alternatively, single-dose intra-amniotic (IA) treatment might be speculated to improve lung maturity. In the goat model, we recently showed that high-dose IA betamethasone (BM) was associated with an increased number of stillbirths and emphysematous changes. The aim of the present study is to expand our previous findings and evaluate the histopathological effects of IA injection of a single high-dose of BM 48 h before induced preterm delivery, using our previously collected specimens.

Material and Methods: Five hair goat fetal lungs that had received 8 mg/kg IA BM at gestational day 118 (term, 150 days) and scheduled for preterm delivery by cesarean section at day 120 of gestation were examined pathologically. Specimens were stained with hematoxylin and eosin (HE) and were interpreted by light microscopy.

Results: The histopathological examination of the fetal lungs revealed edema, hemorrhage, slight inflammatory reaction, marked emphysema, and desquamation of the pneumocytes and bronchiolar or bronchial epithelial cells.

Conclusion: High-dose IA BM administrations to induce lung maturation can paradoxically cause severe pathological lesions in the fetal lungs. These might explain the toxic effects we encountered with this mode of treatment. (J Turkish-German Gynecol Assoc 2012; 13: 242-6)

Key words: Animal models, betamethasone, corticosteroids, intra-amniotic, lung maturation

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Özet

Amaç: Fetal akciğer matürasyonu, yenidoğan sağkalımı için zarurî olan çok önemli bir süreçtir. Kortikosteroitler, mutat olarak preterm fetüste akciğer matürasyonunu sağlamak için anneye uygulanmaktadır. Bunun yerine, tek doz intra-amniyotik (İA) tedavinin de akciğer matüritesini artıracağı ileri sürülebilir. Keçi modelindeki en son çalışmamızda, yüksek doz İA betametazonun (BM) ölü doğumlarda artış ve amfizematöz değişiklikler ile ilişkili olduğunu gösterdik. Mevcut çalışmadaki amacımız; önceki bulgularımızı genişleterek, indüklenmiş preterm doğumdan 48 saat önce tek ve yüksek dozda İA BM sonrasında, elimizdeki materyaller üzerinde histopatolojik değişikliklerin gösterilmesidir.

Gereç ve Yöntemler: Gebeliğin 118. gününde (term, 150 gün) 8 mg/kg İA BM verilen ve 120. günde sezaryen ile preterm doğum planlanan beş kıl keçisi fetüsüne ait akciğerler patolojik incelemeye alındı. Numuneler hematoksilin ve eozin (HE) ile boyanarak ışık mikroskopisi altında değerlendirildi.

Bulgular: Fetal akciğerlerin histopatolojik incelemesinde ödem, kanama, hafif enflamatuvar reaksiyon, belirgin amfizem ve pnömositler ile bronşiyolar veya bronşiyal epitelyum hücrelerinin deskumasyonu saptandı.

Sonuç: Akciğer matürasyonu sağlamak için yüksek doz İA BM uygulanması, beklenmedik şekilde fetal akciğerlerde ağır patolojik lezyonlara neden olabilir. Mevcut bulgular, bu tarz tedaviye bağlı toksik bulguların açıklanmasını sağlayabilir.

(J Turkish-German Gynecol Assoc 2012; 13: 242-6)

Anahtar kelimeler: Hayvan modeli, betametazon, kortikosteroit, intra-amniyotik, akciğer matürasyonu

Geliş Tarihi: 28 Temmuz 2012 **Kabul Tarihi:** 03 Ekim 2012

Introduction

The pulmonary system is among the last of the fetal organ systems to mature, both functionally and structurally. Because the immature pulmonary system may not oxygenate the neonate adequately, preterm birth can lead to significant neonatal morbidity or mortality (1). Reduced lung function during infancy has been shown in association with preterm birth, probably persisting through adulthood (2-4). Immature lungs may increase the risk of respiratory distress and death among both term and preterm infants during the neonatal period (2). Moreover, low birth weight secondary to preterm

Address for Correspondence: *Mekin Sezik, Süleyman Demirel Üniversitesi Tıp Fakültesi, Kadın Hastalıkları ve Doğum Anabilim Dalı 32260 Çünür, Isparta, Turkey*
Phone: +90 246 211 91 83 e.mail: msezik@yahoo.com

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delivery has been associated with reduced lung function and increased death rates from chronic obstructive airway disease in adult life (4).

Animal studies and clinical human data have revealed that fetal lung maturation takes place after maternal corticosteroid administration (5). In line with this, elevated cortisol levels have been shown to enhance lung maturation (6). However, the efficacies of various corticosteroid analogs, total dose, dosing intervals, and the means of administration for fetal lung maturation have not been exactly defined. Alternative routes of administration, such as the IA route might be hypothesized to be more effective than the standard maternal method of administration. Our previous animal study showed that the dose and the application route of betamethasone (BM) are particularly important for fetal lung maturation (7). In the mentioned investigation (7), we proposed that higher (than that of previously used) doses of IA can be an attractive treatment option to induce improved antenatal lung maturation. However, we found that a high (8 mg/kg) dose of intra-amniotic (IA) and fetal i.m. (4 mg/kg) BM was not superior to the standard dose and the maternal route of administration in the goat model. Moreover, IA BM was associated with an increased number of stillbirths and macroscopic emphysematous changes, compared to controls. Despite these toxic effects, detailed histopathological examination of the fetal lungs was not included in this previous paper (7), and we were not able to identify the exact underlying mechanisms of pulmonary injury following the IA route.

Here, we report the detailed histopathological findings in the fetal lungs of kids that we gathered from our previous experimental design. These were antenatally treated with high-dose (8 mg/kg of estimated fetal weight) IA BM. Our aim was to delineate the causes for the unexpected toxic effect we encountered following high-dose IA therapy.

Material and Methods

All the experiments were approved by the institutional animal use and care committee at Süleyman Demirel University and performed in accordance with the National Institute of Health Guidelines for the Care and Handling of Animals. The number of animals was restricted (n=5), as suggested by the animal ethics committee. Details of the procedures were described thoroughly in our previous study (7). Briefly, five female hair goats (Capra hircus), 2 to 4 years old and 40±5 kg in weight were used for the study. Goats were fed on standard feed and tap water ad libitum. A singleton structurally normal ongoing pregnancy was confirmed by ultrasonography (Echo Camera SSD-500, Aloka, Tokyo, Japan) at gestational days 60, 75, and 90 (term pregnancy, 150-155 days). At day 118, amniocentesis with a 21 G needle under ultrasound guidance was performed (Figure 1). Betamethasone disodium phosphate plus betamethasone acetate (Celestone chronodose amp, Schering-Plough Inc, İstanbul, Turkey) equivalent to 8 mg/kg of estimated fetal weight was injected into the amniotic sac. Subsequently, at day 120, excluding the stillborn kids, cesarean section (CS) with paralumbal skin and dorsal curvature uterine incision was performed under sterile conditions at gestational day 120 (corresponding to 31-32 weeks of gestation in human pregnancy). Details of the operation are provided elsewhere (8).

After preterm delivery, the kids were euthanized with high-dose (50 mg/kg) sodium thiopental (Pental Sodyum, IE Ulugay, İstanbul, Turkey) administered via the umbilical catheter, the trachea was clamped for 3 min to maintain airway pressure and necropsy performed. At macroscopic examination, any visible lung rupture or pulmonary interstitial emphysema was recorded. The lungs were excised en bloc, the trachea removed to the bifurcation and their wet weight was measured (Figure 2). The lungs were then fixed by 10% formalin inflation into the airways, and fixation continued in formalin for three days. Five different samples were taken from all of the lungs (left apical, left diaphragmatic, right apical, right cardiac and right diaphragmatic lobes). Then, tissue samples were routinely processed, paraffin embedded and cut into 5 mm sections, and slides were stained with hematoxylin and eosin (HE) to be interpreted by

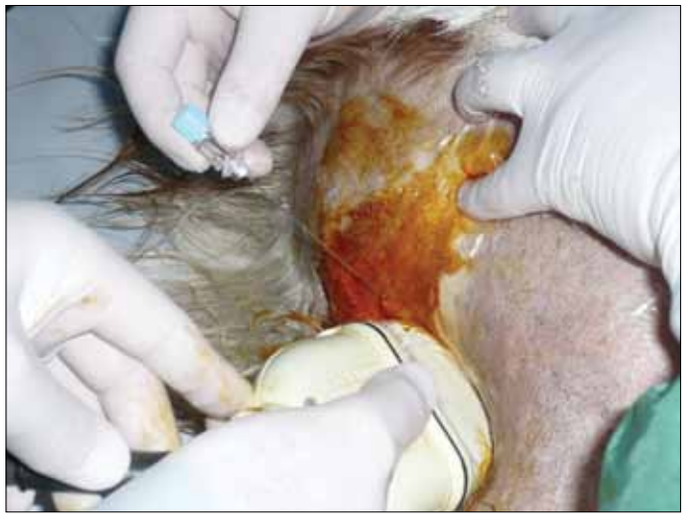

Figure 1. The amniocentesis procedure

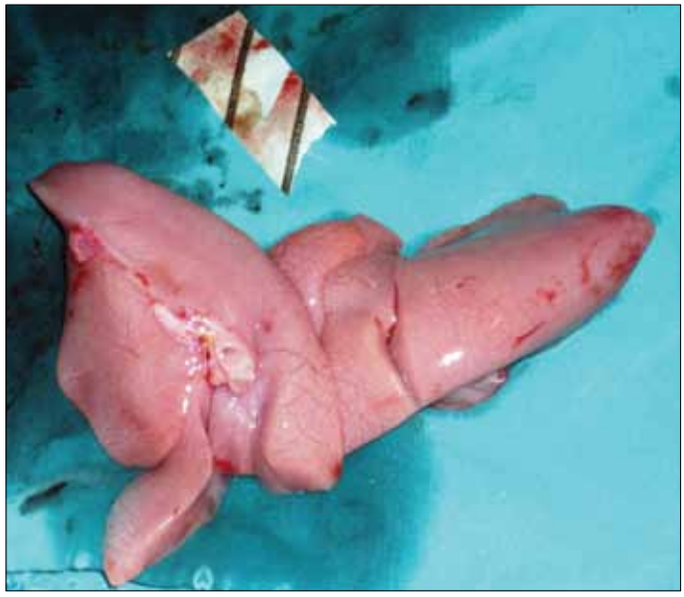

Figure 2. Excised fetal lungs following preterm delivery

light microscopy. Histopathological changes were examined in a blinded manner under the 40x objective of a Nikon E-600 trinocular microscope and microphotography apparatus.

Results

There were 3 stillbirths out of 5 fetuses that were given IA BM. The fetal lungs from one of those kids could not be examined because of a severe autolytic reaction, probably secondary to significant trauma by other goats nearby following stillbirth. The histopathological observation of the remaining fetal lungs (a total of 8 gross specimens from 4 fetuses) revealed edema, slight inflammatory reaction, hemorrhage, marked emphysema, and desquamation of the pneumocytes (Figures 3-5).

Edema was typically localized around the vessels at the interstitial tissue. Protein-rich eosinophilic edema fluid was also present in some alveolar spaces. Slight neutrophilic and mononuclear inflammatory cells were evident around the pulmonary vasculature and around bronchioles. Marked increase in alveolar macrophages was a prominent finding in the alveolar lumens. Severe desquamation was observed in alveolar cells. In addition to alveolar cells, desquamation was also prominent in bronchiolar and bronchial epithelial cells.

Pulmonary lesions were more severe in specimens from stillbirths compared to live births. Marked areas of hemorrhage and edema were common findings in stillborn kids. Thickening at the septal areas due to edema and inflammatory cell infiltrations were also characteristic in these kids. Some of the alveolar spaces were filled with desquamated epithelial cells and inflammatory cells. Atelectatic areas were also commonly encountered in fetuses with stillbirth (Figures 6, 7).

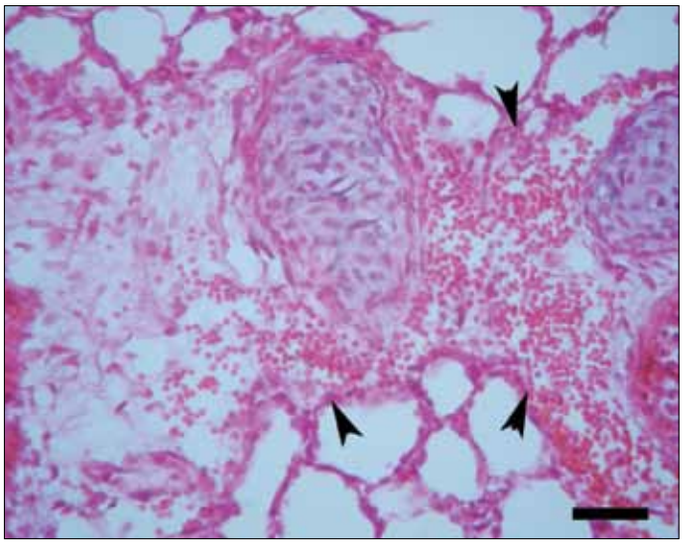

Figure 3. Histopathological appearance of the lungs of a kid showing severe hemorrhage in septal tissue around the bronchi (arrow heads), HE, Bar=50 μm

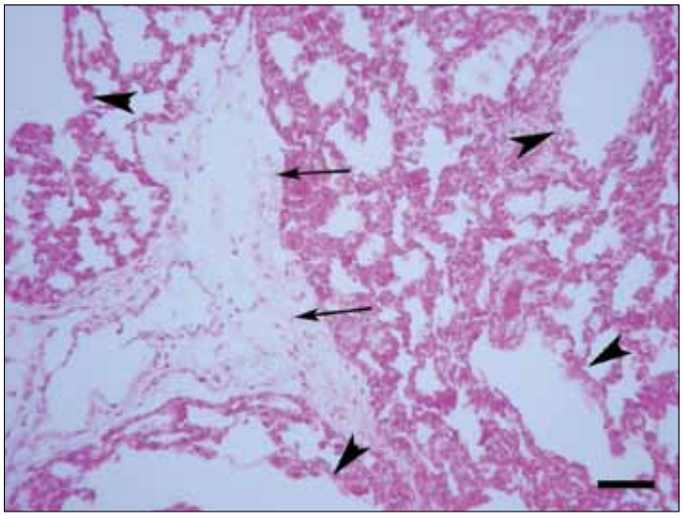

Figure 4. Severe emphysemic areas (arrow heads) and edematous interstitial tissue (arrow) in a kid, HE, Bar=100 μm

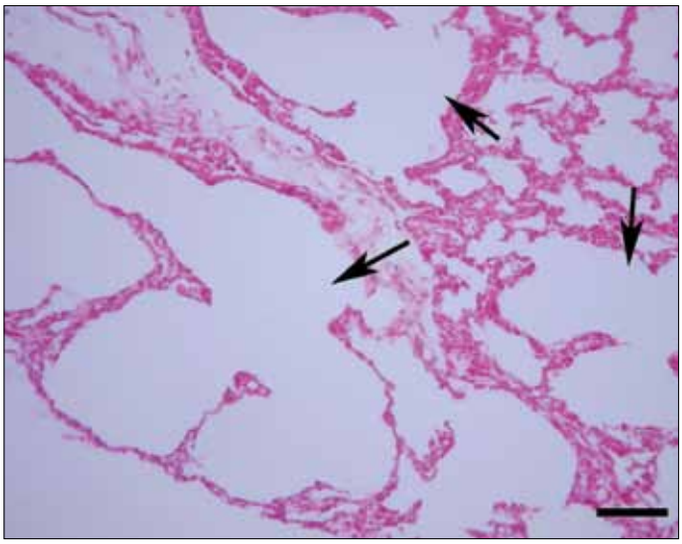

Figure 5. Severe alveolar emphysema (arrows) in the lungs of a kid, HE, Bar=100 μm

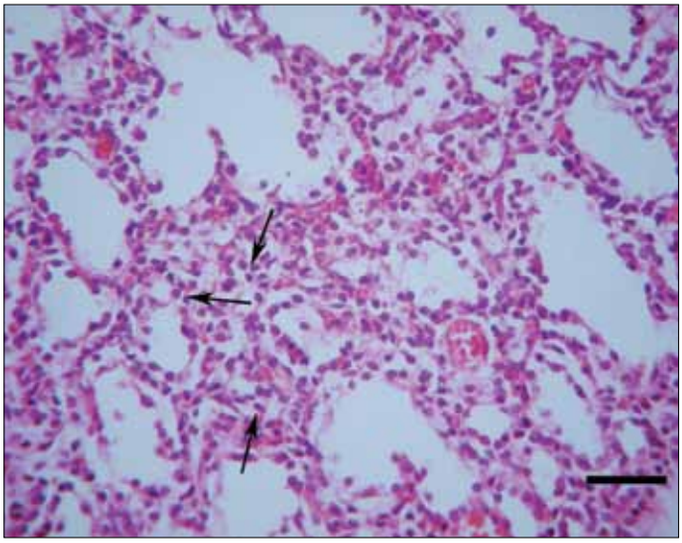

Figure 6. Inflammatory reaction (arrows) in a lung of a stillborn kid, HE, Bar=50 μm

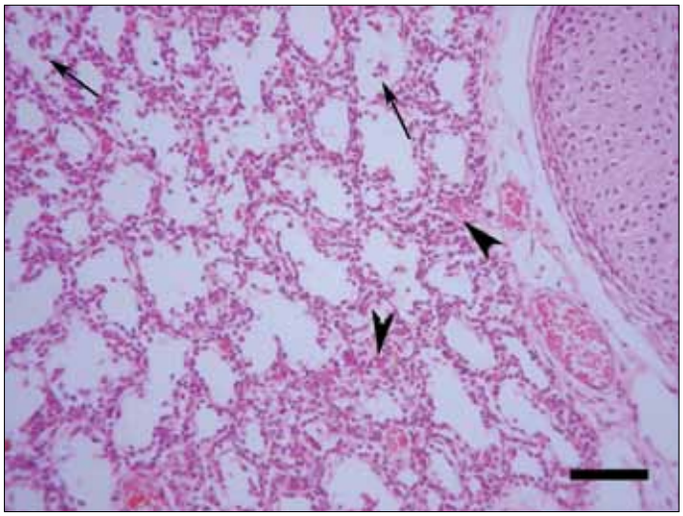

Figure 7. Desquamation of the epithelial cells (arrows) and atelectatic areas (arrow heads) in the lungs of a stillborn kid, HE, Bar=100 μm

Discussion

High-dose IA treatment with BM was unexpectedly associated with fetal losses (60%) and pulmonary injury in our design (7). In fact, scarce small ruminant animal data in the English language literature, disclosing 50% prenatal loss rate with >2 mg/kg estimated fetal weight of intra-amniotically administered BM, confirms our results (9). Interestingly, lower doses (0.5 mg/kg estimated fetal weight) of IA BM were not associated with stillbirths (0/9 lambs) in the mentioned study (9). In another investigation on fetal baboons, 6 mg (fixed dose) IA BM at four and again at three days prior to preterm delivery was reported to significantly increase the amniotic fluid lecithin/sphingomyelin ratio (10). The immature fetal baboon pulmonary system was shown to respond to IA BM with improved pulmonary stability but not with a synchronous increase in tissue distensibility (10).

Although limited available data on low-dose IA BM indicates certain favorable effects, lower doses were shown to induce inadequate fetal lung maturation, especially when compared with the conventional maternal route of administration (9). Therefore, we had speculated that a single high-dose BM administered into the amniotic fluid would lead to effective drug concentrations in the fetal alveolar fluid and pulmonary tissues, which are actually the primary target of treatment. However, our histopathological data indicated toxic effects associated with prominent pulmonary injury at this high dose.

We could locate only one prior investigation on pulmonary histological changes following low-dose (2 mg/kg estimated fetal weight) IA BM or budesonide (11). The authors showed that IA corticosteroids, 2 or 7 days before preterm delivery in lambs, resulted in thinning of alveolar walls with a higher proportion of alveolar ducts and a lower alveolar wall fraction relative to controls. These favorable changes were correlated with improved lung function and increased surfactant (11). As we did not observe similar favorable morphometric changes, the dose we used in our design is probably toxic and conveys no additional benefit.

BM given intra-amniotically at high doses (>2mg/kg) can be speculated to be absorbed by the chorioamniotic membranes and perhaps by the fetal surface of the placenta, leading to reabsorption by the fetus. Following reabsorption in a cyclic manner, BM would be expected to accumulate toxic levels in the fetus. This toxic accumulation probably initiates lung injury and finally causes fetal death. Some fetuses tend to demonstrate an exaggerated response with prominent edema and inflammation, as supported by our findings from stillbirths. Another possibility, however, is the emergence of such histopathological characteristics following fetal loss. This may be unlikely, as the specimens were available in a short time after fetal loss except in one case, which was excluded from analysis.

Septal thickening was an important histopathological feature that we encountered. In contrast, low (2 mg/kg) doses of IA BM were reported to be associated with thinning of alveolar walls, which is a favorable finding for lung maturation. Therefore, IA BM at very high doses (8 mg/kg) not only causes lung injury but also disturbs normal fetal lung maturation. It must, however, be noted that even standard doses of BM administered maternally for fetal lung maturation can induce certain adverse histopathological changes. For example, the results of a pregnant sheep model indicated that 70% of lambs delivered at 128 days of gestation, 24h after a single injection of 0.5 mg/kg maternal i.m. BM developed pulmonary interstitial emphysema, compared with fewer than 5% of control animals (12).

Our data from the present design would be insufficient to explain the exact mechanisms and causes of increased fetal death rate (60%) following high-dose IA BM, as we do not have specimens from other organs. However, toxicity caused particularly by BM in other organ systems, including the fetal central nervous system, can be expected. At this stage, we do not know whether these unfavorable effects on the fetus are specific for BM or whether they can be generalized to other glucocorticoids.

Budesonide is a steroid derivative with minimal systemic absorption and almost no placental transfer. Budesonide is conventionally used as an oral inhalant in the treatment of childhood asthma. Limited data from animal (sheep) experimentation reveals no perinatal loss with relatively high doses of IA budesonide administration; moreover, its pulmonary maturational effects were reported to be comparable to the standard maternal BM therapy (9, 11). Hence, budesonide and other corticosteroids with minimal absorption and limited toxicity can be candidates for fetal therapy, including IA administrations. Further experimental studies will be needed on IA budesonide therapy.

Conclusion

High-dose IA BM causes severe pathological lesions, including edema and hemorrhage in the fetal lungs. These changes may elucidate the toxic effects and increased fetal losses we encountered with this mode of treatment.

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Conflict of interest

No conflict of interest was declared by the authors.

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Health related quality of life among different PCOS phenotypes of infertile women

İnfertil PKOS fenotipleri arasında sağlıkla ilişkili hayat kalitesi

Berna Dilbaz[1], Mehmet Çınar[1], Enis Özkaya[2], Nazan Vanlı Tonyalı[1], Serdar Dilbaz[1]

[1]Department of Gynecology and Obstetrics, Etlik Zübeyde Hanım Maternity & Women's Health Teaching and Research Hospital, Ankara, Turkey

[2]Department of Gynecology and Obstetrics, Faculty of Medicine, Giresun University, Giresun, Turkey

Abstract

Objective: The aim of this study was to evaluate the clinical features and health quality profile differences between infertile women with polycystic ovary syndrome (PCOS) phenotypes and women with unexplained infertility.

Material and Methods: The WHOQOL-BREF were administered in a cross-sectional survey to 132 women diagnosed with PCOS (study group) and 32 women diagnosed with unexplained infertility (control group). Body mass index (BMI), duration of infertility (DOI), type of infertility (TOI) and Ferriman Gallwey scores (FG scores), were compared between the study and control groups and between different phenotype groups of PCOS: Group 1-Hyperandrogenemia (HA)-anovulation (N=34), Group 2-HA-PCO (ovulatory PCOS, (N=34), Group 3-PCO-anovulation (N=32), and Group 4-HA-PCO-anovulation (N=32) and the associations of these parameters with the health quality profile were analyzed.

