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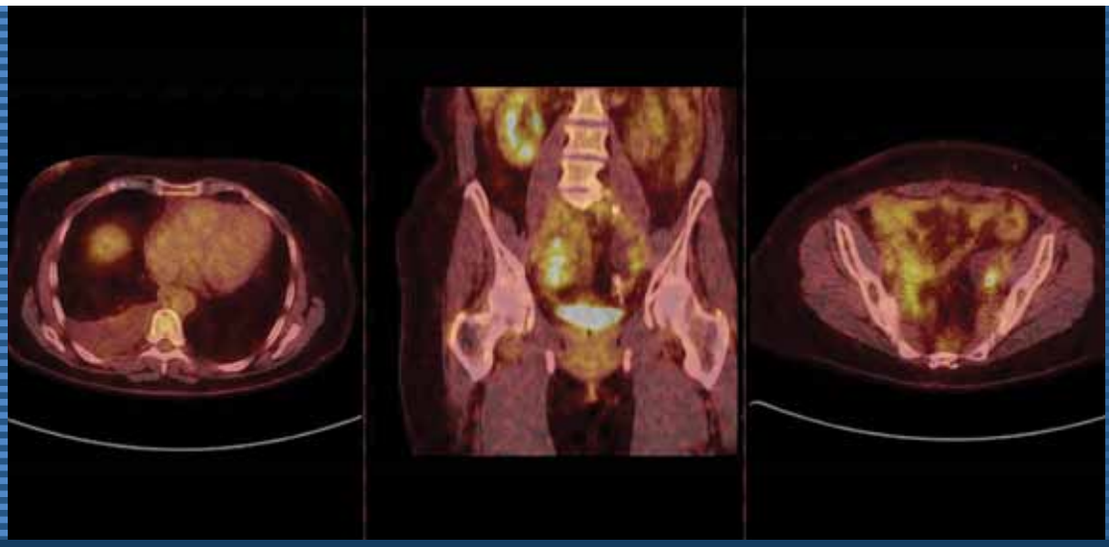

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Aims and Scope

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 November.

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

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Contents

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Editorial

Dear Colleagues,

I have always believed in the importance of maintaining a high standard of scientific quality in scientific journals. However, it is equally important to provide the sustainability of this level. This is the first issue of the 13th year of our journal - JTGGA (Journal of the Turkish-German Gynecological Association), which is the outcome of intense devotion and hard work through long years. We will steadily continue our efforts as the TAJEV family in aiming to raise our journal to an ever better position in the scientific community.

Herein is presented another highly scientific content issue. In this issue, you will read an interesting manuscript by NESA's (New European Surgical Academy) President Prof. Michael Stark, who you all know as the founder of the NOS (Natural Orifice Surgery). Prof. Stark has also submitted an interesting letter regarding the importance and future of laparoscopy and tactile feeling in telesurgery. In this letter, Prof. Stark shared his experiences about the recent NESA project - "Telelap Alf-X", which is a new system providing tactile feeling in robotic surgery. In this issue, you will also find an innovative study on the documentation regarding water birth practice and its effects on the maternal, fetal and the newborn wellbeing through labor.

The editorial process of JTGGA, which is a peer-reviewed and open access journal, is carried out meticulously. Our journal has an increasing level of international acknowledgement and attracts the attention of a wide region with submissions from India to Germany. I hereby thank all co-editors taking part in all processes of the fair evaluation. I would also like to send my special thanks for her sincere efforts to Dr. Yaprak Üstün who joined our editorial team recently.

Dear young colleagues at the beginning of your academic careers,

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 wish all the obstetrics and gynecology community a fruitful and successful academic spell in 2012.

Best regards,

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

Do clinical and laboratory parameters effect maternal and fetal outcomes in pregnancies complicated with hemolysis, elevated liver enzymes, and low platelet count syndrome?

Klinik ve laboratuvar özellikler hemoliz, artmış karaciğer enzimleri ve düşük trombosit sayımı sendromlu gebelerde maternal ve fetal sonuçları etkiler mi?