Results: Physical, Spiritual and Environmental scores were significantly lower ($p<0.05$) in Group 1 patients (HA-AO) in comparison to the other three PCOS groups and the control group, while the same difference was observed in the social scores with a near significance ($p=0.05$). Linear regeression analyses revealed significant associations between type of infertility (beta coefficient: -0.423, $p=0.001$), FG score (beta coefficient: -0.177, $p=0.016$), phenotype 1 (beta coefficient: -0.236, $p=0.002$) and physical scores. Psychological scores were associated with the type (beta coefficient: -0.641, $p=0.001$) and duration (beta coefficient: -0.149, $p=0.009$) of infertility. Scores in the social area were only associated with type of infertility (beta coefficient: -0.443, $p=0.001$). Scores of environmental area were significantly associated again with the type of infertility (beta coefficient: -0.499, $p=0.001$) and FG scores (beta coefficient: -0.195, $p=0.008$). Primary infertility was a risk factor for low physical (odds ratio: 8.100, 95% CI: 3.827-17.142), social (odds ratio: 9.183, 95% CI: 4.084-20.648) and environmental (odds ratio: 9.966, 95% CI: 4.623-21.468) scores determined according to the median level.

Conclusion: FG scores, primary infertility and phenotype 1 PCOS were associated with lower health quality of life scores. Infertile women with Phenotype 1 (HA-AO) had the lowest scores.

(J Turkish-German Gynecol Assoc 2012; 13: 247-52)

Key words: Health related quality of life, polycystic ovary syndrome, phenotypes, unexplained infertility, hirsutism

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Özet

Amaç: Bu çalışmanın amacı polikistik over sendromu olan infertil kadınlar ile açıklanamayan infertilitesi olan kadınlar arasında klinik özeliikler ve hayat kalite profilini karşılaştırmaktır.

Gereç ve Yöntemler: Polikistik over hastalığı tanısı alan 132 infertil hasta ve açıklanamayan infertilitesi olan 32 hastaya Dünya Sağlık Örgütü hayat kalitesi anketi kısa versiyonu uygulandı. Tüm hastalar arasında beden kitle indeksi, infertilite süresi, infertilite tipi, Ferriman Gallwey skoru gruplar arasında ve farklı PCOS fenotipleri arasında karşılaştırıldı. Fenotip grupları şu şekilde idi: Grup 1-Hiperandrojenemi (HA)-anovulasyon (N=34), Grup 2-HA-PCO (ovulatuar PCOS, (N=34), Grup 3-PCO-anovulasyon (N=32), and Grup 4-HA-PCO-anovulasyon (N=32). Bu parametrelerle hayat kalite skorunun ilişkisi irdelendi.

Bulgular: Fiziksel, Spiritual ve çevresel skorlar Grup 1 haslarda diğer PCOS gruplarına ve kontrol grubuna kıyasla anlamlı düşükken ($p<0.05$), aynı farklılık sosyal skorda daha düşük anlamlılıkla gözlendi ($p=0.05$). Lineer regresyon analiz sonucuna göre infertilite tipi (beta katsayısı: -0.423, $p=0.001$), FG skor (beta katsayısı: -0.177, $p=0.016$), grup1 (beta katsayısı: -0.236, $p=0.002$) ve fiziksel skorlar anlamlı ilişkili saptandı. Fiziksel skorlar infertilite tipi (beta coefficient: -0.641, $p=0.001$) ve süresi (beta coefficient: -0.149, $p=0.009$) ile anlamlı ilişkili idi. Sosyal alan skorları sadece infertilite tipi ile ilişkili saptandı (beta coefficient: -0.443, $p=0.001$). Çevresel alana ait skorlar yine infertilite tipi (beta coefficient: -0.499, $p=0.001$) ve FG skorları (beta coefficient: -0.195, $p=0.008$) ilişkili idi. Primer infertilite median değerlere göre saptanan düşük fiziksel (odds ratio: 8.100, 95% CI: 3.827-17.142), sosyal (odds ratio: 9.183, 95% CI:4.084 -20.648) ve çevresel (odds ratio: 9.966, 95% CI: 4.623-21.468) skorlar için risk faktörü olarak saptandı.

Sonuç: FG skoru, primer infertilite ve grup 1 PCOS düşük hayat kalite skoru ile ilişkilidir ve fenotip 1 grubundaki infertil hastalar en düşük değere sahiptir. (J Turkish-German Gynecol Assoc 2012; 13: 247-52)

Anahtar kelimeler: Sağlıkla ilişkili hayat kalitesi, polikistik over sendromu, fenotipler, açıklanamayan infertilite, hirşutizm

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Address for Correspondence: *Enis Özkaya, Department of Gynecology and Obstetrics, Faculty of Medicine, Giresun University, Giresun, Turkey*
Phone: +90 505 474 24 59 e.mail: enozkaya1979@gmail.com

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Introduction

Polycystic ovary syndrome (PCOS) is a multifactiorial and polygenic pathology that manifests itself with a wide spectrum of signs and symptoms that are related to the disturbances of reproductive, endocrine, and metabolic functions. Thus, involvement of various organ systems at different degrees results in a heterogenous presentation of the disease (1). The diagnostic criteria defined for PCOS hava undergone several changes in recent years. While the clinical presentation of chronic anovulation and hyperandrogenism has been stressed as the major diagnostic criteria, the presence of normal ovulatory function in some women with PCOS has been acknowledged in recent years (2, 3). New diagnostic criteria were established in 2004, including all these three factors: presence of chronic anovulation, hyperandrogenism, and polycystic ovaries together, with a special emphasis placed on existence of polycystic ovaries on ultrasonography (4, 5). Polycystic ovary syndrome was diagnosed in the presence of two of the three diagnostic criteria. Using the possible combinations of these three criteria, four different phenotypes of PCOS are identified: hyperandrogenism (clinical or biochemichal) and chronic anovulation; hyperandrogenism and polycystic ovaries but with ovulatory cycles; and chronic anovulation and polycystic ovaries without clinical hyperandrogenism and hyperandrogenism, chronic anovulation and polycystic ovaries. At least 90% of women attending fertility clinics with failure to ovulate have PCOS (6). PCOS is associated with reduced quality of life (QoL) (7). The disorder is associated with biochemical disturbances that can lead to mood disturbances per se (8). Hirsutism, menstrual irregularity, acne and infertility have been shown to be the most distressing symptoms in adults with PCOS (9), while weight gain has been identified as the most distressing symptom in adolescents and young women with PCOS (10-12).

The aim of this study is to evaluate the clinical, endocrine, and health quality profile differences between the main PCOS phenotypes, and compare these findings with women with unexplained infertility in order to eliminate "infertility" as a major source of concern for the patients and indicate other factors that might affect the health quality profile.

Material and Methods

Among 500 women screened for presence of PCOS and found to have PCOS according to the Rotterdam Criteria, 34 consecutive patients from each phenotype were taken as the study group. Thirty-four patients with unexplained infertility were taken as the control group. Standardized screening was approved by the local Institutional Review Board, and signed written informed consent was obtained from all of the participants. According to the Rotterdam (5) criteria, PCOS was diagnosed when at least two of the following criteria were present: oligo/amenorrhea, clinical or biochemical hyperandrogenism, and PCO on ultrasonography. Other etiologies mimicking PCOS, like Cushing's syndrome, late onset adrenal hyperplasia or androgen-producing neoplasm, and thyroid dysfunction or hyperprolactinemia were considered as exclusion criteria. Patients who had taken any medication during the previous 3 months were excluded from the study. Menstrual cycle length shorter than 24 days and longer than 34 days were recorded as abnormal. Oligomenorrhea was diagnosed in patients with cycles longer than 35 days intervals, and amenorrhea was determined as the absence of menstruation for at least 6 months. Anovulation was defined as having a serum progesterone of <3 ng/mL on day 21-24 of the menstrual cycle with normal base-line hormonal values. Primary infertility was defined as failure to become pregnant after at least one year of unprotected intercourse, while secondary infertility refers to women who have been pregnant at least once but failed to conceive after at least one year of unprotected intercourse. Polycystic ovarian morphology was established using the criteria of ten or more peripheral follicular cysts 8mm in diameter or less in one plane along with increased central ovarian stroma, based on the Rotterdam-PCOS criteria (4).

Women with unexplained infertility after at least one year of unprotected regular sexual intercourse were included in the study as the control group. All the patients in the control group had patent fallopian tubes detected by hysterosalpingography and/or laparoscopy, normal ovulation confirmed by a midluteal progesterone level more than 3 ng/mL and normal hormonal profile (follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin, estradiol (E2) and thyroid stimulating hormone (TSH) in the early follicular phase. All the male partners had normal semen analysis according to WHO criteria (13).

Medical history regarding age, race, menstrual cycle pattern, personal and family medical history, type and duration of infertility, any previous or current use of medication, the presence of acne, and hirsutism were recorded. Body mass index (BMI), waist, and hip circumferences were recorded. Basal FSH, LH and E2 levels were measured on day-3 of the menstrual cycle. Progesterone levels were measured on the 21st day of the cycle. Hirsutism was established by using the Ferriman-Gallwey score (≥7) (14). The BMI and hirsutism scores were assessed by a single investigator for all of the subjects.

Transvaginal ultrasonography was systematically performed by the same investigator using the 7.5 MHz transvaginal probe to a logic ultrasound system. Antral follicles were measured in three dimensions, and those with a mean diameter of 2-9 mm counted.

Four different phenotypes were defined as follows: Group 1-Hyperandrogenemia (HA)-anovulation, Group 2-HA-PCO (ovulatory PCOS), Group 3-PCO-anovulation, and Group 4-HA-PCO-anovulation.

Patients in all groups filled in WHOQOL-BREF (Turkish short version) at the Infertility Department of Etlik Zubeyde Hanim Women's Health Teaching and Research Hospital between September 2010 and March 2011. Four-dimensional quality of life scales were calculated: 1-Physical, 2-Spiritual, 3-Social and 4-Environmental. Subjects were selected without intention to balance groups. Sample size was calculated with 95% CI and 80% power according to the previous study by Guestella et al. (15). WHOQOL-BREF is the abbreviated version of the original WHOQOL instrument. While the long form includes 100 items, WHOQOL-BREF has 26 items with a five point Likert type response scales-generic QoL instrument. It was developed

by WHO as a multilingual, multidimensional profile of QoL for crosscultural use (16, 17). WHOQOL was adapted to more than 40 cultures in the world. WHOQOL-BFEF has four broad domains namely: Physical, Psychological, Social Relations and Environmental domains. The instrument assesses satisfaction with life as well as the impact of disease or illness, and it captures positive and negative aspects of QoL. WHOQOL is a profile which has a good underlying theoretical conceptualisation of Qol 22. It was validated for Turkish by Eser et al. (18).

Statistical Analysis

The statistical analyses were performed using the Statistic Package for Social Sciences (ver. 11.0; SPSS Inc., Chicago, IL). For group comparisons, analysis of variance and posthoc Tukey test was used. Binary logistic regression was used to calculate the odds ratio. Correlation analysis was used to calculate degree of associations and linear regression analysis was used to determine associations. The Chi square test was used for categorical variable comparisons. ANCOVA was used for statistical adjustment. A P value smaller than 0.05 was accepted as statistically significant.

Results

Out of 170 patients recruited to the study; two patients from Group 3 and Group 4 and two patients from the control group were lost to follow-up. Overall, 164 patients with primary (N:66, 40.2%) and secondary (N:98, 59.7%) infertility were recruited to the study. One hundred and thirty-two patients had PCOS while 32 patients were in the control group. The distribution of 132 patients in PCOS phenotypes were as follows: Group 1: 34, Group 2: 34, Group 3: 32, Group 4: 32 patients. The distribution of age, duration of infertility, BMI, F/G score, WHOQOL-BREF (Turkish short version) scala is given in Table 1.

Group comparisons

The age, duration of infertility, BMI, distribution of types of infertility were similar in the study and control groups ($p>0.05$).

Table 1. The distribution of age, duration of infertility, BMI, F/G score, WHOQOL- BREF (Turkish short version) scala in PCOS phenotypes and in women with unexplained infertility (Control group)

		PCOS Phenotypes					p values
		Group 1 HA-AO N=34	Group 2 HA-PCO N=34	Group 3 PCO-AO N=32	Group 4 HA-PCO-AO N=32	Control N=32	
Age		24.9±2.6	25.7±2.4	25.3±2.9	24.9±2.7	25.2±2.7	0.78
Duration of infertility		3.2±1.26	3.26±1.6	3.19±1.5	3.19±1.5	3.25±1.5	1.00
Type of Infertility	Primary	9	16	12	15	14	0.38
	Secondary	25	18	20	17	18	
Ferriman-Gallwey Scores		15.8±2.8	13.9±2.2	5.6±1.9	14.9±1.9	5.2±1.9	0.00
BMI		29.7±4.2	27.9±4.5	27.5±4.1	28.6±4.2	27.7±4.0	0.20
Physical Scores		14.2±2.4	16.2±2.2	16.1±1.9	16.1±1.9	16.6±1.7	0.00
	BMI Adjusted	14.3±0.3	16.3±0.3	16.0±0.3	16.1±0.3	16.5±0.3	0.00
	F/G Adjusted	15.4±0.48	17.0±0.39	14.5±0.5	17.1±0.4	14.9±0.5	0.00
	BMI+F/G Adjusted	15.6±0.4	17.0±0.3	14.4±0.5	17.1±0.4	14.8±0.5	0.00
Spiritual Scores		13.8±2.3	15.1±2.2	14.8±2.2	14.9±2.3	15.2±2.5	0.10
	BMI Adjusted	13.9±0.4	15.1±0.4	14.7±0.4	14.9±0.4	15.2±0.4	0.191
	F/G Adjusted	14.9±0.5	15.7±0.4	13.5±0.6	15.7±0.5	13.9±0.6	0.06
	BMI+F/G Adjusted	14.9±0.5	15.7±0.4	13.5±0.5	15.7±0.5	13.9±0.6	0.07
Social Scores		14.5±3.2	15.8±3.1	15.7±2.9	16.2±2.5	16.5±2.5	0.05
	BMI Adjusted	14.5±0.5	15.2±0.5	15.7±0.5	16.1±0.5	16.5±0.5	0.08
	F/G Adjusted	15.6.0±0.6	16.2±0.8	14.3±0.8	17.3±0.5	15.4±0.5	0.06
	BMI+F/G Adjusted	15.6±0.6	16.5.0±0.5	14.3±0.7	17.1±0.6	15.0±0.7	0.07
Environmental Scores		13.1±2.4	14.3±2.3	14.6±1.9	14.5±1.9	15.3±2.3	0.01
	BMI Adjusted	13.2±0.3	14.2±0.3	14.6±0.5	14.5±0.4	15.1±0.6	0.03
	F/G Adjusted	14.2±0.5	14.9±0.4	14.6±0.3	14.5±0.3	15.0±0.3	0.02
	BMI+F/G Adjusted	14.2±0.5	14.9±0.5	13.3±0.5	15.3±0.5	13.6±0.6	0.09

BMI: Body mass index, F/G: Ferriman Gallwey, HA: Hyperandrogenemia, PCO: Polycystic ovary, AO: Anovulation

Ferriman-Gallwey scores were statistically significantly higher in Group 1, Group 2 and Group 4 patients in comparison to Group 3 patients (PCOS-AO) and the control group. Physical, Spiritual and Environmental scores were significantly lower ($p<0.05$) in Group 1 patients (HA-AO) in comparison to the other three groups and control group. Adjusted and unadjusted means among groups were shown in Tables 1 and 2.

Comparison of Categorical variables

Group 1 phenotype was compared to other phenotypes according to the rates of low scores: There were 23 (67%) low physical scores in Group 1 and 52 (40%) in others. The low physical scores were 41% in Group 2 (N:14), 43% in Group 3 (N:14), 45% in Group 4 (N:13) and 34% in the Control Group (N:11) ($p=0.004$). There were nineteen (59%) low social scores in Group 1 and 48 (35%) in others ($p=0.045$). Low environmental scores were observed in 22 (69%) subjects in Group 1 and 58 (43%) in others ($p=0.037$). Altough spiritual scores were lower in Group 1 in comparison to the other groups, the difference was not statistically significant.

Correlations

Type of infertility ($r=-0.464$, $p<0.001$), FG scores ($r=-0.318$, $p<0.001$), BMI ($r=-0.245$, $p=0.002$) and phenotype of PCOS ($r=0.300$, $p<0.001$) were significantly correlated with physical scores. Type of infertility was significantly correlated with psychological scores ($r=-0.677$, $p<0.001$). Type of infertility ($r=-0.462$, $p<0.001$) and phenotype ($r=0.215$, $p=0.006$) were correlated with social scores. Type of infertility ($r=-0.531$, $p<0.001$), phenotype ($r=0.246$, $p=0.001$) and FG scores ($r=-0.274$, $p<0.001$) were correlated with environmental scores.

Table 2. The distribution of physical, spiritural, social, enviromental scores in infertility types

		Type of İnfertility		
		Primary N=66	Secondary N=98	p values
Physical		15±2.2	17.1±1.5	0.00
	BMI Adjusted	15.0±0.2	17.0±0.1	0.00
	F/G Adjusted	15.0±0.1	17.0±0.1	0.00
	BMI+F/G Adjusted	15.0±0.1	17.0±0.1	0.00
Spiritual		13.4±0.2	16.7±1.1	0.00
	BMI Adjusted	13.5±0.1	16.7±0.2	0.00
	F/G Adjusted	13.5±0.1	16.7±0.2	0.00
	BMI+F/G Adjusted	13.5±0.1	16.7±0.2	0.00
Social		14.6±2.9	17.4±2.1	0.00
	BMI Adjusted	14.6±0.3	17.4±0.2	0.00
	F/G Adjusted	14.6±0.3	17.3±0.2	0.00
	BMI+F/G Adjusted	14.9±0.3	17.3±0.2	0.00
Environmental		13.3±2.0	15.8±1.7	0.00
	BMI Adjusted	13.4±0.1	15.7±0.2	0.00
	F/G Adjusted	13.6±0.1	15.7±0.2	0.00
	BMI+F/G Adjusted	13.6±0.1	15.7±0.2	0.00

Regression analyses

Linear regression analyses revealed significant association between type of infertility (beta coefficient: -0.423, $p=0.001$), FG score (beta coefficient: -0.177, $p=0.016$), phenotype 1 (beta coefficient: -0.236, $p=0.002$) and physical scores. Psychological scores were associated with the type (beta coefficient: -0.641, $p=0.001$) and duration (beta coefficient: -0.149, $p=0.009$) of infertility. Scores in the social area were only associated with the type of infertility (beta coefficient: -0.443, $p=0.001$). Scores of environmental area were significantly associated again with type of infertility (beta coefficient: -0.499, $p=0.001$) and FG scores (beta coefficient: -0.195, $p=0.008$)

Odds ratios

Primary infertility was a risk factor for low physical (odds ratio: 8.100, 95% CI: 3.827-17.142), social (odds ratio: 9.183, 95% CI: 4.084-20.648) and environmental (odds ratio: 9.966, 95% CI: 4.623-21.468) scores determined according to the median level.

Discussion

Polycystic ovary syndrome has diverse clinical manifestations that affect the reproductive life (infertility, anovulation, hypernadrogenism) and metabolic features (insulin resistance, impaired glucose tolerance, increased cardivascular disease risk, type 2 diabetes mellitus). The adverse impact of this heterogenous condition on psychological features (increased anxiety, depression and worsened quality of life) has become a new area of research in the last decade (19). The common manifestations of PCOS; infertility, obesity, acne, hirsutism, menstrual irregularities have a negative impact on mood and psychological status. Depression, anxiety, negative body image and psychosexual dysfunction are the most common exacerbations of the negative impact of PCOS on the quality of life (20). Infertility is related with impaired health-related quality of life. A review of 14 studies that investigated the effect of infertility on QoL and health-related quality of life (HRQOL) among infertile women and men revealed that infertile women had more impaired QOL and HRQOL and lower scores in several QOL and HRQOL domains; mainly mental health, social functioning and emotional behaviour in comparison to men (21). A Turkish study on infertile couples revealed that, while physical and psychological health and social relations domain score was similar in infertile men and women; the quality of life in the environmental domain was greater in infertile women when compared to that of infertile men (22). Variables affecting quality of life of infertile individuals were found to affect women and men in similar ways. The authors stressed the importance of awareness of the factors that affect quality of life of the patients among nurses and health care professionals. In our study, the main aim was to analyze the health quality scores of infertile women with and without PCOS and the differences between 4 PCOS phenotypes. We have selected a group of infertile subjects with similar socioeconomical, educational status and

tried to establish homogenous groups according to the known parameters affecting the tests.

In a a recent study by Greil et al. (23), it was stated that, while both primary and secondary infertility is related to fertility specific stress, women with primary infertility are particularly distressed and caregivers should address their emotional needs. Consistent with this result, we have shown that primary infertile individuals differ from secondary infertile women according to their life quality scores. In the presented study, especially the primary infertile women in both PCOS groups and the control group of women with unexplained infertility were found to be under significant distress compared to women with secondary infertility.

In our study, the health quality scores were similar among the 3 PCOS groups and unexplained infertile patients except for the PCOS phenotype 1 that presents itself with clinical hyperandrogenemia and menstrual abnormalities. Phenotype 1 patients, who had significantly higher BMI and FG scores in comparison to the other pheotypes, also showed significant score differences in the WHOQOL-BFEF that can be attributed to these differences. BMI and FG adjusted mean scores were found to be similar among all groups except for physical scores. A recent study form Turkey comparing the psychological health of 226 PCOS patients with 85 BMI-adjusted healthy controls found 8.1 fold increased depression scores in PCOS patients and, similar to our study, menstrual problems and hirsutism were reported to be the most severe concerns on the HRQOL followed by emotional problems (24).

Life quality scores have been studied in several groups of subjects including PCOS patients. However, the presented study is a pioneering study that evaluates the WHOQOL-BFEF scores in infertile subjects with and without the diagnosis of PCOS, and the variations of WHOQOL-BFEF score among different phenotypes of PCOS. A previous study investigated the depression, anxiety scores and quality of life of PCOS subjects before and after laser treatment of hirsutism and compared them to the control group. The study concluded that laser treatment appeared to reduce the severity of facial hair, resulting in improvement of depression and anxiety scores and psychological quality of life in women with PCOS (25). Our results support the idea that FG scores and health related quality of life are interrelated especially in the physical domain.

Kumarapeli et al. compared the scores of women with PCOS with the control group, and they concluded that PCOS occurring in South Asians adversely affected their psychological well-being and health related quality of life. The psychological distress in South Asians was found to be related to hirsutism rather than to obesity, contrary to the white European women with PCOS (26). In our study we also did not find a direct relation between BMI and scores and the presence of hirsutism was a prominent factor affecting quality of life.

Sundararaman et al. applied the “Goldberg’s General Health Questionnaire” (GHQ 28) to PCOS subjects in order to assess the psychological status. The authors stated that women presenting with PCOS had increased psychological distress, which was related to the severity of physical manifestations of the condition, such as hirsutism, obesity and increased waist circumference (27).

Obese subjects were found to have lower physical and psychological scores when compared with the healthy population reference group in some studies. Pan et al. suggested that obesity can cause impaired HRQOL, that can be improved through body weight loss intervention (28). Obesity and health related quality of life were assessed in non-pregnant Turkish women aged betwen 15-49. The authors repoted that, after adjusting for age, level of education and co-morbid illnesses, subjects with a BMI higher than normal value had significantly lower HRQOL scores, compared to normal-weight individuals on each of the domains, except for the environmental domain. The study results suggested that the body weight alone could negatively affect HRQOL. In conclusion, body weight should also be controlled in studies examining HRQOL (29). Therefore in the current study, the means were compared after adjustment for FG, BMI and FG-BMI.

While managing PCOS, the psychological issues accompanying this multifaceted disease should not be underestimated and a special multi-disciplinary approach is crucial.

The weakness of the present study is the limited number of subjects in each phenotype of PCOS and the lack of evidence to explain why phenotype 1 had lower physical scores after adjustment for BMI and FG score, because phenotype 4 with all the criteria for PCOS diagnosis did not differ from other phenotypes. Future studies with larger number of variables are needed to assess health quality of life in phenotype 1.

Conclusion

FG scores, primary infertility and phenotype 1 PCOS were associated with lower health quality of life scores in the physical domain.

Conflict of interest

No conflict of interest was declared by the authors.

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Colpocleisis, patient satisfaction and quality of life

Kolpokleisis, hasta memnuniyeti ve yaşam kalitesi

Mehmet Reşit Asoğlu, Selçuk Selçuk, Çetin Çam, Reyhan Ayaz, Niyazi Tuğ, Ateş Karateke
Department of Gynecology and Obstetrics, Zeynep Kamil Training and Research Hospital, İstanbul, Turkey

Abstract

Objective: The aim of this study was to investigate the impact of colpocleisis operations with or without an anti-incontinence procedure on post-operative objective and subjective outcomes.

Material and Methods: Partial and total colpocleisis cases, with or without concomitant trans-obturator tension-free (TOT) procedure, were analyzed retrospectively. Pre- and post-operative POP-Q, urodynamics, UDI 6 and IIQ 7 scores and the level of patient satisfaction were the outcome measures.

Results: A total of 27 patients with colpocleisis (23 partial and 4 total) were analyzed. Seven women underwent also a concomitant TOT procedure. Of the patients, 66.7%, 25.9% and 7.4% were 'very satisfied', 'satisfied' and 'not satisfied', respectively. UDI-6 and IIQ-7 scores were improved in all patients. Post-operative urinary retention was not observed and prolapse recurred in one patient.

Conclusion: In elderly or medically compromised patients with advanced pelvic organ prolapse, colpocleisis is a safe and effective surgical technique with a high subjective satisfaction rate. A concomitant TOT procedure may be added where indicated.

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Key words: Colpocleisis, transobturatuar tape, pelvic organ prolapse, quality of life, patient satisfaction

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Özet

Amaç: Bu çalışmanın amacı, kolpoklesis operasyonu±anti-inkontinans prosedürünün post-operatif objektif ve subjektif sonuçlara etkisini araştırmaktır.

Gereç ve Yöntemler: Parsiyel ve total kolpokleisis operasyonu± transobturatuar tape prosedürü (TOT) uygulanan olgular retrospektif olarak analiz edildi. Pre-operatif ve post-operatif POP-Q, ürodinami, UDI-6 ve IIQ-7 skorları, hasta memnuniyeti seviyeleri değerlendirildi.

Bulgular: Kolpokleisis operasyonu (23 parsiyel ve 4 total) yapılan toplam 27 hasta değerlendirildi. Yedi hastaya eş zamanlı TOT prosedürü uygulandı. Hastaların; %66.7'si çok memnun, %25.9'u memnun, %7.4'i memnun değildi. Bütün hastalarda UDI-6 ve IIQ-7 skorlarında iyleşme oldu. Hastaların hiçbirinde post-operatif üriner retansiyon izlenmedi ve sadece bir hastada prolapsus nüks etti.

Sonuç: İleri derece pelvik organ prolapsusu olan yaşlı hastalarda veya medikal olarak düşkün hastalarda, yüksek subjektif başarı oranı ile kolpokleisis güvenli ve etkili bir cerrahi tekniktir. Endike olduğu durumlarda eş zamanlı TOT prosedürü eklenebilir.

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Anahtar kelimeler: Kolpokleisis, transobturatuar tape, pelvik organ prolapsusu, yaşam kalitesi, hasta memnuniyeti

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Introduction

Life expectancy is increasing, and consequently there are growing number of elderly women with lower urinary tract symptoms (LUTS) who also suffer from other chronic medical conditions (1). Surgery is one of the main options for advanced prolapse but it carries an inherent risk for re-operation for up to one-third (2). Therefore, it is important to select an optimal surgical technique with high operative success and low risk of complications in these elderly, fragile women with increased peri-operative risks and complications. In women who do not wish to maintain their vaginal coital function, obliterative procedures such as colpocleisis may be the management of choice (1).

Not much is known about management of the LUTS at the time of colpocleisis. Although a concomitant anti-incontinence procedure is suggested to prevent new onset post-operative urinary incontinence, it has been proposed that the weakened detrusor muscle function commonly seen in these elderly women may lead to urinary retention (3-6).

In this study, we retrospectively analyzed the impact of colpocleisis, with or without an anti-incontinence procedure, on post-operative objective and subjective outcomes.

Material and Methods

Study design and patients selection

Data collected from a total of 27 patients who underwent total or partial colpocleisis operations in a tertial referral pelvic reconstructive surgery clinic between 2005 and 2009 were analyzed.

Pre-/post-operative evaluation

All the patients underwent a standard preoperative evaluation, including cervical smear, endometrial sampling, transvaginal

Address for Correspondence: *Mehmet Reşit Asoğlu, Arakiyeci Hacı Mehmet Mah. Burhanettin Üstünel Cad. No:10, 34668, Üsküdar, İstanbul, Turkey*
Phone: +90 216 391 06 80 e.mail: mresitasoglu@hotmail.com

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and renal ultrasonography. The assessment of the prolapse was performed by the principal author (A.K) using the Pelvic Organ Prolapse Quantification (POP-Q) scoring system (7). Pre- and post-operative symptoms and complaints were assessed using the validated versions of the short forms of the Urinary Distress Inventory (UDI-6) and the Incontinence Impact Questionnaire (IIQ-7) (8). Subjective satisfaction of the patient was classified as '*very satisfied*', '*satisfied*', '*not satisfied*' and '*regret*'.
All patients underwent urodynamic evaluation (filling cystometry) pre-and post-operatively. Urodynamic evaluations were performed in accordance with criteria established by the International Continence Society (9). To detect masked stress incontinence (SUI), pre-operative urodynamic testing was performed with the reduction of the prolapsed segment(s) by using ring forceps without any possible compression to the urethra.

Surgical Methods
All the operations were performed by the principal author (A.K.)

Partial Colpocleisis
Rectangular portions of the anterior and posterior vaginal walls were dissected off the underlying fibromuscular tissue, leaving a lateral 2-cm bridge of vaginal mucosa. Special care was taken not to involve the area beneath the urethra. Lateral vaginal canals were created and the anterior and posterior fibromuscular tissue compartments were approximated by delayed absorbable interrupted sutures.

Total Colpocleisis
In hysterectomized patients the vaginal epithelium was completely dissected off as the canals were not necessary. According to the surgeon's preference, levator myorrhaphy and/or perineorrhaphy were performed.
Patients with urodynamic stress incontinence (USI) also underwent a concomitant transobturator procedure.
Post-operatively, post void residue (PVR) measurements less than 100 mL were considered as normal.

Statistical analysis
All values were given as mean±standard deviation. Statistical analysis was performed using SPSS 11.5 software. Student's T, and Paired T tests were performed as appropriate; p=0.05 was accepted as the degree of significance.

Results

Data from 27 patients were eligible for the study. Demographic data of the patients are presented in Table 1 and operative data are given in Table 2. The mean post-operative follow-up period was 27.5±13.7 (min 12, max 78) months. One patient had a history of vaginal hysterectomy for uterine prolapse. The remaining 26 patients did not report any operation for prolapse. One patient with partial colpocleisis received local anesthesia and the remaining 26 operations were performed under spinal anesthesia. After excluding additional operations, mean operation times of partial and total colpocleisis were 25.4±1.78 and 60±5 minutes, respectively. No major per-operative complication was recorded.
Preoperatively, seven patients (25.9%) were diagnosed as having USI and ten (37.1%) women had urodynamical findings of detrusor overactivity (DOA). Postoperatively, 11% (3/27) of patients had symptoms of SUI. Persistence of USI was recorded

Table 1. Demographic data of the patients (n=27)

BMI (kg/m^2) mean±sd	26.70±3.94
Age (years) mean±sd	72.85±6.12
Parity (n) mean±sd	4.70±2.39
Diabetes Mellitus	
%	33.3
(n)	(9)
Hypertension	
%	51.9
(n)	(14)
Stage III Prolapse	
%	7.4
(n)	(2)
Stage IV Prolapse	
%	92.6
(n)	(25)
Prior hysterectomy	
%	3.7
(n)	(1)
BMI: Body Mass Index	

Table 2. Operative data of the patients (n=27)

Partial Colpocleisis	
%	85.2
(n)	(23)
Total Colpocleisis	
%	14.8
(n)	(4)
Concomitant TOT	
%	25.9
(n)	(7)
Concomitant Hysterectomy	
%	11.1
(n)	(3)
Hospitalization (days)	
mean±sd	2.4±0.6
TOT: trans-obturator tension-free	

in one patient who underwent concomitant TOT procedure and two patients without this anti-incontinence procedure were diagnosed as *de novo* USI after colpocleisis. 18.5% (5/27) of patients had DOA, postoperatively. No patient showed urodynamical findings of DOA postoperatively. Anatomical success was evident in 96.3% (26/27) patients but in one patient, recurrence of prolapse was diagnosed with the leading point of the prolapsed segment three centimeters beyond the hymenal ring. No patient in the postoperative period had a PVR value greater than 100 mL, whereas the rate of patients with elevated preoperative PVR value was 22% (6/27).

Pre and post-operative mean total scores of IIQ 7 were 15.07±2.18 and, 2.67±4.09, respectively, in all patients. This difference was statistically significant ($p<0.0001$). In 20 patients without any concomitant anti-incontinence procedure, total IIQ-7 scores and irritative, stress and obstructive subscores of UDI-6 were lower in the postoperative period, compared to the preoperative assessment. These improvements were statistically significant in IIQ-7 scores and in the obstructive subscore of UDI-6 ($p< 000.1$) (Table 3).

In 7 patients with concomitant TOT procedure, total IIQ-7 scores and irritative, stress and obstructive subscores of UDI-6 were lower in the postoperative period, compared to preoperative assessment (15.14±2.27 to 3.14±5.37, 3.00±1.00 to 1.29±1.11, 5.43±0.79 to 1.00±2.24 and 2.14±1.22 to 0.29±0.49; respectively). No statistical calculation was performed.

Among all participants, 92.6% (25/27) were satisfied ((66.7% (18/27) and 5.9% (7/27) '*very satisfied*' and '*satisfied*', respectively) with the operations. Two (7.4%) patients reported having feelings of regret, one because of the recurrence of her prolapse and incontinence, and the other because of the new onset of stress incontinence.

Discussion

This retrospective study showed that women who underwent colpocleisis operations were satisfied with the results of their management. The satisfaction rate was over 90%, with an anatomical success rate over 95%, which is consistent with previously reported anatomical success rates between 91% and 100% (3, 5, 10-12). Furthermore, this study provided some data about the improvement in their quality of life (QoL) using validated condition-specific instruments for pelvic floor symptoms.

Table 3. Comparison of pre-and post-operative UDI-6 subscores and IIQ-7 scores of patients without any additional incontinence operation

	Pre-operative (mean±sd)	Post-operative (mean±sd)	p
UDI-6 irritative	1.70±1.49	1.65±1.42	0.914
UDI-6 stress	1.65±1.78	0.95±1.54	0.192
UDI-6 obstructive	2.85±1.95	0.35±0.59	0.000
IIQ 7	15.05±2.21	2.50±3.69	0.000

UDI-6: Urinary Distress Inventory, IIQ-7 Incontinence Impact Questionnaire

For women with bothersome symptoms, surgical therapy is an effective option. Treatment choice depends on age and medical co-morbidities, desire for sexual function and risk factors for recurrence. The main goal should be to improve the QoL of the sufferers and the benefits of the management should always outweigh the risks. Anatomical success may satisfy the surgeon but little is known about the impact of colpocleisis on patient satisfaction and on their LUTS. Shortage of this information limits the clinicians in counselling the patients to consider colpocleisis for their prolapse.

Trouble with stress incontinence and/or urge symptoms is common in these patients (13). In this study, IIQ-7 and UDI-6 questionnaires, which were both developed and combined to assess the impact of urinary incontinence on QoL, were used to measure this . Our retrospective cohort consisted of two groups of patients, one with colpocleisis and other with concomitant TOT procedure. Unfortunately, this retrospective study was not powered to assess the efficacy of an added anti-incontinence procedure. Although Total IIQ-7 scores and irritative, stress and obstructive subscores of UDI-6 were lower in the postoperative period in these patients, compared to preoperative assessment, with such small numbers it was impossible to draw any conclusion or make any statistics. On the other hand, when all patients are considered as a whole, pre and post-operative mean total scores of IIQ-7 were significantly better, showing a clear benefit from colpocleisis whether an anti-incontinence procedure is added or not. This improvement in QoL was also evident in the Total IIQ-7 score and the obstructive subscore of UDI-6 of patients without any concomitant anti-incontinence procedure. It is known that patients with advanced POP show a high rate of urinary retention secondary to compromised urethral flow. Adding anti-incontinence surgery in these patients may raise concerns about worsening o fthe obstructed urinary outflow, but adequate repair of the prolapse resolves the obstruction (6). One fifth of our cohort had preoperative PVR values greater than 100 mL but no patient had a PVR value suggesting outflow obstruction in the postoperative period. These data may be interpreted as being in agreement with the findings that colpocleisis and concomitant mid-urethral sling interventions improve urinary symptoms without causing significant urinary retention and may be offered to elderly women with SUI who are undergoing colpocleisis regardless of preoperative PVR (14). Even when no concomitant anti-incontinence operation is planned; we believe that special care has to be taken not to involve the area beneath the urethra for the possibility of a sling operation, in case *de novo* SUI occurs after the operation.

Although colpocleisis is offered to elderly women without sexual function, some concern exists that such an obliterative procedure which significantly alters female genital anatomy and vaginal function may adversely affect body image, cause patient dissatisfaction and give rise to feelings of regret about the treatment of their prolapse (13). The rate of regret after colpocleisis ranges between 3% and 10% and in this study only two (7.4%) patients reported feelings of regret (15). However, it should be noted that these two patients were not mainly concerned about their lost sexual image, one reporting her reason of feelings of regret as recurrence of her prolapse and incon-

tinence, and the other as new onset (*de novo*) stress incontinence. Overall, 92.6% of this retrospective cohort were satisfied (66.7% and 5.9%, '*very satisfied*' and '*satisfied*', respectively) with the operations.

Conclusion

Colpocleisis offers a safe and effective option in resolving prolapse and pelvic symptoms and improves the QoL of elderly, fragile women who do not seek vaginal coital function. It is associated with high patient satisfaction and most causes of regret can be corrected with re-operation. Although the number of concomitantly treated patients is insufficient to draw any firm conclusion in this study, it seems to work well when combined with a suburethral sling operation.

The number of older women seeking care for their prolapse is increasing and colpocleisis is becoming more important than ever. Nevertheless, further studies are still needed to establish the management of concomitant anti-incontinence surgery for overt or masked stress urinary incontinence.

Conflict of interest

No conflict of interest was declared by the authors.

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Effects of intravaginally inserted controlled-release dinoprostone and oxytocin for labor induction on umbilical cord blood gas parameters

Doğum eylemi indüksiyonunda kullanılan intravajinal dinoproston ve oksitosin'in umblikal arter kan gazı parametreleri üzerine etkilerinin karşılaştırılması

Hüseyin Levent Keskin, Gökalp Kabacaoğlu, Elçin İşlek Seçen, Işık Üstüner, Gülin Yeğin, Ayşe Filiz Avşar

Department of Obstetrics and Gynecology, Ankara Atatürk Education and Research Hospital, Ankara, Turkey

Abstract

Objective: To compare the effects of oxytocin and dinoprostone used in labor induction on fetal blood gas parameters.

Material and Methods: This prospective randomized trial involved 108 women who completed 37 gestational weeks and who required labor induction prior to normal vaginal birth. Labor was induced in 57 women with an intravenous low dose oxytocin regimen and in 51 with intravaginal dinoprostone (PGE_2). Following childbirth, umbilical artery blood gas was analyzed, with pH, pCO_2, pO_2, HCO_3 and base excess (BE) compared in the two groups.

Results: Mean age and obstetrical data (gravidity, parity, gestational weeks and birthweight) were similar in the two groups ($p>0.05$). All infants had 1 and 5 minute APGAR scores ≥7. Umbilical artery blood pH was similar in the oxytocin and dinoprostone groups (7.31±0.07 vs. 7.31±0.05, $p=0.780$), as were the other blood gas parameters (pCO_2, pO_2, base excess and HCO_3; $p>0.05$ each).

Conclusion: Induction of labor with either oxytocin or dinoprostone in women with uncomplicated term pregnancies had no adverse effects on umbilical artery blood gas parameters.

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Key words: Oxytocin, dinoproston, umbilical artery blood gas, labor induction, pH

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Özet

Amaç: Bu çalışmada amacımız, doğum indüksiyonunda kullanılan iki metod olan oksitosin ve dinoprostonun fetal kan gazı parametreleri üzerine olan etkilerinin karşılaştırılmasıdır.

Gereç ve Yöntemler: Bu prospektif randomize çalışmaya 37 gebelik haftasını doldurmuş ve doğum eylemi indüksiyonu uygulanan ve vajinal yoldan doğumu gerçekleşen 108 olgu alındı. Eylem indüksiyonu için 57 olguya intravenöz yolla oksitosin, 51 olguya ise intravajinal dinoproston ($PG\ E_2$) uygulandı. Doğumu takiben umblikal arter kan gazı analizi yapıldı ve her iki indüksiyon metodu için bazı parametreler (pH, pCO_2, pO_2, HCO_3 and baz açığı (BE)) incelendi.

Bulgular: İki grup arasında ortalama yaş ve obstetrik değişkenler (gravida, parite, gebelik haftası ve doğum ağırlığı) benzer saptandı ($p>0.05$). Tüm olgularda 1. ve 5. dakika Apgar skoru ≥7 idi. Umbilikal arter kan pH değeri oksitosin grubunda 7.31±0.07 iken ; dinoproston grubunda 7.31±0.05 olarak izlendi ($p=0.780$). Diğer kan gazı parameterleri değerlendirildiğinde de (pCO_2, pO_2, baz açığı ve HCO_3) iki grup arasında belirgin farklılık saptanmadı ($p>0.05$).

Sonuç: Komplike olmayan term gebeliklerde doğum eylemi indüksiyonununda kullanılan iki metod olan oksitosin ve dinoprostonun fetal kan gazı parametreleri üzerine olumsuz etkisi yoktur.

(J Turkish-German Gynecol Assoc 2012; 13: 257-60)

Anahtar kelimeler: Oksitosin, dinoproston, umblikal arter kan gazı, doğum eylemi indüksiyonu, pH

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Introduction

Induction of labor refers to the process whereby uterine contractions are initiated by mechanical or pharmacological methods before the onset of spontaneous labor (1). Induction of labor is advised in situations when the pregnancy is dangerous for the mother or fetus; or when induction is beneficial for both. Induction of labor decreases operative labor and minimizes risks to the fetus (2). Labor is induced in 20% of all pregnancies (3).

Among the factors influencing the method used to induce labor are cervical and membrane status, parity, and patient and provider preferences (4). The ideal method should be safe, painless, inexpensive, comfortable and effective. The most common pharmacological agents are oxytocin and prostaglandins (PGE_1 and PGE_2) (5). Oxytocin is a safe and efficient starter of uterine contractions, but its success is associated with the condition of the cervix at the start of the labor. Dinoprostone (PGE_2) is used to turn an unfavorable to a favorable cervix before the induction of labor (6, 7).

Address for Correspondence: *Elçin İşlek Seçen, Mutluköy Mah., Ağaçseven Sok., Elit Apt., No: 7, Ümitköy, Ankara, Turkey*
Phone: +90 506 763 79 03 e.mail: elcinislek@hotmail.com

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If labor is not induced under acceptable indications and surroundings, the uterus may be overstimulated, causing it to contract too frequently. Too many contractions may lead to changes in fetal heart rate and result in fetal distress (2). We therefore compared the effects of oxytocin and dinoprostone on fetal blood gas parameters.

Material and Methods

This study involved women pregnant for ≥37 weeks who did not experience spontaneous uterine contractions, had a cervical Bishop score ≤3, and were in need of labor induction. Women with fetal distress before labor induction, as determined by fetal monitoring; with a pathological umbilical artery or pathological findings on Doppler examination; with intrauterine growth retardation; or with maternal disease (e.g. thyroid dysfunction, diabetes, hypertension, preeclampsia, or cardiac disorders) were excluded. We enrolled 160 consecutive pregnant women fulfilling the above criteria; these women were randomized 1:1 to labor induction with oxytocin or dinoprostone using numbers generated randomly by a computer. Indications for labor induction were postterm pregnancy (n=81), premature rupture of membranes (n=50), non-assurance on non-stress tests (n=11), and oligohydramnios (amniotic fluid index <60 mm in all four quadrants; n=18). Seven women withdrew consent prior to the initiation of medication and were removed from the study.

Labor was induced in one group with intravenous low dose oxytocin (Synpitan® amp) and in the other with Propess®, a hydrogel polymer matrix containing 10 mg dinoprostone, administered intravaginally. Each woman in the oxytocin group received an intravenous infusion of 2 mU/min oxytocin, which was doubled at 15-min intervals until the appropriate contraction pattern (at least 200 Montevideo Units) was achieved. The infused dose was increased to a maximum of 20 mU/min and afterward kept constant, even after an ideal pattern was reached. If a contraction pattern had not been induced after 18 hours or if spontaneous labor did not start within 18 hours of receiving oxytocin, a cesarean section was performed.

In patients randomized to dinoprostone, fetal heart rate and uterine activity were monitored continuously, starting 15 to 30 minutes before treatment. A polymer matrix containing dinoprostone was placed intravaginally on the posterior fornix, releasing PGE_2 at a constant rate of 0.3 mg/h over 12 hours. As hyperstimulation may occur after placement of the insert, fetal heart rate and uterine activity were monitored continually, from the time of insertion to 15 minutes after removal. The insert was removed by pulling the cord at the start of active labor or at the time of uterine hyperstimulation. If active labor did not start within 12 hours of insertion, the insert was removed and oxytocin infusion was begun.

Five patients in the dinoprostone group were removed from the study, 3 due to spontaneous dislocation of the insert and 2 due to hyperstimulation. Three patients in the oxytocin group elected to stop oxytocin infusion and were removed from the study. Eleven patients in the dinoprostone group were switched to oxytocin for progression of labor. An additional 11 patients were removed from the dinoprostone group, 6 for fetal distress and 5 for cephalopelvic disproportion and malpresentation; and 15 patients were removed from the oxytocin group, 7 for fetal distress, 5 for labor dystocia and 3 for non response to oxytocin. All 26 of these patients underwent cesarean sections. The remaining 108 patients, 51 in the dinoprostone group and 57 in the oxytocin group, were included in the study. The study flow chart is shown in Figure 1.

Following vaginal birth, the umbilical cord was clamped, and a 2 cc blood sample was drawn from the umbilical artery within 30 seconds. Blood gas samples were analyzed for pH, pCO_2, pO_2, HCO_3 and base excess (BE), while obeying the rules of cold chain. One and 5 minute APGAR scores of each newborn were recorded.

This study protocol was approved by the Local Ethics Committee of our Hospital, and all subjects provided informed consent.

Power and Statistical Analyses

The primary end point of our study was a comparison of blood gas parameters in the two groups. We calculated the minimal sample size for this trial using G*power ver. 3.1.3 software (Germany). We estimated a minimum of 49 patients per group, assuming an effect size of 0.6, a Type I error (α) of 0.05 and a statistical power of 90%. Assuming an overall loss rate of 40%, however, with 20% of patients undergoing cesarean section and 20% lost for other reasons, we planned to enroll 160 subjects (80 per group).

Data were analyzed using SPSS ver. 17.0 software. Before statistical analysis, the normal distribution of continuous data was tested using the Shapiro-Wilk test. All the parameters except for gravidity and parity were distributed normally ($p>0.05$). Normally distributed parameters were expressed as mean±SD and compared using independent sample T tests, non-normally distributed parameters were expressed as median (IQR) and compared using Mann-Whitney U tests. A p value ≤0.05 was considered statistically significant.

Results

The mean age of all women enrolled in this study was 26.0±5.1 years, and the mean gestational age was 39.8±1.0 weeks.

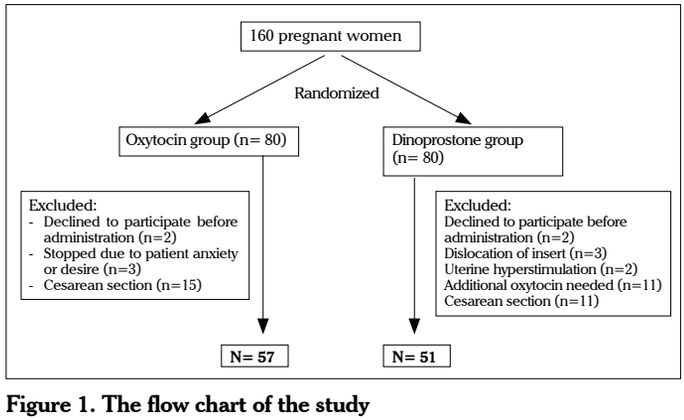

Figure 1. The flow chart of the study

Indications for labor induction in the dinoprostone group included postterm pregnancy in 27 women, premature rupture of membranes in 18, oligohydramnios in 3 and non reassuring NST in 3; whereas indications in the oxytocin group were postterm pregnancy in 29, premature rupture of membranes in 22, oligohydramnios in 4 and non-reassuring NST in 2. The demographic and obstetrical data of the two groups were comparable ($p>0.05$ each; Table 1). All infants had 1 and 5 minute Apgar scores ≥7.

Comparisons of umbilical arterial blood gas pH, pCO_2, pO_2, HCO_3 and BE showed no differences between the oxytocin and dinoprostone groups ($p>0.05$ each; Figure 2).

Discussion

The initiation of labor has become a routine procedure in gynecology and obstetrics clinics. Indications for labor induction include postterm pregnancy, early rupture of membranes, fetal distress, intrauterine growth restriction, fetal death, placenta detachment, preeclampsia, maternal disease and chorioamnionitis (2). We compared the effects of two different methods of labor induction on fetal blood gas parameters. To exclude the effects of fetal distress, we excluded women with chronic maternal disease, complications of pregnancy or fetal distress, and included only uncomplicated pregnancies ending with vaginal birth. Evaluations included Apgar scores and umbilical artery blood gas parameters of the newborn to determine whether acidemia had occurred.

Table 1. Demographic, clinical and blood gas characteristics in the ocytocin and dinoprostone groups

	Oxytocin (n=57)	Dinoprostone (n=51)	P value
Age*	26.4±5.9 (18-37)	25.5±4.3 (19-40)	0.576
Gravidity†	2.0 (2) (1-8)	1.0 (1) (1-5)	0.034
Parity†	1.0 (1) (0-3)	0 (1) (0-2)	0.195
Gestational weeks*	39.7±1.2 (37.1-41.3)	40.0±0.8 (38.1-41.7)	0.469
Birthweight (g)*	3382±457 (2555-4490)	3473±385 (2820-4320)	0.335
pH*	7.31±0.07 (7.10-7.47)	7.31±0.05 (7.18-7.38)	0.780
pCO_2 (mmHg)*	43.14±8.67 (22.1-65.2)	44.16±7.74 (22.1-59.7)	0.579
pO_2 (mmHg)*	18.84±8.29 (4.1-41.2)	18.70±7.40 (6.7-40.1)	0.981
BE (mmol/L)*	-3.76±2.50 (-12-0)	-3.28±2.92 (-13-2)	0.479
HCO_3 (mmol/L)*	21.71±1.19 (19.8-25.6)	21.86±1.68 (19.2-25.4)	0.620

*Mean±SD (min-max), †Median (Interquartile range) (min-max)

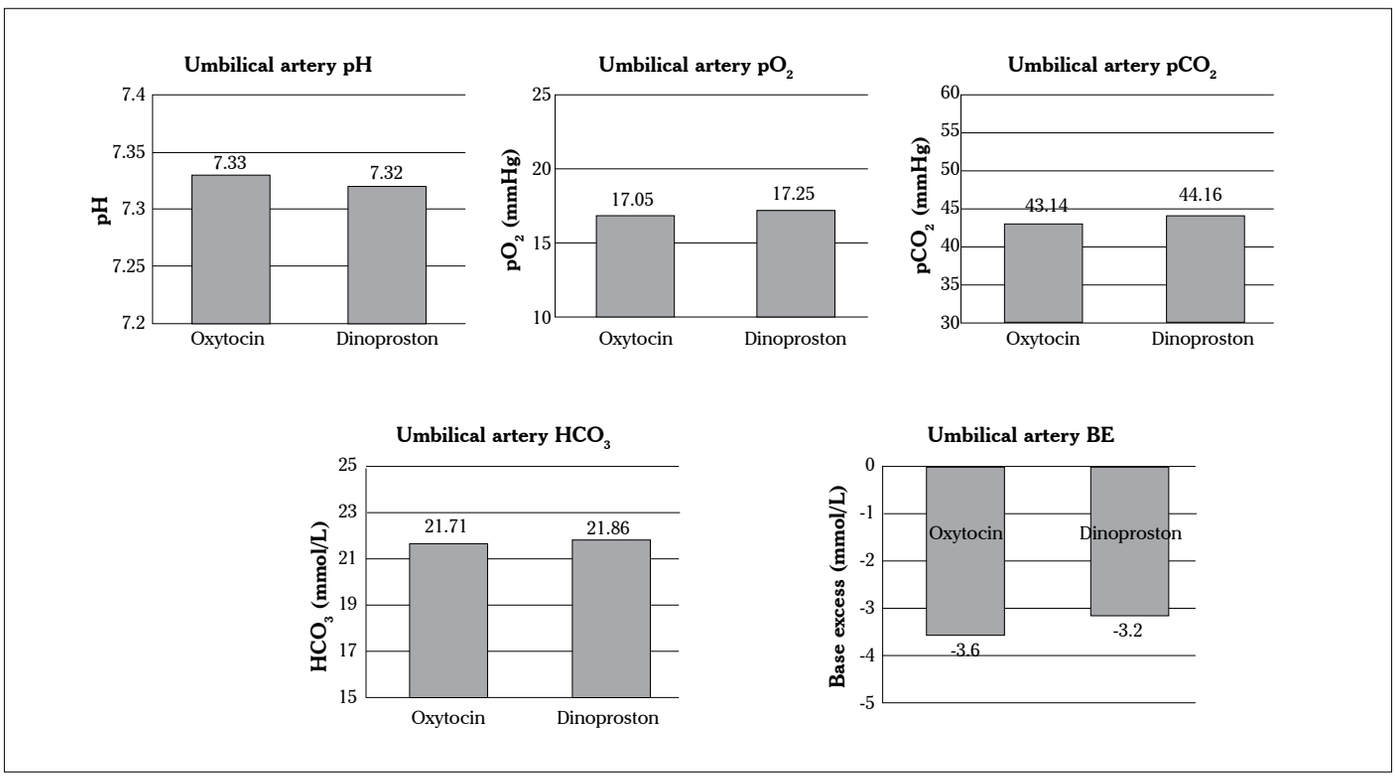

Figure 2. Umbilical artery blood gas parameters in the oxytocin and dinoprostone groups. (A) pH, (B) pO_2, (C) HCO_3 concentration, (D) base excess (BE)

Low Apgar scores are indications of fetal depression and hypotonicity and can be affected by many other parameters other than asphyxia. The acid/base status of umbilical artery blood gas may be an alternative to Apgar score in evaluating neonatal status and in managing any neonatal assistance required. Umbilical artery pH has been shown to be an objective measurement of fetal acid/base balance (8). Fetal acidemia has been defined as pH ≤7.20 and pathological fetal acidemia as pH ≤7.0. Risks of fetal morbidity and mortality were found to be increased at umbilical artery pH ≤7.0, whereas no morbidity was observed in term babies with umbilical artery pH >7.0 (9). Intravenous administration of oxytocin is the method most frequently used to induce labor (10). Although treatment with oxytocin does not increase fetal morbidity, uterine hyperstimulation may result in fetal distress, uterine rupture and/or water intoxication (10). Uterine perfusion decreases during contractions, and increased uterine activity has negative effects on uteroplacental and fetoplacental circulation (11). Intravenous oxcytocin was shown to result in hyperstimulation in 8.3-11.1% of women and fetal distress in 15.9-18%, suggesting that oxytocin application during labor may trigger fetal oxidative stress (5, 12). However, oxytocin did not have any negative effects on pH and did not increase perinatal risk (11, 13). When we investigated the effects of oxytocin-induced labor on fetal acid-base status, we observed an acid-base balance in the umbilical cord, a finding supported by intrapartum cardiotocographic findings and Apgar scores. Thus, the use of oxytocin to assist labor does not have negative effects on the fetus (11, 13).

Intracervical or intravaginal application of dinoprostone (PGE_2) is also frequently used to induce labor (5, 6, 10). Long term treatment with low-dose controlled dinoprostone was well tolerated by both the mother and the fetus (14), with uterine hyperstimulation rates of 7.4-7.8% and fetal distress rates of 10.9-26% (5, 12). In comparison, we observed lower uterine hyperstimulation (2.6%) and fetal distress (7.7%) rates in our dinoprostone group, whereas the fetal distress rate in our oxytocin group was 9.3%. In addition, about 19% of women in whom cervical maturation was established with dinoprostone have been reported to require oxytocin for the progression of labor (12, 15). In comparison, we found that this rate was 14.1%. Few studies to date have compared the effects of oxytocin and dinoprostone on fetal umbilical artery blood gas parameters. A comparison in 91 pregnant women showed no differences in methods of delivery or neonatal Apgar scores (16), although that umbilical artery blood pH was lower after PGE_2 than after oxytocin treatment (16). Arterial and venous umbilical cord gas parameters have been found to be unaffected by oxytocin augmentation or induction (11, 13). Moreover Apgar scores, which indicate the well being of newborn infants, were shown to be statistically similar in women induced with dinoprostone and oxytocin (5, 12). We found that all Apgar scores were ≥7, all umbilical artery pHs were above 7.10, and none of the newborns experienced fetal acidosis. Umbilical artery blood gas parameters did not differ significantly in our oxytocin and dinoprostone groups, indicating that their safety and effectiveness were similar for labor induction.

Conclusion

We found that both labor induction agents, oxytocin and dinoprostone, did not have any unfavorable effects on fetal blood gas in uncomplicated term pregnancies.

Conflict of interest

No conflict of interest was declared by the authors.

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Osteoprotective effect of hormone therapy on bone microarchitecture before impaired bone mineral density in ovariectomized rats

Ooferektomili ratlarda kemik mineral dansitesi etkilenmeden önce, hormon tedavisinin kemik mikromimari üzerine koruyucu etkisi

Hasan Terzi[1], Teksin Çırpan[2], Rabia Terzi[3], Ahmet Özgür Yeniel[2], Hüseyin Aktuğ[4], Onur Bilgin[2]

[1]Department of Gynecology and Obstetrics, Derince Training and Research Hospital, Kocaeli, Turkey

[2]Department of Gynecology and Obstetrics, Faculty of Medicine, Ege University, İzmir, Turkey

[3]Department of Physical Medicine and Rehabilitation, Derince Training and Research Hospital, Kocaeli, Turkey

[4]Department of Histology, Faculty of Medicine, Ege University, İzmir, Turkey

Abstract

Objective: We aimed to determine the effect of hormone replacement therapy on bone microarchitecture in ovariectomized rats.

Material and Methods: In the Animal Ethics Committee approved-study, the effect of treatment with 17 β-estradiol 50 μg/kg and medroxyprogesterone 2.5 mg/kg on bone architecture and bone mineral density in rats versus ovariectomized control rats over the course of 20 days were evaluated. Femoral and lumbar bone mineral density levels and morphometric measurements were performed.

Results: There were no significant differences in the femoral and lumbar bone mineral density levels between the groups. In the intact control group, the trabecular structures were significantly superior to those in the other groups. Additionally, the osteoblast count was significantly higher while the osteoclast count was significantly lower than in all other groups. Two parameters reflecting trabecular bone microarchitecture, which include the trabecular count and the trabecular area, demonstrated significant improvement in the hormone replacement group when compared to the ovariectomized control group. In the hormone replacement groups, the osteoblast count was significantly higher while the osteoclast count was significantly lower than in the ovariectomized control group.

Conclusion: We suggest that offering estrogen alone or in combination with progestogen can be a beneficial approach in preventing early postmenopausal bone loss regardless of bone mineral density.

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Key words: Hormone replacement therapy, bone density, ovariectomy, menopause, bone microarchitecture

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Özet

Amaç: Çalışmamızda ooferektomili sıçanlarda hormon replasman tedavisinin kemik mikromimari üzerine etkisini değerlendirmeyi amaçladık.

Gereç ve Yöntemler: Çalışmanın hayvan etik komite onayı alındı. 17 β-östradiol 50 μg/kg ve medroksiprogesteron 2.5 mg/kg tedavisi ile; ooferektomili ve ooferektomi uygulanmayan sıçanlarda 20 günlük tedaviyi takiben kemik mikromimarisi ve kemik mineral yoğunluğa etkileri araştırıldı. Femoral ve lumbal kemik mineral yoğunluk ölçümleri yapıldı.

Bulgular: Gruplar arasında kemik mineral yoğunluk ölçümleri değerlendirildiğinde anlamlı fark bulunmadı. Ooferektomi uygulanmayan kontrol grubunda trabeküler yapılar diğer guplara göre anlamlı yüksek bulundu. Ayrıca ooferektomi uygulanmayan kontrol grubunda, diğer gruplara göre; osteoblast sayısı anlamlı yüksek ve osteoklast sayısı anlamlı düşük bulundu.

Sonuç: Tek başına östrojen yada progesteron ile kombine hormon tedavisinin; erken postmenapozal dönemde kemik mineral dansitesine yansımayan kemik kayıplarının önlenmesinde etkili olduğunu düşünmekteyiz. (J Turkish-German Gynecol Assoc 2012; 13: 261-6)

Anahtar kelimeler: Hormon replasman tedavisi, kemik yoğunluğu, ooferektomi, menopoz, kemik mikromimari

Geliş Tarihi: 24 Haziran 2012 **Kabul Tarihi:** 27 Ekim 2012

Introduction

Osteoporosis is defined as a reduction in bone mass associated with impaired bone microarchitecture (1). Postmenopausal osteoporosis is the most common type of osteoporosis and causes an imbalance between osteoclastic activity and osteoblastic function; therefore, trabecular continuity and connectivity of the trabecular bone structure are decreased resulting in increased bone fragility and increased fracture risk (2). Impaired bone microarchitecture occurs with the conversion of normal plate-like trabeculae into thinner rod-like structures (3).

Address for Correspondence: *Hasan Terzi, Department of Gynecology and Obstetrics, Derince Training and Research Hospital, Kocaeli, Turkey*
Phone: +90 262 317 80 17 e.mail: drhterzi@yahoo.com

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Bone mineral density (BMD) measurement is commonly used in practice for the diagnosis and management of postmenopausal osteoporosis (4). However, it has limitations, such as not allowing for the assessment of microarchitecture, bone geometry, mineralization and intracortical porosity (5). Whether increases in BMD contribute to bone fragility, fracture risk, and the therapeutic efficacy of osteoporosis agents is controversial (6, 7). Thus, factors other than BMD, such as bone microarchitecture, should be evaluated for management of postmenopausal osteoporosis and assessment of effects of therapuetic agents. Although new techniques have been developed for more comprehensive evaluation of bone turnover and quality, such as imaging techniques with high-resolution peripheral computerized tomography (CT); whether these novel techniques will be useful in daily practice remains to be seen (8). Clinical trials evaluating the changes of bone microarchitecture during postmenopausal osteoporosis and the effects of therapuetic agents on these changes are needed.

The hormone replacement therapy (HRT) is known to prevent accelerated bone loss (9, 10) and improve bone mass in postmenopausal osteoporosis (11). In addition to improvement in BMD, fractures were decreased with hormone therapy (12). In the conjugated ethinyl estradiol (CEE) medroxyprogesterone (MPA) arm of the Women's Health Initiative (WHI) study, active therapy significantly reduced fractures; however, the WHI study population consisted of women who were older than 70 years of age and who had undergone menopause more than 20 years previously (13). On the other hand, patients in the early stages of postmenopause with no complications are usually asymptomatic, leading to underdiagnosing and undertreatment of potential osteoporosis, and patient noncompliance to treatment. Tiihonen et al. (14) reported that women using HRT need more information about the advantages and risks of HRT to increase compliance with the treatment. This information is especially important for women who are hesitant to use HRT.

Therefore it is necessary to determine the effect of widely used HRT on markers other than and with respect to BMD such as bone microarchitecture in early postmenopausal osteoporosis.

We hypothesized that bone microarchitecture is impaired before impairment of BMD in postmenopausal osteoporosis and HRT has favorable effect on bone microarchitecture before its well-known effect on BMD. Demonstration of the positive effect of HRT on bone microarchitecture in animal models would provide a basis and preliminary data for further clinical studies to implement the use of HRT starting with the early stages of postmenopausal period. Therefore, in this study, we aimed to investigate the effect of HRT on bone microarchitecture with respect to its effect on BMD in a rat model with t surgically induced early menopause.

Material and Methods

In the present study, 20 adult female Sprague-Dawley rats weighing between 190 and 250 grams were used. Approval was obtained from the Animal Ethics Committee. Fifteen rats underwent bilateral dorsal ovariectomy (OVX) under combined intramuscular 10 mg/kg xylazine (Bayer Health Care, Monheim, Germany) and 60 mg/kg ketamine hydrochloride (Parke Davis, Istanbul, Turkey) anesthesia. Five rats did not undergo oophorectomy. Rats were kept at the postmenopausal period for three weeks and were divided into four groups:

Group 1, control group with no OVX and no hormone therapy (n=5)

Group 2, control group with OVX and no hormone therapy (nut oil as placebo) (n=5)

Group 3, with OVX and receiving 17β estradiol (n=5)

Group 4, with OVX and receiving 17β estradiol and continuous MPA (n=5)

The following medications were administered intra-peritoneally for twenty days: nut oil 1 mL/kg, 17β estradiol 50 micrograms/kg (Sigma, Germany), MPA 2.5 mg/kg (Sigma, Germany). The intra-peritoneal route provided optimization and certainty of the hormone therapy dose. Twenty days later, BMD of experimental animals under general anesthesia were measured by Hologic QDR-4500A and a "small animal" program. Measurements were taken with high resolution in two different regions: the left extremity distal femoral diaphysis and the lumbar vertebrae. Intracardiac perfusion was applied to the rats under general anesthesia. Following a thoracic incision, a 20G catheter was inserted into the left ventricle, and a 10% formaldehyde fixative was given at a rate of 1 cc/sec/g into the systemic circulation. Following the perfusion procedure, the left femurs of the animals were dissected and kept at room temperature in a 10% formaldehyde fixative for 24 hours for histomorphometric analysis. Following fixation, specimens were placed in 10% formic acid. After decalcification was completed within 7 days, they were taken into routine light microscope follow-up. From the prepared paraffin blocks, transverse sections were obtained in 3-micron thicknesses with a Leica MR 2145 microtome. For morphometric analysis, hematoxylenen-eosin dyed preparations were used (15). Finally, one drop of entellan was added to preparations dried at room temperature and kept at 37°C for at least ten days to dry.

Morphometric analyses

For morphometric analyses, five sections were serially obtained from the left hind extremity distal femoral metaphysis in the paraffin blocks of all animals included in the study. Sections were dyed with hematoxylene-eosin, and digital pictures were taken by 10x objective zoom using an Olympus microscope. The semi-automatic digital system UTHSCSA Image Tool for Windows Version 1.28 was used to measure cortex thickness, trabecular count, trabecular thickness and trabecular area. Osteoblast and osteoclast counts were obtained with 40x magnification 0.5 mm from the epiphysis plaque (16). Trabecular measurements were obtained from the distal 0.46 mm of the epiphysis plaque and equal distances from both sides of the cortex in femur preparations (17). The lengths were calculated as pixels with the aid of a program (1 pixel=128x10-8 mm). All measurements were taken in accordance with the article by Parfit et al. (17).

Morphometric measurements

For trabecular thickness (μm), measurements were taken at a minimum of fifty different points for every trabecula, and measurements continued to be taken until the mean values became constant (17, 18). The trabecular count was obtained by counting all trabeculae and each trabecula parallel to each

other at 0.46 mm distal to the epiphysis plaque at equal distances from both sides of the cortex (16, 17). The trabecular area (mm^2) was calculated by determining the borders of the trabeculae in the region where the trabecular count was determined (17-19). Cortical thickness (μm) was calculated by mean values of fifty measurements from 3-micron sections in digital pictures of each preparation (17-19). Osteoblast and osteoclast counts were calculated in hematoxylene-eosin dyed preparations with 40x objective zoomed digital pictures using an image analysis program and counting cells around trabeculae 0.5 mm under the epiphysis plaque (17-19).

Statistical analyses

All statistical analyses were performed using the Microsoft SPSS 11.0 Windows program. Data were expressed as mean±standard deviation and were analyzed by the Chi-square test, the Bonferroni test and the Duncan test. A p-value of <0.05 was considered significant.

Results

There was no significant difference among the groups according to lumbar and femoral BMD values (p>0.05). The mean BMD values are shown in Table 1.

When the trabecular count was compared between the control groups (group 1, which is the group with no OVX and no hormone therapy and group 2, which is the group with OVX and no hormone therapy), it was found to have decreased significantly in the OVX control group without hormone therapy (group 2) (p=0.008). When the trabecular count was compared among the hormone replacement groups (group 3, which is the group with OVX and receiving 17β estradiol and group 4, which is the group with OVX and receiving 17β estradiol and continuous MPA) and in the OVX control group without hormone therapy (group 2), the trabecular count was significantly higher in hormone treatment groups (p<0.001, p=0.008 for groups 3 and 4; respectively). When the trabecular area was compared between the control groups, it was significantly higher in the control group with no OVX (group 1) than the control group with OVX (group 2) (p<0.001). Additionally, the trabecular area was significantly lower in the control group with OVX (group 2) when compared to the hormone treatment groups (p<0.001, p<0.001 for groups 3 and 4; respectively). The distribution of the trabecular structures among the groups is demonstrated in Figure 1.

The trabecular thickness was significantly higher in the control group with no OVX (Group 1) when compared to all other groups (p<0.001). With regard to trabecular thickness, there was no significant difference among the OVX groups. The cortical thickness was significantly higher in the control group with no OVX (Group 1) than in all other groups (p<0.001), whereas there was no significant difference with respect to cortical thickness among the OVX groups (p>0.05). The results of the morphometric analyses are shown in Table 2 and Figure 2.

According to the morphometric measurements, the osteoblast count was significantly higher in the control group with no OVX (Group 1) than in all of the OVX groups (p<0.05). When the osteoblast count was compared among the OVX groups, it was found to be significantly higher in groups 3 and 4 (hormone treatment groups) than in group 2 (control group with OVX) (p<0.01). The osteoclast count was also significantly higher in the control group with no OVX (Group 1) than in the OVX HRT groups, whereas there was no significant difference with respect to the osteoclast count between the control groups with or without OVX (p>0.05). However, when the osteoblast count was evaluated, the osteoclast count ratio was found to be significantly lower in the OVX control groups than in all other groups. In addition, there was no significant difference with respect to this ratio between the intact control group and the OVX HRT groups. The morphometric measurements are provided in Figure 3.

Discussion

In the present animal experiment, we found that in ovariectomized rats, bone microarchitecture, which was assessed with

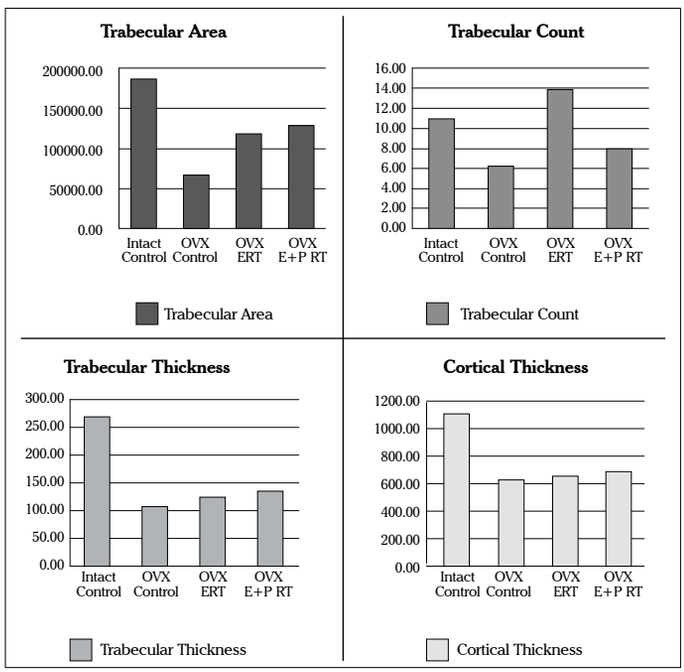

Figure 1. Histological appearance of trabecular structures under Olympus microscope (hematoxylene-eosin, x10). Intact control: control group with no OVX and no hormone therapy, OVX control: control group with OVX and no hormone therapy (nut oil as placebo), OVX ERT: with OVX and receiving 17β estradiol, OVX E+PRT: with OVX and receiving 17β estradiol and continuous MPA

Table 1. Lumbar and femoral BMD values of study groups (mean±SD)

	Intact control	OVX control	OVX ERT	OVX E+P RT
Lumbar BMD	0.15±0.03	0.12±0.03	0.15±0.02	0.14±0.01
Femoral BMD	0.21±0.06	0.19±0.04	0.22±0.02	0.24±0.08

Table 2. Results of morphometric analyses of study groups (mean±SD)

	Intact control	OVX control	OVX ERT	OVX E+P RT
Trabecular count	11.13±1.4	6.28±1.34	14.0±2.3	8.04±1.26
Trabecular thickness	272.66±29.65	110.64±18.79	127.10±5.41	135.58±3.15
Trabecular area	186731.2±5026.1	67367.8±2106.3	121156.8±5627.8	129912.8±6062.9
Cortex thickness	1104.9±202.7	622.6±85.44	667.2±69.87	693.5±51.39

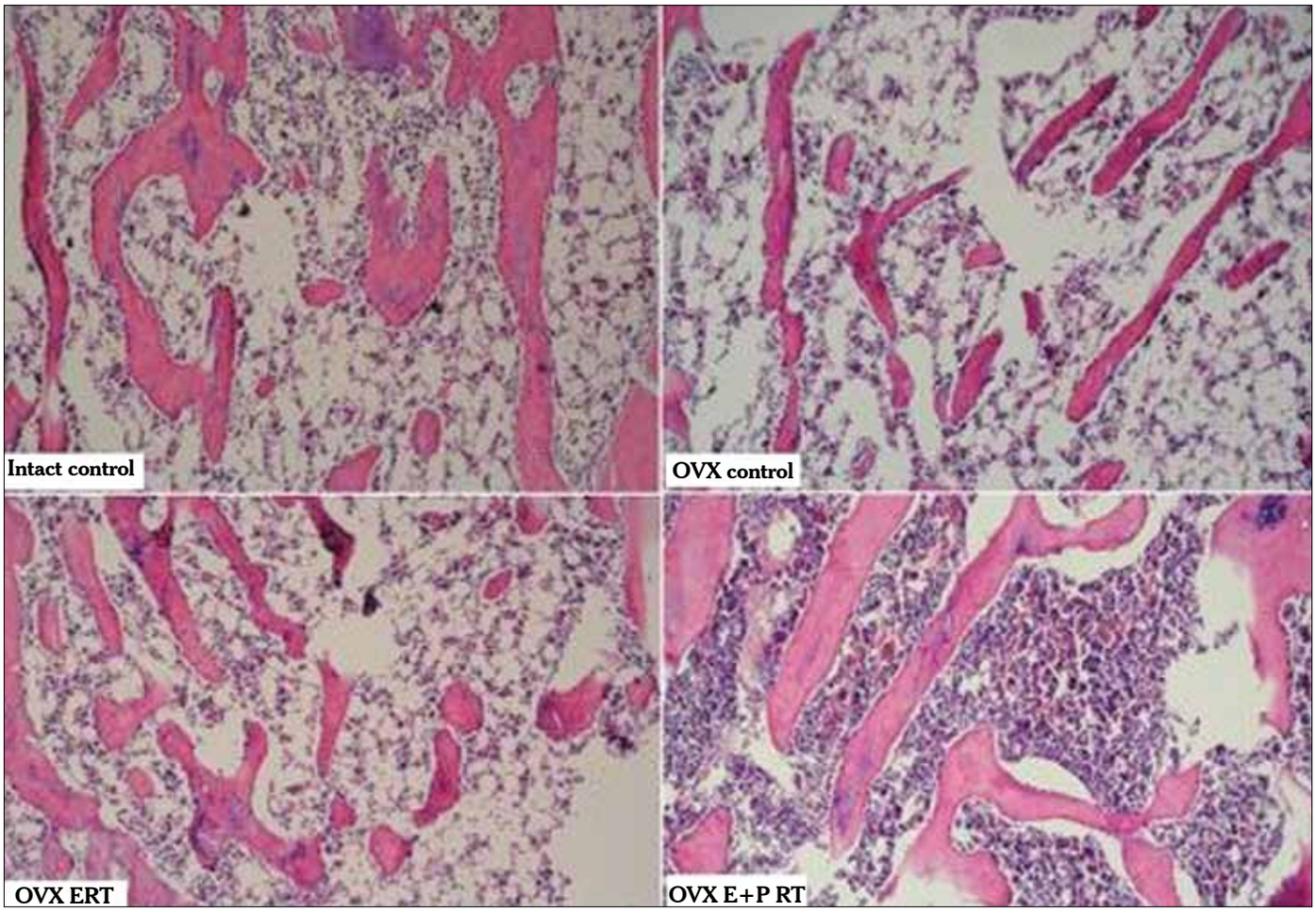

Figure 2. Results of morphometric analyses as mean values. Intact control: control group with no OVX and no hormone therapy, OVX control: control group with OVX and no hormone therapy (nut oil as placebo), OVX ERT: with OVX and receiving 17β estradiol, OVX E+PRT: with OVX and receiving 17β estradiol and continuous MPA

morphometric studies, deteriorates before impairment of BMD and this deterioration of bone microarchitecture was corrected with hormone therapy (17β estradiol with or without MPA).

Osteoporosis is a disease with significant medical and socioeconomic costs. It is characterized by an increase in the tendency for fragility fractures and an enhanced risk of other complications, such as pneumonia or thromboembolic disease due to prolonged hospitalization. Prolongation of life has made osteoporosis an important health problem (2, 20). Macroscopically, there are two types of bones in the human body skeleton: cortical bone, which constitutes 80%, and trabecular bone (cancellous). The cancellous to cortical bone ratio is approximately 50:50 in the femoral head (21). When bone loss starts due to menopause, aging, etc., cancellous bone is affected earlier than cortical bone. Osteoporosis is described as a reduction in bone mass associated with impaired bone architecture, disruption of trabecular continuity by trabecular perforation, increased bone fragility, increased fracture risk, and thinning and increased porosity of the cortices with the conversion of normal plate-like trabeculae into thinner rod-like structures (3). These changes are the result of the combination of increased osteoclastic activity and reduced osteoblast function that characterizes postmenopausal osteoporosis. The view of affected trabecular bone can be described as stair steps that have decreased in size or been lost (1).

In the present study, the intact control group's mean femoral histomorphometric parameters, such as trabecular count, tra-

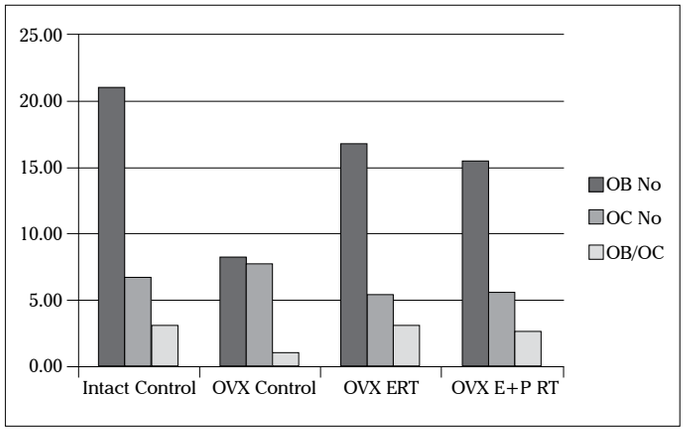

Figure 3. Results of morphometric measurements as mean values. Osteoblast and osteoclast counts were calculated using an image analysis program. Intact control: control group with no OVX and no hormone therapy, OVX control: control group with OVX and no hormone therapy (nut oil as placebo), OVX ERT: with OVX and receiving 17β estradiol, OVX E+PRT: with OVX and receiving 17β estradiol and continuous MPA. OB no: osteoblast count, OC no: osteoclast count,OB/OC:osteoblast:osteoclast count ratio

becular thickness, trabecular area, and cortex thickness, were found to be significantly superior to the OVX control group. In addition, morphometric measurements, such as the osteoblast and osteoclast count, in groups 1 and 2 support an impaired microarchitecture in the OVX rat models. In their study, Bagi et al. (22) investigated the effect of oophorectomy on bone mass and endurance. The bone masses of the femoral neck of rats were evaluated by Dual Energy X-Ray Absorptiometry (DEXA) and histomorphometric parameters using various dynamic and static methods. They determined that the muscular and capsular structures of pelvic articular cartilages in rats resembled those of humans. While endocortical and cancellous bone restructuring following oophorectomy was particularly affected, the ovariectomized group was observed to have a significant decrease in trabecular count, cortical thickness and bone endurance compared with the control group. We found no correlation between impaired bone microarchitecture and femoral BMD. Similarly, in recent years, bone microarchitecture has been increasingly used to determine bone loss or fracture risk when compared to BMD. Ladinsky et al. (23) reported that structural measures obtained at the distal radius in vivo by magnetic resonance imaging (MRI) explained a significant portion of the variation in the total spinal deformity burden in postmenopausal women independent of area BMD. The National Osteoporosis Risk Assessment (NORA) study found that more than one half of women who sustained osteoporotic fractures had BMD T-scores ≥-2.0, and a significant portion had BMD levels in a range considered to be normal (24). New specialized techniques have been developed, such as high-resolution CT (hrCT), micro CT, high-resolution MR (hrMR) and microMR, that are able to provide structural information about local and systemic skeletal health, the propensity to fracture and the pathophysiology of bone fragility. While quantitative assessment of bone macrostructure can be obtained by DEXA and CT, assessment of trabecular bone microstructure may be obtained by hrCT, microCT, hrMR, and microMR (1). The relationship between osteoporosis and postmenopausal hormone replacement therapy has been widely studied. Several studies from the 1970s reported that treatment with estrogen alone or estrogen plus progestogen at the time of menopause prevented accelerated bone loss (9, 10). According to a meta-analysis published in 2002, preparations of estrogen with or without progestogen were significantly more effective than placebo in preserving and increasing BMD (11). Several follow-up studies demonstrated that discontinuation of estrogen therapy caused bone loss similar to that seen in early menopause (24). Early menopause and ovariectomy before age 45 are associated with a lower BMD and a higher osteoporotic fracture rate (25).

Data on the effect of estrogen on bone are inconsistent and range from preserving the existing bone structure (26, 27) to having a strong anabolic effect (19, 28). Lindsay et al. (29) reported that the effect of progesterone on bone is unclear. In the present study, two parameters reflecting trabecular bone microarchitecture, which include the trabecular count and trabecular area, demonstrated significant improvement in the hormone replacement group when compared to the ovariectomized control group. There was no significant difference between the two groups with respect to other parameters including trabecular and cortical thickness. In addition, there was no significant difference between treatment with estrogen with or without progesterone. Although the action of osteoprotective estrogen remains unclear, it has been suggested that, during estrogen deficiency, bone remodeling is impaired because of an increase in some cytokines, such as TNF-a, IL-1, IL-6, and IL-8. This indirect effect leads to bone resorption through stimulation of osteoclastogenesis (30). In the present study, we found that impaired bone microarchitecture and an imbalance between osteoblasts and osteoclasts in the OVX rats were improved by HRT independent of BMD. This finding suggests that the effect of estrogen deficiency on bone starts in the early period of menopause and that HRT reverts these changes. In a novel study, Komm et al. (31) examined the effect of daily treatment with bazedoxifene, conjugated estrogens, or both treatments combined on bone mass, bone architecture, bone strength, and the biochemical markers of bone turnover in ovariectomized rats over the course of 12 months. The investigators reported that treatment with conjugated estrogens alone or in combination with bazedoxifene completely prevented the ovariectomized-induced loss of BMD at the lumbar spine and proximal femur (31). Batukan et al. (32) found that estrogen in combination with simvastatin increased the BMD of proximal femur and lumbal vertebra effectively in rats. In addition, the WHI studies have demonstrated that estrogen with or without progestogen can prevent hip and vertebral fractures in an unselected population of women (level of evidence: A) (25).

Taking into account the duration of treatment in the present study, the main finding of this study is that the bone microarchitecture was improved in the HRT group without loss of BMD.

This study had some limitations. First, the number of rats in each study group was considerably small. We kept the total number of rats low for ethical reasons. Second, biochemical markers of bone metabolism could not be measured due to technical inadequacy in our hospital. In spite of these limitations, this animal study showed that hormone therapy produces

improvement on bone microarchitecture before its known effect on imparired bone mineral density.

Conclusion

We found that HRT corrects impaired bone microarcitecture which develops before impairement of BMD in a rat model with surgically induced early menopause. Therefore, estrogen alone or in combination with progestogen can be a beneficial approach to preventing early postmenopausal bone loss.

Conflict of interest

No conflict of interest was declared by the authors.

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Medicine in stamps: history of Down syndrome through philately

Pullardaki tıp: filateli aracılığıyla Down sendromu tarihi

Ahmet Doğan Ataman[1], Emine Elif Vatanoğlu-Lutz[2], Gazi Yıldırım[3]

[1]Department of History of Medicine, Vienna Medical Faculty, Vienna, Austria

[2]Department of Medical History and Ethics Medical Faculty, Yeditepe University, İstanbul, Turkey

[3]Department of Gynecology and Obstetrics Medical Faculty, Yeditepe University, İstanbul, Turkey

Abstract

Down syndrome (DS) is one of the most common chromosomal disorders with mental retardation and some spesific physical and physiological defects. Recently, many advances have been made in pre-natal screening and detection; and the hope is that identification of more genes will lead to a better understanding of the molecular mechanisms underlying the pathologies, and hence to more effective therapy. This paper provides an overview on the discovery of Down syndrome through philately.

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Özet

Down sendromu zihinsel gelişme geriliği ve kendine özgü fiziksel ve fizyolojik defektlerle seyreden, en sık rastlanan kromozom hastalıklarından biridir. Son dönemlerde hastalığın prenatal tarama ve tanısında birçok ilerlemeler kaydedilmiştir. Genlerin ve gen patolojilerinin altında yatan moleküler mekanizmaların daha iyi anlaşılaması daha etkili tedavi yöntemleri konusunda umut vermektedir. Bu çalışma, Down sendromunun tarihine filateli yoluyla ışık tutmaktadır.

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Anahtar kelimeler: Down sendromu, zihinsel gerilik, genetik, tarih, filateli

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Introduction

Down syndrome (DS), also known as trisomy 21, is caused by the presence of all or part of a third copy of chromosome 21 (Figure 1a, b). It is named after John Langdon Down, the British physician who described the syndrome for the first time in 1866. In 1866 he wrote a paper entitled “Observations on the Ethnic Classification of Idiots” in which he put forward the theory that it was possible to classify different types of conditions by ethnic characteristics. He listed several types including the Ethiopian type. He is most famous for his classification of what is known as Down syndrome, named after him, but which he classified as the Mongolian type of Idiot. As a result, Down syndrome was also known as “Mongolism” and people with Down syndrome referred to as “Mongoloids” but the use of the word ‘mongolism’ is now stopped after having so many criticisms about referring a racist title. Thus down syndrome occurs in all human populations, and analogous conditions have been found in other species such as chimpanzees (1).

The chromosome aberration was discovered in 1959 by the French human geneticist Jérôme Jean Louis Marie Lejeune (1926-1994). Dr. Jérôme Lejeune discovered that Down syndrome was caused by an extra chromosome on the 21st pair while working in Raymond Turpin’s laboratory In 1958. The French Academy of Sciences published his scientific work on January 26, 1959. For the first time in world history, his discovery established a link between an intellectual disability and a chromosomal abnormality. After this discovery, an enormous field of investigation was opened up for modern genetics and a new discipline was founded: cytogenetics. Until then, the knowledge about human heredity had been unable to explain Trisomy 21 and other anomalies in hereditary material (2).

Nowadays, down syndrome can be identified in a baby at birth, or by prenatal screening.

Genetic Background

Down syndrome is a complex set of pathologies caused by an extra copy of human chromosome 21 (Hsa21). DS occurs in about one in 750 live births and is the most frequent cause of learning difficulties (Figure 2). The underlying genetic cause, trisomy Hsa21, is the same in most individuals with DS, but the penetrance of the resulting pathologies (3).

Genes on an extra copy of chromosome 21 are responsible for all characteristics associated with Down syndrome. Normally, each human cell contains 23 pairs of different chromosomes. Each chromosome carries genes, which are

Address for Correspondence: *Emine Elif Vatanoğlu-Lutz, İnönü Mah. Kayışdağı Cd. 26 Ağustos Yerleşkesi Kayışdağı 34755 İstanbul, Turkey*
Phone: +90 216 578 00 00/3078 e.mail: drvatanoglu@yahoo.com

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Figure 1. a) Stamp issued in Romania in 2011, for the 21st of March Down Syndrome Day; b) First Day Cover from Romania in 2011, for the 21st of March Down Syndrome Day

Figure 2. Stamp issued in 1981 by Netherlands Antilles, in support of handicapped children, for the International Year of the Disabled

needed for proper development and maintenance of our bodies. At conception, an individual inherits 23 chromosomes from the mother (through the egg cell) and 23 chromosomes from the father (through the sperm cell) (3).

However, sometimes a person inherits an extra chromosome from one of the parents. In Down syndrome, an individual most often inherits two copies of chromosome 21 from the mother and one chromosome 21 from the father for a total of three chromosomes 21 (Figure 3). Because Down syndrome is caused by the inheritance of three chromosomes 21, the disorder is also called trisomy 21. About 95% of individuals with Down syndrome inherit an entire extra chromosome 21 (4).

Approximately 3% to 4% of individuals with Down syndrome do not inherit an entire extra chromosome 21, but just some extra chromosome 21 genes, which are attached to another chromosome (usually chromosome 14). This is called a *translocation*. Most of the time, translocations are random events during conception (Figure 4). In some instances however, a parent is a balanced carrier of a translocation: The parent has exactly two copies of chromosome 21, but some of the genes are distributed to another chromosome. If a baby inherits the chromosome with the extra genes from chromosome 21, then the child will have Down syndrome (two chromosomes 21 plus extra chromosome 21 genes attached to another chromosome) (3, 4).

Figure 3. A stamp issued in Denmark in 2002

Figure 4. A special cancellation from Luxembourg in 2003, emphasising Trisomie 21

Figure 5. A special cancellation from Tahiti in 2007, emphasising Trisomie 21

About 2% to 4% of people with Down syndrome inherit additional genes from chromosome 21, but not in every cell of the body. This is known as *mosaic Down syndrome*. These individuals may, for example, have inherited extra genes from chromosome 21 in their muscle cells, but not in any other type of cell. Because the percentage of cells with extra genes from chromosome 21 varies in people with mosaic Down syndrome, they often don't have all the typical physical characteristics and may not be as severely intellectually impaired as people with full trisomy 21 (Figure 5). Sometimes, mosaic Down syndrome is so mild that it will go undetected. On the other hand, mosaic Down syndrome can also be misdiagnosed as trisomy 21, if no genetic testing has been done (3).

Signs and Symptoms

Most individuals with DS have memory and learning difficulties, craniofacial alterations and muscle hypotonia, but only some have congenital heart malformations, leukaemia or gut abnormalities. The severity of the defects is variable. For example, the extent of cognitive impairment varies widely between individuals with DS (5).

The signs and symptoms of Down syndrome are characterized by the neotenization of the brain and body to the fetal state. Down syndrome is characterized by decelerated maturation (neoteny), incomplete morphogenesis (vestigia) and atavisms. Individuals with Down syndrome may have some or all of the following physical characteristics: microgenia (abnormally small chin), oblique eye fissures with epicanthic skin folds on the inner corner of the eyes (formerly known as a mongoloid fold), muscle hypotonia (poor muscle tone), a flat nasal bridge, a single palmar fold, a protruding tongue (due to small oral cavity, and an enlarged tongue near the tonsils) or macroglossia, "face is flat and broad", a short neck, white spots on the iris known as Brushfield spots, excessive joint laxity including atlanto-axial instability, excessive space between large toe and second toe, a single flexion furrow of the fifth finger, a higher number of ulnar loop dermatoglyphs and short fingers (5).

Growth parameters such as height, weight, and head circumference are smaller in children with DS than with typical individuals of the same age. Adults with DS tend to have short stature and bowed legs. Individuals with DS are also at increased risk for obesity as they age (5).

Conclusion

Many children with Down syndrome who have received family support, special therapies and education manage to graduate from high school and are able to do paid work, and some participate in post-secondary education as well. Early childhood intervention, screening for common problems, medical treatment where indicated, a conducive family environment, and vocational training can improve the overall development of children with Down syndrome. As individuals with DS continue to experience longer lives, the need to understand their aging and associated health conditions becomes more critical. Education and proper care will improve quality of life significantly, despite genetic limitations. Especially adults with DS should be provided with appropriate information to better understand, and counseling to cope with, changes in their own level of ability or health.

Conflict of interest

No conflict of interest was declared by the authors.

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Clinical value of DNA fragmentation evaluation tests under ART treatments

Yardımcı Üreme Teknikleri tedavilerinde sperm DNA fragmentasyonu değerlendirilmesinin klinik etkileri

İlkay Şafak Tavukçuoğlu, Tahani Al-Azawi, Amir Afshin Khaki, Arash Khaki, Ahmed Khalil, Safaa Al-Hasani

Reproductive Medicine Unit, University of Schleswig-Holstein, Luebeck, Germany

Abstract

Male reproductive health has been under scrutiny recently. Many studies in the literature have concluded that semen quality is declining and that the incidence of testicular cancers is increasing. The reason for this change has been attributed to damage in sperm chromatin. During in vivo reproduction, the natural selection process ensures that only a spermatozoon with normal genomic material can fertilize an oocyte. However, the assisted reproduction technique (ART) is our selection process, leading to the possibility that abnormal spermatozoa could be used to fertilize an oocyte. We could avoid this by quantifying the amount and type of genomic damage in sperm using well-accepted laboratory methods. The sperm deoxyribonucleic acid (DNA) integrity is important for success of natural or assisted fertilization as well as normal development of the embryo, fetus and child. Intra cytoplasmic sperm injection (ICSI) is bypassing natural sperm selection mechanisms, which increases the risk of transmitting damaged DNA. The significance of required investigations and multiple techniques is that they could evaluate DNA defects in human spermatozoa. The ability of these techniques to accurately estimate sperm DNA damage depends on many technical and biological aspects. The aim of this review is to evaluate the most commonly used methods.

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Key words: Tunel, Comet, Acridine Orange staining technique (AOT), Sperm Chromatin Dispersion (SCD), Sperm Chromatin structure (SCSA)

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Özet

Erkek üreme sağlığıyla ilgili son zamanlardaki çalışmalar semen kalitesi ve sıklığı artan testiküler kanser vakaları üzerinde yoğunlaşmaktadır. Normal üreme şartlarında spermlerin doğal seleksiyonu söz konusu olmakta ve normal genetik yapıdaki spermlerle oositlerin fertilizasyonu gerçekleşmektedir. Ancak yardımcı üreme teknikleri ile gerçekleştirilen tedavilerde sperm seçimi semen kalitesine bağlı olarak da anormal spermlerle geçekleştirilebilmektedir. Bu durumlarda kabul görmüş birtakım laboratuvar testleri uygulayarak hasarlı genetik yapıdaki spermlerden korunabilmek mümkün olabilmektedir. Sperm deoksiribonükleik asid (DNA) bütünlüğü; doğal ve yardımcı yöntemlerle fertilizasyonun başarısı aynı zamanda embriyo, fetüs ve çocuğun normal gelişimi için önemlidir. İntrasitoplazmik sperm enjeksiyonunda (ICSI) doğal sperm seçim mekanizmaları devre dışı kalmakta, bu da hasarlı DNA'nın transfer riskini artırmaktadır. Bu derlemede sperm DNA fragmantasyonu oranı belirlemede en sık kullanılan testler değerlendirilmektedir. (J Turkish-German Gynecol Assoc 2012; 13: 270-4)

Anahtar kelimeler: Tunel, Comet, Acridine orange boyama tekniği (AOT), sperm kromatin dağılımı testi (SCD), sperm kromatin yapı testi (SCSA)

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Introduction

Sperm quality is ferquently used as an indirect measure of male infertility. The parameters that have been used historically as indicators of male infertility potential include sperm count, motility and morphology, all of which are evaluated in fertility clinics as a part of routine semen analyses (1). Assisted reproductive techniques such as conventional in vitro fertilization (IVF) and intra cytoplasmic sperm injection (ICSI) allow couples whose sperm parameters are impaired to achieve a pregnancy. Among these factors which are involved in the failure to obtain embryos and pregnancies, the impaired sperm genom is frequently incriminated (2, 3). To assist in the risk assesment of ICSI, it would be appropriate to develop methods to measure deoxyribonucleic acid (DNA) damage in the sperm and to correlate this with biological outcomes. DNA abnormalities in sperm are well documented. Cytogenetic analysis of sperm chromosomes has demonstrated sperm aneuploidy, which, although low in frequency, is assosciated with infertility and adverse pregnancy outcome (4, 5). Several techniques and investigations are proposed in order to study these abnormalities. Those which are currently used are; the Tunel test, which allows the evaluation of the sperm DNA fragmentation (6, 7), the Comet test, which represents another way of evaluating the DNA integrity (8, 9) and DNA staining by acridine orange (AO), which differenti-

Address for Correspondence: *Safaa Al-hasani, Reproductive Medicine Unit, University Of Schleswig-Holstein, Luebeck, Germany*
Phone: +4945150002145 e.mail: sf_alhasani@hotmail.com

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ates between single and double stranded DNA based on their reactive colors under fluorescence and thus allows the degree of DNA denaturation to be evaluated (3). Other tests identify the packaging defects of sperm chromatin: aniline blue staining, toluidine blue staining, and chromomycin A3 staining (10).

Sperm DNA and Abnormalities

Deoxyribonucleic acid of sperm is organized in a special way that keeps the nuclear chromatin compact and stable (11). This DNA not only permits the tightly packaged genetic information to be transferred to the oocyte but also ensures that the DNA is delivered in a physical and chemical form that allows the developing embryo to easily access the genetic knowledge. Fertile and normal sperm have stable DNA, which is able to undergo decondensation at the same time in the fertilization process and transmit the DNA without defects.

Defective genomic material in sperm may cause the formationof condensation or nuclear maturity defects, DNA breaks, DNA integrity defects, or sperm chromosomal aneuploidies (12). The causes of these defects have been attributed to disease, drug use, high fever, more than normal testicular temperature, smoking, and advanced age. DNA damage's molecular mechanism in these different conditions is under intense investigation. The most important mechanisms for sperm DNA damage are abnormal chromatin packaging, reactive oxygen species (ROS) (13), and apoptosis (14, 15). It is likely that multiple mechanisms are involved, based on the clinical diagnosis responsible for DNA damage.

Comet Assay

Comet assay uses single cell gel electrophoresis (SCGE) to analyze DNA fragmentation in individual cells, was first introduced in 1984 by Ostling and Johanson (16) who used neutral buffer conditions to study double-stranded DNA breaks (17). This assay is extensively used in somatic cells to measure genotoxic damage, especially single and double strands breaks and was originally applied to sperm by Singh (18). The Comet assay may therefore be used to study single or double stranded DNA breaks in somatic cells or germ cells and is useful because it allows for the distinction between the different kinds of DNA fragmentation necrotic and appoptotic cells. Appoptotic cells produce teardrop shape comets during electrophoresis (19). The shape is due to the migration and accumulation of the short DNA fragments and the intensity of the tail represents the amount of DNA fragments present (20).

Tunel Test

This test was originally described by Garvrieli, Sherman, and Ben-Sasson in 1992 (21). Tunel has become one of the main methods for detecting apoptotic programmed cell death. However, there has been a debate about its accuracy, due to problems in the original assay, which caused necrotic cells to be inappropriately labeled as an apoptosis (22). The method has subsequently been improved dramatically to identify only cells in the last phase of apoptosis (23, 24). New methods incorporate the dUTPs modified by fluorophores or haptens, including biotin or bromine, which can be detected directly in the case of a fluorescently-modified nucleotide (fluorescein-dUTP), or indirectly with streptavidin or antibodies, if biotin-dUTP or BrdUTP are used, respectively.

The TUNEL assay detects both single- and double-stranded DNA breaks by labeling the free 39-OH terminus with modified nucleotides in an enzymatic reaction with terminal deoxynucleotidyl transferase (TdT) and can be analyzed microscopically or by using flow cytometry.

Acridine orange staining technique (AOT), sperm chromatin dispersion (SCD) and sperm chromatin structure (SCSA) tests

The acridine orange staining technique (AOT) is a simple microscopic procedure based on the same principle as the sperm chromatin structure assay (SCSA) but indistinct colours, rapid fading of fluorescence, and heterogeneous staining of slides makes AOT a test of questionable value in clinical practice (25). The SCSA is fluorescence activated cell sorter test, measures the susceptibility of sperm DNA heat or acid induced DNA denaturation in situ followed by staining with acridine orange (26).

Recently, a new method, the sperm chromatin dispersion test (SCD), was introduced for evaluating sperm DNA fragmentation (27-37). The SCD test is based on the principle that sperm with fragmented DNA fails to produce the characteristic halo of dispersed DNA loops that is observed in sperm with non-fragmented DNA following acid denaturation and removal of nuclear proteins.

Evaluation of tests used under ART treatments

Several authors were included to diagnose with the tests results of their differently based researches.

Table 1 shows that some authors had reported a significant relationship between sperm DNA fragmentation index and pregnancy rate (3, 32, 34-37). On the other hand, many others revealed no significant relationship (9, 28-31). However, these controversial results may be attributed to different principles of the techniques of the analytical methods used, as represented in Table 2. Each assay method has their advantages and disadvantages.

Conclusion

Sperm DNA integrity is associated with male infertility potential in vivo and in vitro. There are increased levels of fragmented sperm DNA in a high percentage <40% of men presenting as clinically subfertile. Especially semen with a high percentage of damaged spermatozoa has a very low potential for natural fertility. DNA damage in sperm does not preclude IVF as there is still a chance that samples in which sperm have damaged DNA can be used to achieve a pregnancy. ART studies mentioned that the reproductive parameters that could be affected by the integrity of the DNA in ejaculated spermatozoa include fertilization, blastocyst development and pregnancy rates. In fact, pregnancy rates using conventional IVF and ICSI treatments are significantly reduced in couples with a high percentage of sperm with DNA damage.

All literature shows that sperm DNA damage influences the fertility outcome to different degrees, but there is no consensus

Table 1. The statistical relationship between sperm DNA fragmentation index (DFI %) and pregnancy rate with different analytical tests under ART treatments as reported by some authors

Authors	DFI %	ART Procedure	Patient #	Statistical Results	Analysis
Chohan et al. (28)	<30	IVF or ICSI	52	Not Significant	SCSA, Tunel, SCD
Larson et al. (3)	<27	ICSI	21	Significant	SCSA
Check et al. (29)	<30	ICSI	106	Not Significant	SCSA
Morris et al. (9)	Low DNA Damage	IVF or ICSI	52	Not Significant	Comet
Bungum et al. (30)	<27	IVF	109	Not Significant	SCSA
Bungum et al. (30)	>27	IVF-ICSI	66	Not Significant	SCSA
Larson-Cook et al. (31)	<27	IVF	55	Not Significant	SCSA
Larson-Cook et al. (31)	<27	ICSI	26	Not Significant	SCSA
Virro et al. (32)	<30	IVF	249	Significant	SCSA
Spano et al. (33)	<30	In-vivo	215	No Result	SCSA
Everson et al. (34)	<30	In-vivo	147	Significant	SCSA
Henkel et al. (35)	<36.5	IVF	208	Significant	Tunel
Henkel et al. (36)	<36.5	IVF	167	Significant	Tunel
Caglar et al. (37)	> 4	ICSI	56	Significant	Comet
Caglar et al. (37)	> 4	ICSI	56	Significant	Tunel

DFI: Deoxyribonucleic acid Fragmentation Index, ART: Assisted Reproductive Techniques, SCSA: Sperm chromatin structure assay, SCD: Sperm chromatin dispersion test, IVF: In vitro fertilization, ICSI: Intra Cytoplasmic Sperm Injection, DNA: Deoxyribonucleic acid

Table 2. Evaluation of different analytical tests (principles, detection method, advantages and disadvantages) used in ART treatments

Assay	Principle	Detection method	Adventages	Disadvantages
Tunel	Single & double strand DNA breaks	Fluorescence microscopy, Flow cytometry	Clinically significant high sensivity and specificity large number of spermatozoa counted by flow cytometry	Special equipment, more expensive
Comet	Single & double strand DNA breaks or only double strand DNA breaks	Fluorescence microscopy	Related to Tunel assay, cheap, high sensitivity qualification of DNA damage in individual cells, evaluation of different type of DNA damage	Special equipment and experienced observer
Acridine Orange	Differentiates between single & double stranded DNA	Fluorescence microscopy	Easy to perform, cheap	Special equipment distiction between differenty labelled spermatozoa, not always easy
Sperm Cromatin Decondensation	Evaluation of DNA decondensation halo	Fluorescence microscopy, Optical microscopy	Easy to perform, cheap	Clinical relevance not yet proven
Sperm cromatin stracture assay	Susceptibility of DNA to acid denaturation	Flow cytometry	Clinically significant high sensivity and specificity large number of spermatozoa counted by flow cytometry, unbiased quantitative assessment of DNA bound acridine orange	Special equipment, more expensive

DNA: Deoxyribonucleic acid

on the technique that should be used to measure sperm DNA in subfertile patients. The methods used to detect sperm DNA damage should be standardized to allow comparison among different studies and to permit routine use of tests in clinical laboratories. The results of degrees of DNA damage could give better decision facilitation to physicians on infertile couples about their chances of having a live birth. New researchs aim to identify the type of DNA defects that affect fertility regardless of the quantity of damaged DNA and to identify and isolate spermatozoa with intact DNA for ART. The TUNEL, AOT, and SCD are simple, less expensive procedures and can be performed in a short period of time to assess the levels of DNA fragmentation in sperm from infertile men and donors of known fertility.

Conflict of interest

No conflict of interest was declared by the authors.

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A rare cause of virilization; Ovarian steroid cell tumor, not otherwise specified (NOS)

Nadir rastlanılan virilizasyon sebebi; overyan steroid hücreli tümor

Nicel Taşdemir[1], Cem Çelik[1], Remzi Abalı[1], Erson Aksu[1], Meltem Öznur[3], Murat Yılmaz[2]

[1]Department of Gynecology and Obstetrics, Faculty of Medicine, Namik Kemal University, Tekirdağ, Turkey

[2]Department of Endocrinology and Metabolism, Faculty of Medicine, Namik Kemal University, Tekirdağ, Turkey

[3]Department of Pathology, Faculty of Medicine, Namik Kemal University, Tekirdağ, Turkey

Abstract

Sex cord–stromal tumors account for 5% of ovarian tumors and 2% of malignant ovarian tumors. Steroid cell tumors (SCT), not otherwise specified (NOS), are rare sex cord–stromal tumors of the ovary and account for less than 0.1% of all ovarian tumors. We report a rare case of a postmenopausal woman presented with hirsutism, virilism and with findings of hyperestrogenism. (J Turkish-German Gynecol Assoc 2012; 13: 275-7)

Key words: Hirsutism, steroid cell tumor, virilization, hyperandrogenism, sex cord-stromal tumor

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Özet

Sex-cord hücreli tümörler, tüm over tümörlerinin %5 'ini, malign over tümörlerinin %2'sini oluşturmaktadır. Steroid hücreli tümörler nadir sex kord –stromal tümörler olup, tüm over tümörlerinin %0.1'ini oluşturmaktadır. Biz hirsutizm, virilizasyon ve hiperöströjenizm bulguları mevcut postmenapozal bir bayan olgusunu sunmak istedik.

Anahtar kelimeler: Steroid hücreli tümör, virilizasyon, hirsutizm, hiperandrojenizm, sex cord- stromal tümör

(J Turkish-German Gynecol Assoc 2012; 13: 275-7)

Geliş Tarihi: 01 Nisan 2012 **Kabul Tarihi:** 23 Haziran 2012

Introduction

Sex cord-stromal tumors account for 5% of ovarian tumors and 2% of malignant ovarian tumors (1, 2). SCTs account for less than 0.1% of all ovarian tumors (3). The terms 'lipid cell tumor' and 'lipoid cell tumor' have been used to designate a group of morphologically similar ovarian neoplasms of diverse cellular origin. These tumors are composed exclusively of cells-i.e., lutein cells, leydig cells, and adrenal cortical cells. The use of the above terms is misleading, however, as some tumors in this category contain little or no lipid. In view of this inaccuracy, the term 'steroid cell tumor' is proposed for these neoplasms, which can be divided into several subtypes according to their cells of origin. The designation 'steroid cell tumor' is appropriate not only because of the morphological features of the neoplastic cells, but also because of their propensity to secrete a variety of steroid hormones that often produce characteristic clinical syndromes (3). We report a rare case of postmenopausal woman presenting with hirsutism, virilism and also findings of hyperestrogenism.

Case Reports

A 51-year-old, gravida 5, para 4, abortus 1 woman (age of onset of menopause 42 years) presented with rapidly progressing hirsutism, receding hairline, male-pattern baldness, alopecia and voice deepening (Figure 1). She was diagnosed with hypertension for six years and diabetes mellitus for seven years. She had had a laparoscopic cholecyctectomy operation 3 years earlier and she was diagnosed for depression 2 years previously. Physical examination revealed hirsutism involving the face, chin, upper back, chest, upper and the lower abdomen giving a score of 44 from modified Ferriman and Gallwey scoring system (Figure 2). Gynecologic examination revealed cliteromegaly which had developed over the past 6 years.

Pelvic ultrasound-scan revealed a solid ovarian tumor of 35x36 mm in the left ovary. Markedly elevated serum testosterone level (8.3 ng/mL), elevated serum estradiol level (85.86 pg/mL) and suppressed gonadotrophin levels (FSH: 0.606 mIU/mL, LH<0.01 mIU/mL) were observed. Dehydroepiandrosteronesulfate level was normal (131.4 μg/dL). The levels of tumor markers were normal. Computed tomography and magnetic resonance imaging of the abdomen and pelvis revealed a 40x25mm solid tumor in the left adnexia. Adrenal glands were normal. The patient underwent total abdominal hysterectomy bilateral salpingo-oophorectomy. Frozen section of the left ovary revealed thecoma. Gross pathological examination of the left ovary revealed an 3x2.2 cm well-circumscribed, yellow-orange mass. In the uterine cavity a 0.6x0.8 mm endometirial polyp was observed.

Address for Correspondence: *Cem Çelik, Department of Gynecology and Obstetrics, Faculty of Medicine, Namik Kemal University, Tekirdağ, Turkey*
Phone: +90 532 420 53 65 e.mail: cemcel@yahoo.com

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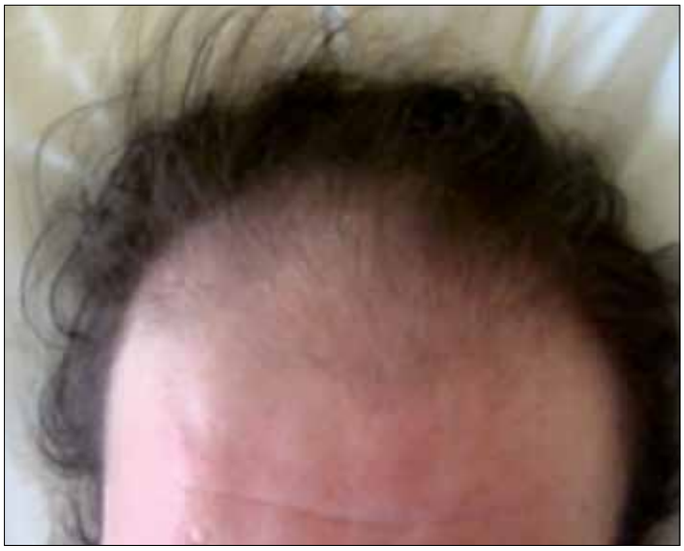

Figure 1. Findings of virilism. Male pattern baldness

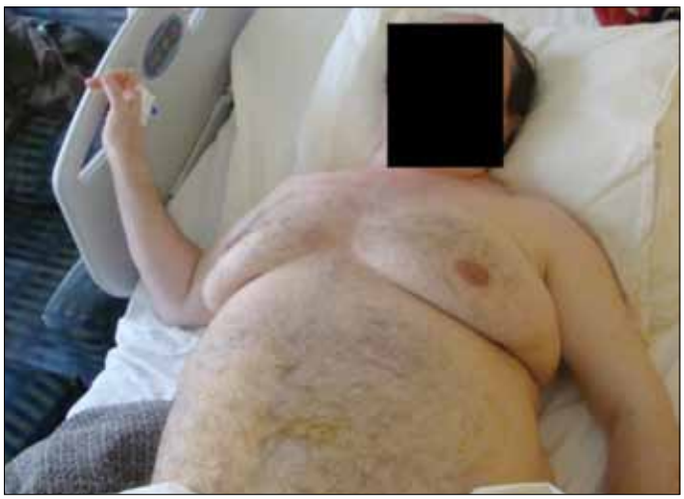

Figure 2. Hirsutism involving the face, chin, upper back, chest, upper and the lower abdomen giving a score of 44 from modified Ferriman and Gallwey scoring system

Microscopic examination revealed steroid cell tumor, not otherwise specified for the mass in the left ovary and endometrial proliferative findings with endometrial polyp for the uterus. Total testosterone level was normal on the postoperative first month.

Discussion

Ovarian SCTs account for 0.1-0.2% of all ovarian tumors, and usually present with the findings of virilization (3, 4). There are three subtypes: stromal luteoma, Leydig-cell tumor, and steroid cell tumor, not otherwise specified (NOS). Steroid cell tumors, NOS, must be distinguished from other tumors in the steroid cell category -luteinized thecomas, pregnancy luteomas and carcinomas, both primary clear cell carcinoma and metastatic renal cell carcinoma. Both the hilus cell tumor and the rare Leydig cell tumor, nonhilar type, can be identified with certainty only by demonstrating the presence of crystals of Reinke in the cytoplasm of the neoplastic cells (5-8). Since testicular Leydig cell tumors lack these inclusions in 60-65% of cases (9, 10), an unknown proportion of tumors in the steroid cell tumor, NOS, category are almost certainly Leydig cell tumors in which crystals have not been identified. The luteinized thecoma can be identified by the presence of a predominant spindle cell background. It is posible, however, that the steroid cell tumor, NOS, is a fully luteinized thecoma (11), since some luteinized thecomas show extensive luteinization (8, 12) and a rare steroid cell tumor, NOS, contains small areas of spindle cell proliferation (13). The focal presence of nonluteinized granulosa cells in a predominantly luteinized granulosa cell tumor helps to distinguish it from a steroid cell tumor. Electron-microscopical examination of the tumor may be of additional help in distinguishing a steroid cell tumor from a clear-cell carcinoma by demonstrating the typical abundant smooth endoplasmic reticulum in the cytoplasm of the neoplastic steroid cells (14).
Steroid cell tumor, not otherwise specified, accounts for 60% of SCTs, 25-45% of which are clinically malignant. This subtype is associated with androgenic changes. SCTs often present as unilateral solid tumors but the size of tumors may be as small as 2-3 cm, thus it would be difficult to diagnose. Clinical and laboratory findings are usually exaggerated according to its dimension. Several medications such as oral contraceptives, cyproteroneacetate and spironolactone were prescribed for the presented case for hirsutism for 2 years. Also laser hair removal treatment was performed. However, symptoms of virilization did not improve. Delayed diagnosis would be important for the tumors with malignant potential. Interestingly, pathologically benign tumors can behave in a clinically malignant fashion.
Estradiol secretion by these tumors is not uncommon (6-23%) (15). The presented case had endometrial hyperplasia and polyp as a result of elevated estradiol level. Excess estrogen production can result in menorrhagia, postmenopausal bleeding and rarely adenocarcinoma. SCTs should be kept in mind for the patients presenting with virilization and high serum androgen level. Meticulous clinical evaluation should be carried out before initiation of medical therapy for such patients.

Conflict of interest

No conflict of interest was declared by the authors.

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Virginity sparing surgery for imperforate hymen: report of two cases and review of literature

İmperfore himende bekaret koruyucu cerrahi, iki olgu ile literatürün gözden geçirilmesi

Osman Temizkan[1], Suna Kabil Kucur[2], Sema Ağar[1], İlay Gözükara[2], Atıf Akyol[1], İnci Davas[1]

[1]Department of Gynecology and Obstetrics, Sisli Etfal Training and Research Hospital, İstanbul, Turkey

[2]Department of Gynecology and Obstetrics, Nene Hatun Gynecology and Obstetrics Hospital, Erzurum, Turkey

Abstract

Imperforate Hymen (IH), an obstructive congenital anomaly of the female genital tract, is seen in 1 in 2000 female births. Treatment of IH is hymenotomy or hymenectomy. Different types of incisions are mentioned in the literature. We reported two cases of IH with different clinical presentations and described a simple virginity preserving and socially acceptable procedure to protect the virginity of the patient. In cultures and religions where the destruction of the hymen is a social problem in unmarried girls, virginity sparing surgery should be chosen in gynecological practice. Here we described a simple procedure without need for prophylactic antibiotic treatment and foley catheter application to form an intact annular hymen in two cases.

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Key words: Virginity, imperforate hymen, genital anomaly, primary amenorrhea, adolescence

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Özet

İmperfore himen (İH), kadın genital sisteminin obtrüktif doğumsal anomalisi olup, 2000 kız doğumda bir sıklıkta görülür. Tedavisi himenotomi veya himenektomidir. Literatürde IH tedavisinde farklı tiplerde cerrahi yöntemler tariflenmiştir. Farklı klinik tablolarla başvurmuş iki İH olgusu ile himen bütünlüğünü koruyucu, sosyal olarak kabul edilebilir basit bir yöntem tariflenmiştir. Bekareti korumanın önemli olduğu kültürlerde İH olgularında himen bütünlüğünün korunabildiği prosedürler seçilmelidir. İki İH olgusuyla antibiyotik profilaksisi ve foley kateter kullanmadan intak anüler bir himen oluşturduğumuz basit bir yöntem tanımladık.

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Anahtar kelimeler: Bekaret, imperfore himen, genital anomali, primer amenore, adolesan

Geliş Tarihi: 02 Nisan 2012 **Kabul Tarihi:** 03 Temmuz 2012

Introduction

Imperforate Hymen (IH), an obstructive congenital anomaly of female genital tract, is seen in 1 in 2000 female births (1). Although it is congenital, in most cases it is diagnosed in adolescence when it prevents passage of blood, causing accumulation of menstrual products in the vagina or uterus. Patients are usually brought to pediatric emergency services with the complaint of cyclic lower abdominal pain, dysuria and, rarely, acute urinary retantion. Treatment is a simple surgical procedure with a few types of incisions on the imperforate hymenal membrane. As it is evidence of virginity, an intact hymen is important in some cultures and religions. Patients and families have fears about loosing virginity after surgical interventions, so the surgeon should choose the technique that provides the best natural annular intact hymenal architecture under those circumstances.

The aim of this case report is to show different presentations of IH and a simple virginity preserving socially acceptable procedure to provide an annular intact hymen.

We report 2 cases of IH admitted to Sisli Etfal Training and Research Hospital Emergency Service.

Case Reports

Case 1

A 15 year old girl was admitted to the pediatric emergency department with severe abdominal pain for 5 hours. She had a history of vague lower abdominal and back pain for the previous 15 days. On physical examination there was no rebound tenderness but suprapubic and bilateral lower abdominal pain. In the complete blood count, white blood cell: 12.5x109/L, hemoglobin: 13.2 g/dL, and hematorit level 38.1%. Urinalyses revealed no specific finding. Physical exam revealed a healthy girl with signs of adrenarche and thelarche. On transabdominal ultrasonography, the vagina and uterine cavity were filled with blood suggesting hematometra and hematocolpos. She was referred to our obstetric and gynecology outpatient clinic. On genital exanination, a bulging imperforate hymen was seen. The patient and her family were informed about the situation. The family stated their desire for conservation of virginity. A small central hymenotomy was performed under general anesthesia and about 700 ml of blood was evacuated. The next day she was discharged from the hospital. She had regular mensruation

Address for Correspondence: *Suna Kabil Kucur, Department of Gynecology and Obstetrics, Nene Hatun Gynecology and Obstetrics Hospital, Erzurum, Turkey*
Phone: +90 532 355 90 47 e.mail: dr.suna@hotmail.com

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at follow up for 8 months. Now she has an intact annular hymen (Figure 1).

Case 2

A 13 year old premenarcheal girl presented to the pediatric emergency department with the complaint of urinary retention for 24 hours and constipation for 3 days. She had a history of lower abdominal pain for two months. She had no nausea, vomiting or diarrhea. She was referred to the pediatric urology service with the diagnosis of glob vesicale. In the urology service, after catheterisation of the bladder she was referred to our obstetric and gynecology clinic. On transabdominal ultrasonography, the uterine cavity was filled with blood and a cystic mass of 11x9x13 cm in diameter (hematocolpos) was detected (Figure 2, 3). On physical examination, she had well developed secondary sexual characteristics with no history of menarche. There was no rebound tenderness but there was suprapubic pain on abdominal examination. Her vital signs were normal. A bluish imperforate hymen was seen on genital examination. All blood and urine laboratory tests, including β-HCG, were normal. The diagnosis was hematocolpos with hematometra. The hematocolpos was so severe as to cause obstruction of the urethra. The patient and her family were informed about the situation and the procedure. We performed a small central circular hymenotomy, forming an intact annular hymen . Following drainage of about 1100 mL of blood, we performed vaginal washing. The patient was discharged on the following day. Follow up of the girl during 6 months was normal with regular menstrual cycles and no restenosis of the hymen.

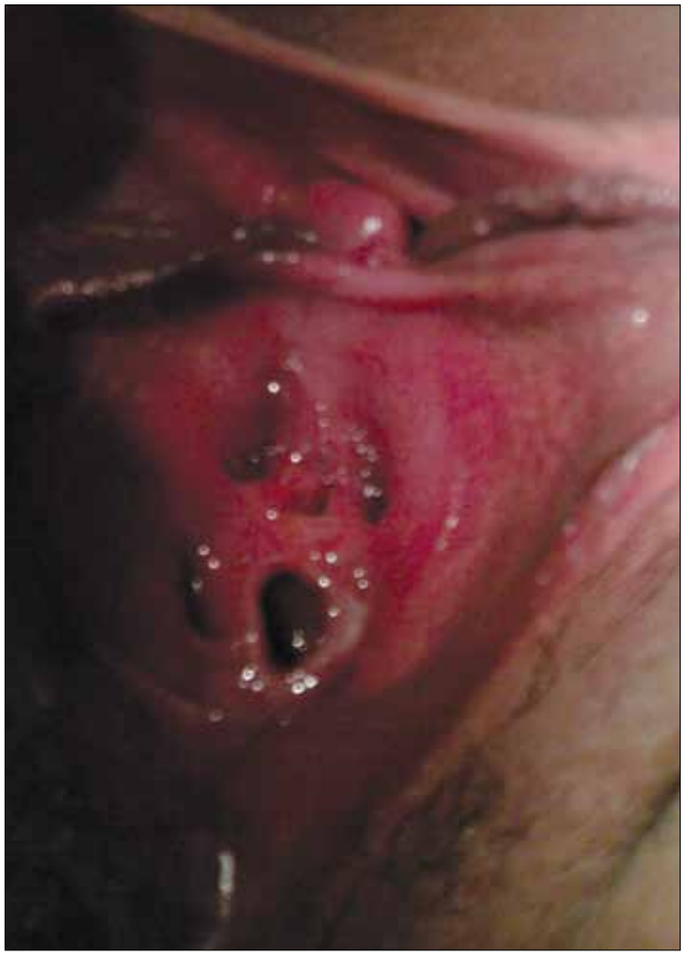

Figure 1. Intact annular hymen after operation

Discussion

IH is not an uncommon entity and diagnosis is easy with thorough history taking and genital examination. IH is usually sporadic, but some familial cases have been reported so far (2, 3). Despite the ease of diagnosis with simple inspection of the external genitalia, the detection of IH can be delayed or missed. Possible complications of delayed diagnosis include endometriosis secondary to retrograde menstruation and ruptured hematosalpynx. As IH is asymptomatic and until the onset of mentruation it usually remains undiagnosed. However, after menarche when blood begins to accumulate behind the imperforate hymen, hematocolpos, hematometra and hematosalpinx occur, causing cyclic symptoms. Hematocolpos can be so severe to cause obstruction of urethra as in our second case. Urinary retention caused by hematocolpos has been

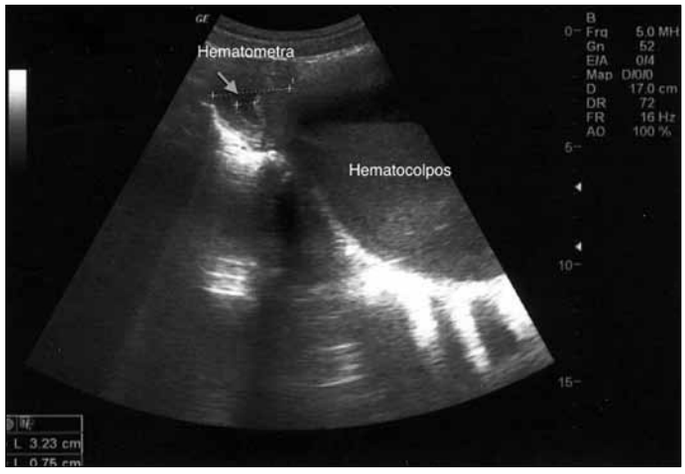

Figure 2. Hematocolpometra

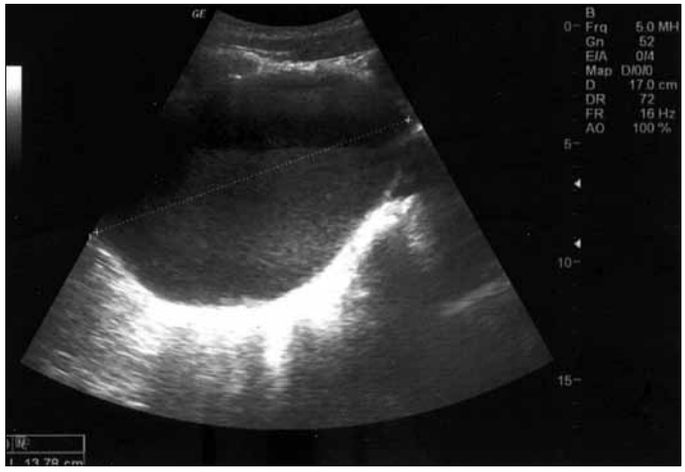

Figure 3. A cyctic mass of 11x9x13 cm in diameter (hematocolpos)

mentioned in a few cases in the literature (4). IH should be kept in mind when evaluating a girl with urinary retention.

It can easily be misdiagnosed as appendicitis or adnexial masses because patients come to the emergency department with nonspecific symptoms such as lower abdominal pain, dysuria, urinary retention, and constipation (5, 6). Menstrual history and secondary sexual characteristics should be investigated in such adolescents. The most important clue for diagnosis is absence of menarche. The diagnosis can be confirmed with the detection of a bluish bulging imperfotare hymen on perineal examination. Therefore, careful perineal examination should be performed in premenarchal girls presenting with abdominal pain and obstructive urinary symptoms. The two cases we reported above both had a history of primary amenorrhea despite well developed secondary sexual characteristics.

Treatment of IH is hymenotomy or hymenectomy. Different types of incisions are mentioned in the literature. Some prefer cruciate or vertical incisions, while the others prefer simple central excision of the hymen making an annular-intact hymen using a foley catheter for 2 weeks in order to prevent restenosis (7, 10).

In the cases we reported above, we placed a clamp at the center of the imperforate membrane after the patients were positioned in the lithotomy position. Then we excised a 1 cm central portion with a circular incision around the clamp, forming an annular intact hymenal ring. The vagina was irrigated with saline solution after drainage of the blood. Although general anesthesia is not needed for this simple intervention, we performed the procedure under general anesthesia because of the patients' preference. Patients were discharged from the hospital on the next day after the procedure. We advised patients to clean the vulva with povidone iodine for one week. There was no complication such as bleeding, infection or restenosis in the follow up of patients for 6-8 months.

The techniques mentioned above have similar restenosis risks, so incision type depends on patient's desire or surgeon's preferance. In cultures where the destruction of the hymen is a social problem in unmarried girls, it is important to preserve the annular structure of the hymen in gynecological practice. We prefer the simple central circular excision of the hymen leaving an intact annular hymen as described by Acar et al. (11) However, we did not use a foley catheter after the procedure. There was no restenosis in the follow up of our patients. It is so uncomfortable especially for such young girls having foreign material protruding from the vagina for two weeks. Also there is no need to give prophylactic antibiotic treatment to patients. The procedure is less invasive than other methods described in the literature. and more comfortable for the patients. The result of two cases is not sufficient to provide a conclusion, but studies with large numbers of cases would show the efficacy of the procedure so that it will have wolrdwide acceptance.

Conflict of interest

No conflict of interest was declared by the authors.

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Fetal cardiac circulation in isolated aortic atresia assessed with ultrasound

İzole aortik atrezide fetal kardiyak dolaşımın ultrason eşliğinde değerlendirilmesi

Aslı Tanrıvermiş Sayit[1], Ali İpek[1], Aydın Kurt[2], Bayan G. Aghdasi[3], Halil Arslan[1], Mehmet Gümüş[1]

[1]Department of Radiology, Ankara Ataturk Education and Research Hospital, Ankara, Turkey

[2]Department of Radiology, Ankara Etlik Ihtisas Education and Research Hospital, Ankara, Turkey

[3]Medical Student, David Geffen School of Medicine at University of California, Los Angeles, USA

Abstract

Congenital heart diseases are common, with an incidence of more than 8 in 1000 live births. Aortic atresia is a rare diagnosis and its prognosis is very poor. In this article, we present a case of isolated aortic atresia, a very rare cardiovascular anomaly, and its fetal ultrasound findings which include blood flow at foramen ovale from left to right, right deviation of the interventricular septum, dysfunction of the mitral valve and cardiomegaly. Aortic stenosis should be considered in the differential diagnosis of aortic atresia. However, in the case of severe aortic stenosis and/or accompanying ventricular septal defect, differential diagnosis may not be done.

(J Turkish-German Gynecol Assoc 2012; 13: 281-3)

Key words: Aortic atresia, cardiovascular anomaly, congenital heart disease, color Doppler ultrasound, fetus

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Özet

Konjenital kalp hastalıkları çok sık görülmekte olup insidansı 1000 canlı doğumda 8' den fazladır. Aortik atrezi nadir görülmekte olup prognozu kötüdür. Biz bu olgu sunumunda, ultrasonografi bulguları arasında foramen ovaleden soldan sağa şant, interventriküler septumda sağa deviasyon, mitral kapakta disfonksiyon ve kardiyomegalinin izlendiği nadir görülen bir kardiyovasküler anomali olan aort atrezisini tartışmayı amaçladık. Aort atrezisinin ayırıcı tanısında mutlaka aort stenozu göz önünde bulundurulmalıdır. Ancak şiddetli aort stenozuna eşlik eden VSD varlığında ayırıcı tanı yapılamayabilir.

(J Turkish-German Gynecol Assoc 2012; 13: 281-3)

Anahtar kelimeler: Aort atrezisi, fetüs, kardiyovasküler anomali, konjenital kalp hastalığı, renkli Doppler ultrason

Geliş Tarihi: 04 Nisan 2012 **Kabul Tarihi:** 12 Mayıs 2012

Introduction

Congenital heart diseases (CHD) are common, and are seen with an incidence greater than 8 in 1000 live births. These anomalies can cause severe morbidity and mortality in the fetus and neonate (1). Among CHD, atrioventricular septal defect, hypoplastic left heart syndrome, coarctation of the aorta, tricuspid dysplasia/Ebstein's anomaly and ventricular septal defect are the most common (2). In this article, we present a case of isolated aortic atresia, which is a very rare cardiovascular anomaly, and the fetal color Doppler ultrasound (USG) findings and assessment of secondary hemodynamic changes.

Case Report

At the fetal USG of a 24 year old pregnant woman (gravida 1, para 0) a 25 weeks and 4 days old male fetus was seen. According to her last menstrual cycle, the mother had been pregnant for 25 weeks. This was her first visit to our clinic. There was no family history of CHD. In the fetal USG, the position and axis of the heart were normal, and upon visualization of three vessels, the localizations of the pulmonary artery (PA) and superior vena cava were also normal. There was no vascular outflow tract at the left ventricle. The ascending aorta had a ligamentous appearance and its diameter was measured as 1 mm (Figure 1a and b). Blood flow at the foramen ovale from left to right, right deviation of the interventricular septum, dysfunction of the mitral valve and cardiomegaly were detected on the color Doppler USG (Figure 2 and 3). The diameter of the PA was measured to be wider than normal (7.2 mm), and the ductus arteriosus (DA) was dilated. Because of the obstruction at the left ventricular outflow tract and the dysfunction of the mitral valve, blood flowed back to the left atrium from the left ventricle and went to the right atrium by passing through the foramen ovale. Most of the blood passing to the right ventricle was going to the systemic circulation by the DA. Blood coming to the left atrium by the pulmonary veins was going to the right atrium by the foramen ovale too. No atrioventricular

Address for Correspondence: *Aslı Tanrıvermiş, Sayit, Department of Radiology, Ankara Ataturk Education and Research Hospital, 06800 Ankara, Turkey*
Phone: +0090 5324 94 90 82 e.mail: draslitanrivermissayit@gmail.com

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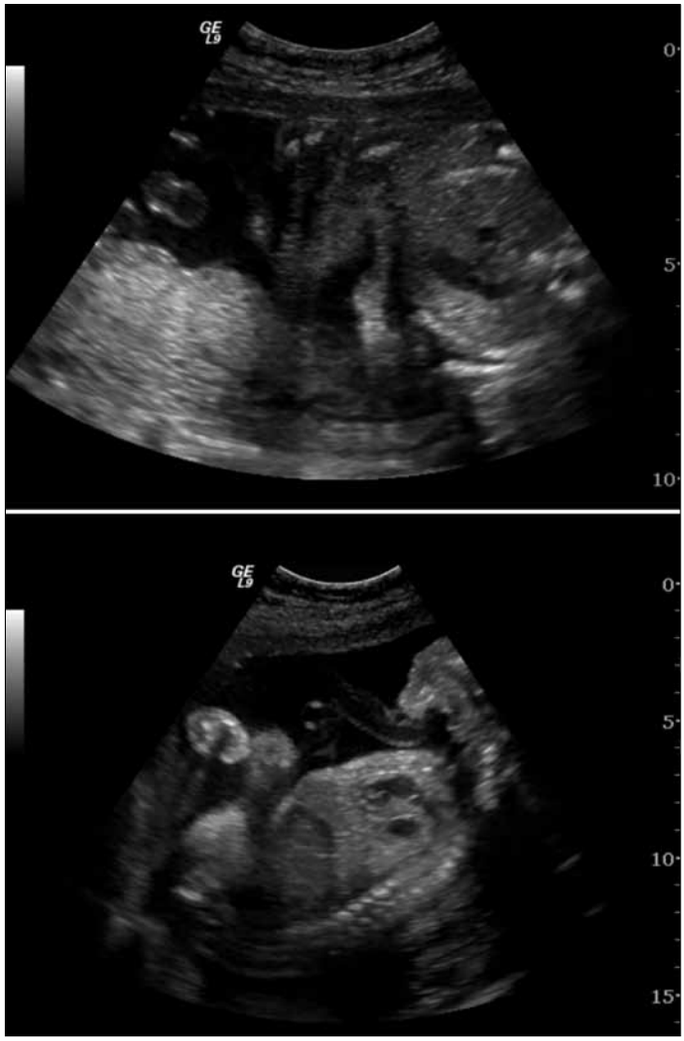

Figure 1. a) Axial three-vessel view; ascending aorta is not seen b) Sagittal sonography; Pulmonary artery behind the aortic root was not observed

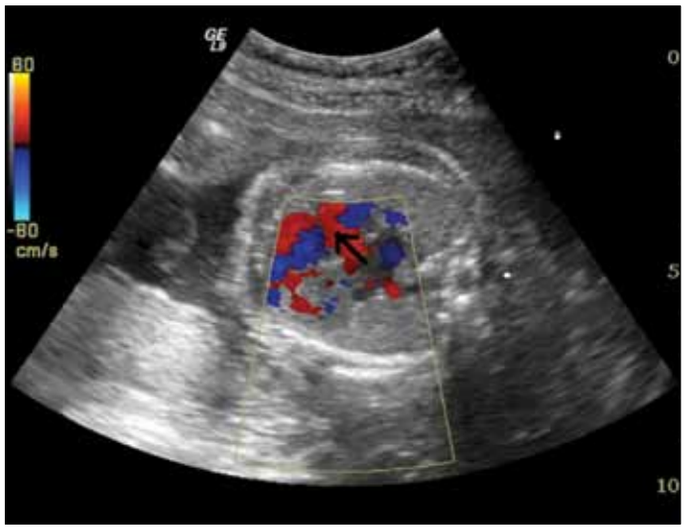

Figure 2. Blood flow from left to right through the foramen ovale is seen in color Doppler ultrasound

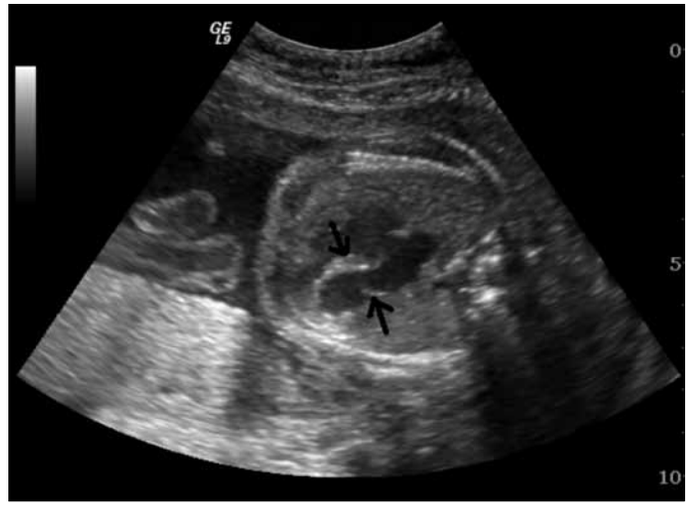

Figure 3. Mild right deviation of the interventricular septum and relaxation of the mitral valve are seen in axial sonography

septal defect or other organ anomalies were detected. After a pediatric cardiology consultation, the patient was offered to be delivered, but she did not accept the procedure. The fetus was born at term, but died just 6 hours after. The family did not agree to a postmortem autopsy.

Discussion

Fetal cardiac USG is a very simple and sensitive diagnostic procedure for the diagnosis of CHD. A four-chamber view of the heart has been proposed as a routine portion of the fetal USG to be obtained at visits from 18 weeks to term. The position of the heart, approximately equal size of the ventricles, the foramen ovale opening to the left atrial cavity, and the positioning of both atrioventricular valves are important items to look for. However, if only the four-chamber assessment is made, ventricular outflow tracts or the great artery anomalies may be missed. The relationship between the aorta and the left ventricle is assessed by a long axis view of the ventricle (1). The three vessels are assessed by longitudinal scans to the level of fetus's uppermost mediastenium transverse scan. The trunk of the PA, ascending aorta and superior vena cava are all arranged in a straight line in this view. The imaging of these three-vessels gives important information about potential abnormal diameters and positioning of the vessels, and pathological relationships between the ascending aorta and trunk of the PA (3). Absence of flow out of the left ventricle through the aortic valve is suggestive of aortic atresia. Reverse flow from the DA into the aortic arch and a hypoplastic ascending aorta is an indirect sign of severe left ventricular outflow tract obstruction.

It is diagnostic of aortic atresia when the internal diameter of the ascending aorta is less than 5 mm. It is seen with a hypoplastic mitral valve, and rarely with mitral atresia and a hypoplastic left ventricle or rudimentary left ventricle without a left atrioventricular connection. If the mitral valve is patent, the size of the ventricles may be different (4, 5). In our case, the diameter of the ascending aorta was 1mm and it was ligamentous in appearance. Because of the mitral valve insufficiency, there

was a mild dilatation of the left ventricle and cardiomegaly. Tometzki et al. presented a fetus with a normal size ventricle with aortic atresia, pulmonary stenosis, VSD and truncus arteriosus (6). In our case, the output of the PA from the ventricle was normal, whereas the diameter of the PA was 7.2 mm. The aorta was in the normal position and of a normal size at the level of the DA. There was no additional cardiac pathology such as atrial or ventricular septal defects.

Altough the myocyte number is increased initially in fetuses with aortic atresia and a patent mitral valve, later the proliferative phase stops and a hypoplastic left ventricle may develop (7). Hypoplastic left heart syndrome is a wide-spectrumed malformation associated with a small left ventricle and aortic atresia and an atresic or hypoplastic mitral valve. Andrew et al. reported a monochorionic twin pregnancy in which both of the fetuses had hypoplastic left heart syndrome. They detected a small left ventricular cavity and hypoplastic aortic arch in the first fetus and no ventricular cavity and a bright ecogenity in its place in the second fetus. The gestation was terminated at 17 weeks, hypoplastic left heart syndrome was verified and mitral and aortic atresia were detected at autopsy (7, 8). In this case, because mitral valve failure developed with aortic atresia, mild dilatation of the left ventricle, diffuse cardiomegaly, and a mild left deviation of the interventricular septum were seen. Blood flow from left to right through the foramen ovale due to increased pressure in the left atrium was visualized with color Doppler USG. In this case, because of the fetus's own special circulation, there was cardiomegaly, and hypoplastic left heart syndrome did not seem to apply. Gembruch et al. (4) reported a 33 week old fetus with aortic atresia and mitral valve insufficiency, left ventricular hypertrophy and premature closing of the foramen ovale. Because of this, volume overload in the right ventricle and congestive cardiac failure developed.

Early closing of the foramen ovale causes hydrops fetalis. Also, fetuses with severe aortic obstruction and mitral valve insufficiency with a predominant shunt from left to right have overloading of the right heart and hydrops. Fetuses with aortic atresia but without VSD require decompression of the left atrium provided by an interatrial shunt from left to right. In our case, because of the absence of a VSD, flow from the left to right atrium was occuring through the foramen ovale. Because of a predominantly left to right shunt by the foramen ovale, mild growth and overloading of the right heart was detected, but non-immune hydrops was not seen.

Reverse flow into the aortic arch through the ductus arteriosus and a hypoplastic ascending aorta together point to severe left ventricular outflow tract obstruction. The absence of flow out of the left ventricle through the aortic valve should draw attention to aortic atresia. Absence of forward flow at color Doppler USG, reverse flow in the hypoplastic aortic tract and mitral regurgitation are important diagnostic criteria for aortic atresia. Expansion of the left atrium and ventricle, mitral valve insufficiency and hypofunction of the left ventricular myocardium are seen in complete left ventricle outflow tract obtruction. In such cases, there is a high risk of hydrops fetalis.

Aortic atresia and aortic stenosis have similar findings in two-dimensional imaging. However, unlike aortic atresia, aortic stenosis causes acceleration of prestenotic blood flow in the left ventricular outflow tract, and poststenotically an antegrade jet with very high velocities and turbulence in the ascending aorta is seen in color Doppler USG. If the stenosis is very severe and/or there is a VSD, in the situation of a patent aortic valve antegrade stenotic jet flow will not be seen and the differential diagnosis of aortic atresia can not be made. If a critical aortic stenosis exists, the degree of ascending aorta hypoplasia and the anatomic situation of the left ventricle wall will indicate inoperability (4, 9).

The ductus arteriosus closes 48 hours after birth. The systemic circulation obliterates due to closing of the ductus arteriosus and that results in hypoxia. As a result the neonate dies (10). In our case we thought the systemic circulation was obliterated due to the closing of the ductus arteriosus and that this caused cyanosis and death. Because the family did not consent to an autopsy, histopathological diagnosis could not be used to confirm the cause of death.

Conclusion

Cardiovascular anomalies have an important place in obstetric USG findings. In a routine fetal cardiac USG of the four chambers of the heart, the great vessels and ventricular outflow tracts must be seen.

Conflict of interest

No conflict of interest was declared by the authors.

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Recurrent familial hydatidiform mole - a rare clinical problem

Rekürren ailesel hidatidiform mol - nadir bir klinik problem

Lavanya Rai, Hebbar Shripad, Shyamala Guruvayare, Adiga Prashant, Anjali Sunil

Department of Gynaecology and Obstetrics, Kasturba Medical College, Manipal University, Manipal, India

Abstract

Familial recurrent hydatidiform mole is a rare event; here we report an unusual case of a gravida 5 aged 29 years, with five recurrent hydatidiform moles and no normal pregnancy. After the fourth molar pregnancy, she developed persistent trophoblastic disease that required 7 cycles of single agent chemotherapy. Two years after the treatment, she presented with her fifth molar pregnancy. Her elder sister had seven hydatidiform moles from two different unrelated male partners. As this is familial, and recurrent, with no viable conceptions in both the sisters, it is likely to be biparental in origin. Unlike androgenetic moles, biparental moles arise due to a global inherited failure of maternal imprinting. It is an autosomal recessive defect in the female germ line. Genetic analysis is essential, although it is not available in all centers. Donor Oocyte IVF is the only option for women with biparental moles to have normal offspring.

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Key words: Recurrent hydatidiform mole, persistent trophoblastic disease, familial mole, donor oocyte invitro fertilisation, preimplantation genetic diagnosis.

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Özet

Ailesel rekürren hidatidiform mol nadir bir olaydır; biz burada beş rekürren hidatidiform molü olan ve normal gebeliği olmayan gravidası 5, yaşı 29 olan olağan dışı bir olgu bildiriyoruz. Dördüncü molar gebelikten sonra hasta tekli ajan ile 7 döngü kemoterapi gerektiren persistan trofoblastik hastalık geliştirdi. Tedaviden iki yıl sonra hasta beşinci molar gebelik ile başvurdu. Hastanın ablasında birbiri ile ilişkisiz iki farklı erkek eşten toplam yedi hidatidiform mol gebelik olmuştu. Bu durumun ailesel ve rekürren olması ve her iki kız kardeşte de canlı konsepsiyonun olmaması nedeniyle biparental orijinli olması muhtemeldir. Androjenik mollerin aksine biparental moller, global kalıtsal maternal imprinting yetmezliğinden kaynaklanmaktadır. Bu, dişi germ hattında otozomal resesif bir defekttir. Tüm merkezlerde ulaşılabilir olmamasına rağmen genetik analiz zorunludur. Biparental molü olan kadınların normal çocuğa sahip olması için tek seçenek donör oosit IVF'dir.

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Anahtar kelimeler: Rekürren hidatidiform mol, persistan trofoblastik hastalık, ailesel mol, donör oosit in vitro fertilizasyon, preimplantasyon genetik tanı

Geliş Tarihi: 12 Aralık 2011 **Kabul Tarihi:** 14 Nisan 2012

Introduction

Hydatidiform mole is the result of abnormal fertilization and is most often a sporadic event. Recurrent moles account for 2% of all hydatidiform moles (1). Some of these recurrent moles are familial, with more than one member of the family having hydatidiform moles and often from different partners (2). The genetic origin of these moles is biparental (BiCHM) and is different from the androgenetic (AnCHM) origin of the usual hydatidiform mole. Besides the risks of persistent trophoblastic disease (PTD), women with recurrent biparental moles are unable to have normal pregnancies.

Case Report

Our index case was a gravida 5, para 0, muslim lady aged 29 years, with 4 previous hydatidiform moles who presented at 8 weeks of gestation with a diagnosis of a 5th hydatidiform mole for termination. Her scan showed typical anechoic spaces suggestive of hydatidiform mole. Ovaries did not show any theca lutein cysts. Her blood group was B positive. Her liver, renal function tests and chest X-ray were normal. Preevacuation serum beta hCG (β hCG) was 113527mLU/mL. Histopathology following suction evacuation revealed a complete hydatidiform mole (CHM). Post evacuation serum β hCG regressed in 6 weeks and at present she is under postmolar survelliance.

Her previous 4 molar pregnancies occurred at intervals of 2 years. They were evacuated elsewhere and were reported as CHM. Following the 4th molar evacuation, PTD was diagnosed with a rising trend of serum β hCG after 2 months of evacuation. She received chemotherapy consisting of methotrexate with folinic acid rescue for low risk PTD (WHO score 4). Serum β hCG had become normal after the 5th cycle and 2 more cycles of chemotherapy were given for residual disease. Her husband is not related to her. The karyotype of the couple is normal.

Address for Correspondence: *Lavanya Rai, Department of Gynaecology and Obstetrics, Kasturba Medical College, Manipal University, 576104 Manipal, India*
Phone: 0850 29622176 e.mail: lavanya.rai@manipal.edu - railalavanya@yahoo.com

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Her elder sister had 7 consecutive hydatidiform moles with no normal pregnancy. Four hydatidiform moles occurred with her first husband after which she was divorced. She had 3 more molar pregnancies from her second husband. The pedigree chart of this family is depicted in Figure 1. There is no relevant past or family history of genetic disease.

Due to the familial, recurrent nature of these moles with no viable conceptions in both the sisters, it is likely to be biparental in origin. As molecular techniques to detect the origin of these moles was not feasible in our set up, we advised donor oocyte in-vitro fertilization (IVF). Adoption was also suggested as an alternative option

Discussion

The majority of CHMs (80%) have a diploid set of paternal chromosomes due to fertilization of an anucleate oocyte by sperms, leading to reduplication of the paternal haploid set of chromosomes (1). This is termed as Androgenetic (AnCHM) origin. Recurrent moles may be sporadic, occurring in a single individual in a family or may be familial as in biparental moles (3). Biparental moles have both a maternal and a paternal component. These are due to an autosomal recessive defect in the female germ line (4).

The hydatidiform moles in our case occurred in sisters married to unrelated men. Their parents are 3° descendents from common parents as depicted in the pedigree chart (Figure 1). BiCHM are seen in families where ≥2 individuals have recurrent molar pregnancies (4). Since the women themselves are affected with the autosomal recessive mutation, paternal genotype does not contribute to the pathogenesis. Dysregulation of imprinting occurs due to the methylation defect during oogenesis in the female germ line (2, 4). This is believed to be a global methylation defect leading to a switch from maternal to paternal methylation pattern, resulting in BiCHM (5). Women with this methylation defect in the germline are unable to establish a normal female imprinting pattern. Initially, Mogalbey et al. (6) mapped this maternal recessive locus to chromosome 19 q13.4. This defect is now seen in several genes in different chromosomes (5). However, recent literature suggests mutation of NLRP7 gene as a major contributor to familial biparental moles (7).

NALP7 gene has a role in cytokine secretion, particularly interleukin 1B (IL-1B), which is necessary for inflammation and apoptosis. This is also essential for folliculogenesis, ovulation, decidualization and trophoblast invasion. Mutation in this gene is said to cause biparental moles and other forms of reproductive loss (1, 7).

AnCHM can also recur more than twice when there are consanguinous marriages in families. However, the risk of recurrence is much lower than BiCHM and they have some chance of having normal pregnancy, unlike BiCHM, and hence it is suggested that genetic analysis should be done after 2 or more moles (3). Anucleate oocytes caused by defective meiosis are the result of extrusion of the maternal nuclear genome into one of the polar bodies leaving an anucleate ovum. Another hypothesis for post zygotic diploidisation (PDT) has been postulated for recurrent moles because triploid conceptions occur far more frequently than anucleate oocytes (3) According to this concept, all non BiCHM moles can be the result of dispermic fertilization. These triploid conceptions are unstable at first mitosis and give rise to daughter cells that could develop AnCHM.

The risk of PTD is higher (50%) in recurrent molar pregnancy. Histology and degree of invasiveness also increases in successive molar pregnancies (8). Although in our index case, PTD developed after 4 moles, her sister with 7 moles did not have this problem. Incidence of recurrent mole was 0.7% in the Sheffield Trophoblastic centre. They noted that the Asian women, particularly of Indian/ Pakistan origin, and those with blood group B had a higher incidence (9). Our patient also had the same blood group.

The majority of recurrent moles are reported from Muslim countries such as Egypt, Lebanon etc (2). Our patient also was a Muslim. Seoud et al. (10) reported familial recurrent moles in a family with extensive intermarriage.

Molar tissue should be genotyped with polymorphic DNA markers to determine the parental origin as this helps to plan therapeutic options. If it is BiCHM, conception with a donor oocyte is the only option. Tuncer et al. (11) have reported a successful pregnancy through ovum donation in a lady with 3 recurrent molar pregnancies with 2 different partners. The report is not clear whether it was a biparental mole.

Sensi et al. (12) attempted a pregnancy with ovum donation which failed, as the repeat molar pregnancy showed it was established by fertilization of the maternal ovum. It is also believed that, despite fertilization with ovum donation, implantation may fail due to an abnormal inflammatory response in the endometrium as result of mutation of the NLRP7 gene (7) (Figure 2).

If the origin of a recurrent mole is heterozygous androgenetic, then Intracytoplasmic Sperm injection (ICSI)/preimplantation diagnosis (PGD) with Fluorescent in situ hybridization (FISH) is appropriate (3). ICSI ensures monospermic fertilization. FISH for male preselection of embryo, ensures that fertilization occurs with a Y chromosome so that androgenesis with X sperms is avoided. If a female embryo is required, preimplantation determination of parental origin by DNA typing is required (8). Standard IVF procedure and transfer of an embryo presumed to be normal can still result in an hydatidiform mole. As women with BiCHM cannot have their own genetic offspring, counseling has an important role.

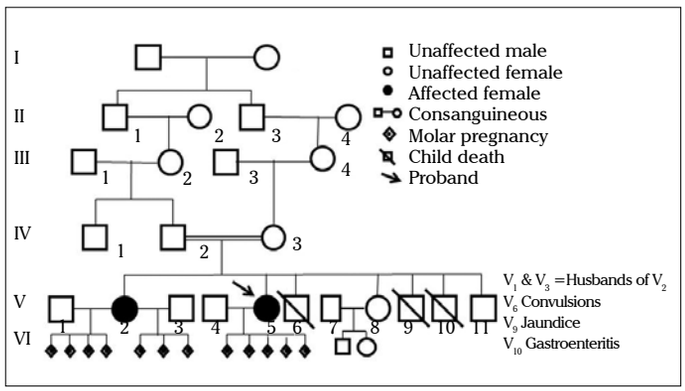

Figure 1. Pedigree Chart of Mrs S

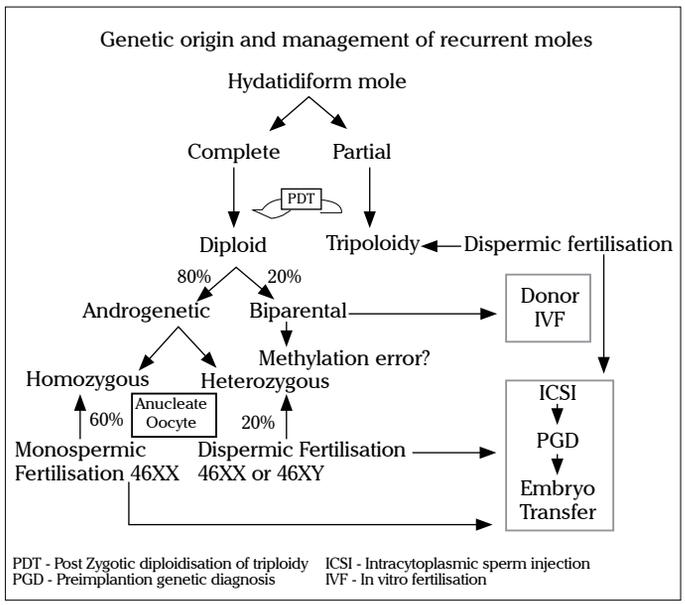

Figure 2. Flow Chart

Conflict of interest

No conflict of interest was declared by the authors.

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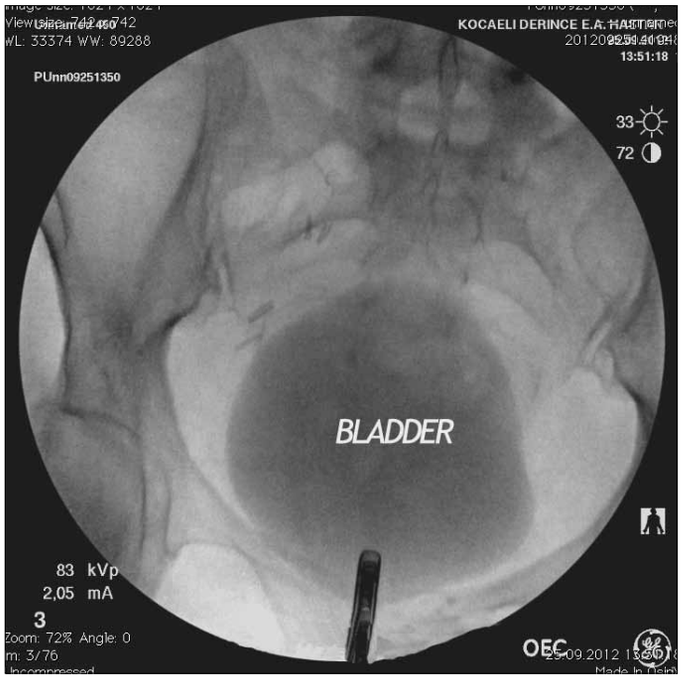
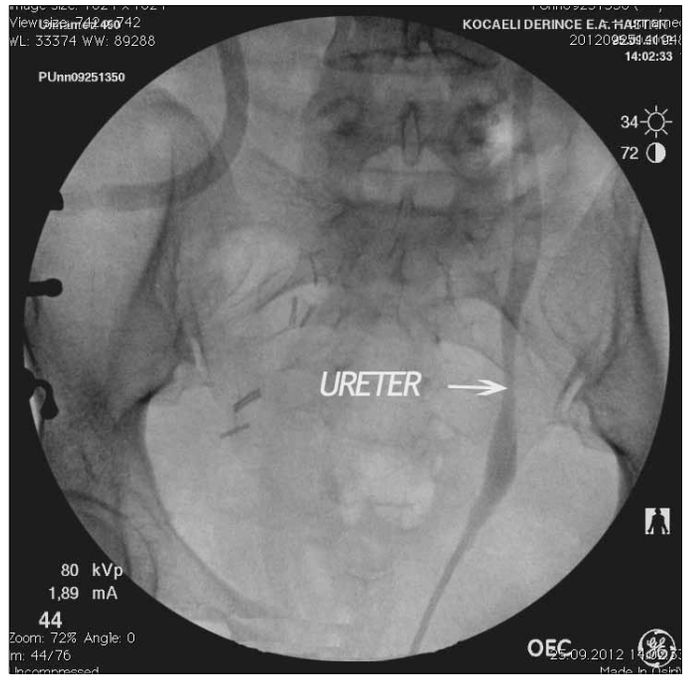
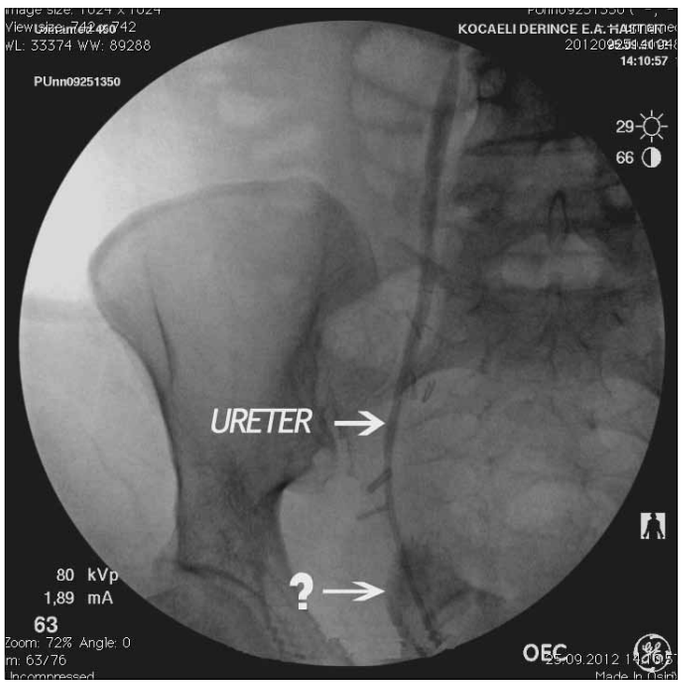
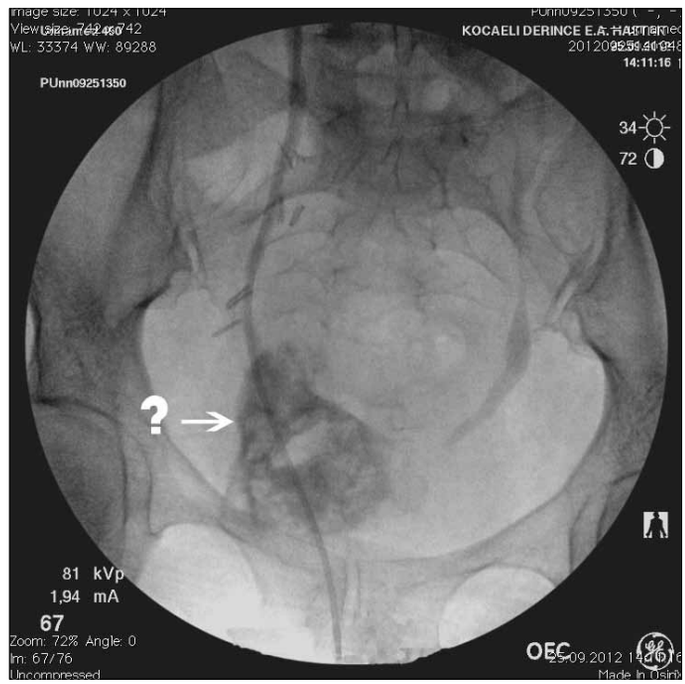
What is your diagnosis?









Address for Correspondence: *Hasan Terzi, Clinic of Gynecology and Obstetrics, Derince Training and Research Hospital, Kocaeli, Turkey*
Phone: +90 262 317 80 17 e.mail: drhterzi@yahoo.com

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In laparoscopic hysterectomy procedures, typically the distal ureters are injured while ligation of the uterine arteries. Also dissection of the cardinal ligaments and division below the uterine vessels causes ureter injuries (1).

Such procedures are trouble some in the case of an abnormal anatomy. However preoperative intravenous pyelography (IVP) or other studies do not help to prevent these injuries (2).

Most of the patients are asymptomatic for a couple of days, however when an abdominal pain, a flank pain or a costovertebral angle tenderness exist this should alert the surgeon. Typically fistulization take place 3 days to 4 weeks if ureteral leak persists; the urine makes its way to the vaginal cuff (3).

In the case of vaginal leakage first step may be a double dye test for differential diagnosis of vesico-vaginal fistule (VVF) or uretero-vaginal fistule (UVF) (4, 5). Vagina is packed and intravenous methylene blue is administered, while intravesical carmine red instilled. Red stained vaginal pack indicates a VVF while blue indicates UVF. Next step is an IVU that may demonstrate hydronephrosis, location and severity of the leakage. If IVU is not helpful a retrograde ureterogram may be diagnostic and therapeutic at the same time with bypassing the fistulated segment of ureter. Also both MRI and multi-slice CT are valued imaging techniques for fistula detection (6).

Treatment options are internal drainage with ureteral double J (DJ) stent, external drainage with percutaneous nephrostomy, surgical repair, or even nephrectomy. If DJ stent bypass the fistula spontaneous healing is likely without a further surgical intervention (7). A close follow-up is mandatory, because of ureteral structuring seen in most cases.

If it is needed timing of surgical repair is controversial, either immediate or delayed ureteral repair are advised (8-10).

Depending on the location, degree and severity of the injury there are several surgical treatment options. Most of the cases successfully repaired with an ureteroneocystostomy further more uretero-ureterostomy, psoas hitch Boari flap, transureteroureterostomy techniques may be applied when it's indicated (9, 11).

Answer; patient who was diagnosed with early-stage cervical cancer ureterovaginal fistula which was developed after laparoscopic radical hysterectomy.

Hasan Terzi[1], Tayyar Alp Özkan[2], Ahmet Kale[1]

[1]Clinic of Gynecology and Obstetrics, Derince Training and Research Hospital, Kocaeli, Turkey

[2]Clinic of Urology, Derince Training and Research Hospital, Kocaeli, Turkey

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We continue to see an increase in the number of submissions to JTGGA as well as the quality. JTGGA is clearly becoming the journal of choice for obstetrics and gynecology healthcare issues in our region. We can afford to be somewhat more selective, and our acceptance rate is 36.9% in 2012 and (and 51.7% since the beginning-2000) approaches that of other major medical journals. The reviews submitted by you are among the best that we have seen among a number of major medical journals. The office regularly receives letters from authors thanking JTGGA for such thorough and helpful reviews, which enables them to produce much better manuscripts.

That fulfills one of our primary missions of teaching authors, especially young authors, how to write better manuscripts. We have several new and exciting programs under review for implementation during the coming year, and we certainly look forward to your ongoing support, suggestions and recommendations as to how to continue to improve the overall quality of JTGGA.

To become a JTGGA peer reviewer, please contact the Editor Cihat UNLU, Prof., M.D., cunlu@ada.net.tr and provide your full contact information and areas of interest.

Best regards.

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Saved	0	0	0	0	0	0	0	0	0	0	0	0	1	1
Waiting for Secretary	0	0	0	0	0	0	0	0	0	0	0	0	0	0
At Editor	0	0	0	0	0	0	0	0	0	0	0	0	0	0
On Review	0	0	0	0	0	0	0	0	0	0	0	0	21	21
Reviewed	0	0	0	0	0	0	0	0	0	0	0	0	0	0
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Rewriting Needed	0	0	0	0	0	0	0	0	0	0	0	0	4	4
Waiting for Final Decision	0	0	0	0	0	0	0	0	0	0	0	0	0	0
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Rejected	0	0	0	0	0	20	53	61	69	64	73	56	52	448
Refused	0	0	0	0	0	0	4	2	10	26	16	8	20	86
Withdrawn	0	0	0	0	0	0	12	35	8	5	8	5	20	93
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Submitted Total	8	9	11	46	51	100	142	177	171	143	178	132	195	1363

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Questions on the article within the scope of CME/CPD

1. Which one is a DNA fragmentation test?
   a) Comet test
   b) Tunel test
   c) Acridine orange staining
   d) Sperm Chromatin Dispersion
   e) All

2. Which sperm detection method has relationship with DNA fragmentation?
   a) IMSI
   b) Kruger strict criteria
   c) WHO criteria
   d) Tunel test
   e) Self experience

3. Which sperm test results affect your medical decisions?
   a) Rutin semen analysis
   b) Sperm FISH tests
   c) DNA fragmentation tests
   d) Semen fructose test
   e) All

4. Which sperm DNA fragmentation evaluation tests do you use in your laboratory easily?
   a) Comet
   b) Sperm Fructose
   c) Acridine orange staining
   d) Tunel
   e) None

5. Which cases do need DNA fragmentation tests?
   a) Recurrent implantation failure
   b) Fertilization failure
   c) Recurrent abortus
   d) Oligoasthenoteratospermia
   e) All

6. Which DNA fragmentation test method does need optical microscopy for detection?
   a) Comet test
   b) Tunel test
   c) Acridine orange staining
   d) Sperm Chromatin Dispersion
   e) Sperm chromatin structure assay

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7-8 December 2012	**2nd International Meeting "Principles and Controversies in the Treatment of Deep Infiltrating Endometriosis (DIE)"** Athens/Greece http://www.erasmus.gr/
1 January 2013	**38th Annual Meeting of the IUGA by International Urogynecological A.** Dublin/Ireland http://www.iuga.org/
16-20 January 2013	**All India Congress on Obstetrics & Gynecology (AICOG 2013) by Federation of Obstetric & Gynaecological Societies of India** Mumbai/India http://www.aicog2013.com/home
28 Feb- 3 March 2013	**3. Kadın Doğum Günleri (İ.Ü)** Harbiye Askeri Müze ve Kültür Sitesi-Istanbul http://istanbulkadindogum2013.org/
7-10 March 2013	**11. Uludağ Jinekoloji ve Obstetri Kış Kongresi** Karinna Otel, Uludağ, Bursa, Türkiye www.uludagkadindogum.org
13-16 February 2013	**EuroAmerican MultiSpecialty Summit VI Laparoscopy & Minimally Invasive Surgery** Disney's Contemporary Resort , Orlando, FL USA http://www.sls.org
24-28 April 2013	**Annual Middle East Society for Gynecologic Endoscopy Congress / Susesi Hotel/ Antalya Turkey** http://www.sls.org