İncim Bezircioğlu, Ali Baloğlu, Burcu Çetinkaya, Betül Pirim

Department of 1st Gynecology and Obstetrics, İzmir Atatürk Training and Research Hospital, İzmir, Turkey

Abstract

Objective: The aim of the study was to investigate whether the clinical features and laboratory parameters affect maternal and fetal outcomes in pregnancies complicated with HELLP syndrome.

Material and Methods: The medical records of pregnant patients complicated with HELLP syndrome were analyzed retrospectively between June 01, 2003 and June 01, 2010. The demographic data, medical history, admission symptoms, clinical and laboratory findings and recovery time were evaluated. The adverse maternal outcomes including eclampsia, placental abruption, disseminated intravascular coagulation, postpartum hemorrhage, pulmonary complications, cerebral edema and visual loss were recorded. Fetal growth restriction, necessity for neonatal intensive care unit admission and perinatal mortality were recorded as an adverse fetal outcome.

Results: The incidence of HELLP syndrome was 0.52%. The mean age of the patients was 28.93±7.90 (range 17-45). HELLP syndrome was diagnosed on average in the 33.68±4.41th (ranged 24-40) week of gestation. Eighteen cases (40.9%) were nullipara and twenty-six cases (59.1%) multipara. The most common complications were eclampsia (40.9%) and abruption placenta (15.9%). Pregnancy was terminated within 48 hours in all patients. The rate of cesarean section was 90.9%. Perinatal mortality rate in HELLP syndrome was 31.8%. There was no maternal mortality.

Conclusion: Neither clinical characteristics nor laboratory parameters was found effective for prediction of adverse maternal and fetal outcomes. (J Turkish-German Gynecol Assoc 2012; 13: 1-7)

Key words: HELLP syndrome, preeclampsia, maternal outcome, fetal outcome

Received: 22 June, 2011 **Accepted:** 28 September, 2011

Özet

Amaç: Çalışmanın amacı HELLP sendromlu gebelerde klinik özellikler ile laboratuvar belirteçlerinin maternal ve fetal sonuçları etkileyip etkilemediğini araştırmaktır.

Gereç ve Yöntemler: 01 Haziran 2003 ile 01 Haziran 2010 tarihleri arasında HELLP sendromlu gebe kadınların tıbbi kayıtları geriye dönük olarak incelendi. Demografik verileri, tıbbi öyküsü, başvuru yakınmaları, klinik ve laboratuvar bulguları ve iyileşme süreleri değerlendirildi. Eklampsi, plasenta dekolmanı, yaygın damar içi pıhtılaşma, postpartum kanama, akciğer komplikasyonları, beyin ödemi ve görme kaybını içeren olumsuz maternal sonuçlar kaydedildi. Fetal büyüme kısıtlılığı, yenidoğanın yoğun bakıma kabulü, perinatal mortalite olumsuz fetal sonuçlar olarak kaydedildi.

Bulgular: HELLP sendromu insidansı %0.52, olguların ortalama yaşı 28.93±7.90 (aralık 17-45) idi. HELLP sendromu gebeliğin ortalama 33.68±4.41 (aralık 24-40) haftasında tanındı. 18 olgu nullipar (%40.9), 26 olgu (%59.1) multipar idi. En sık komplikasyon eklampsi (%40.9) ve ablasyo plasenta (%15.9) oldu. Tüm hastalarda gebelik 48 saat içerisinde sonlandırıldı. Sezaryen hızı %90.9, perinatal mortalite %31.8 idi. Hiç maternal mortalite görülmedi.

Sonuç: Ne klinik özellikler ne de laboratuvar belirteçler olumsuz maternal ve perinatal sonuçları öngörmede etkili bulunmadı.

(J Turkish-German Gynecol Assoc 2012; 13: 1-7)

Anahtar kelimeler: HELLP sendromu, preeklampsi, maternal sonuç, fetal sonuç

Geliş Tarihi: 22 Haziran 2011 **Kabul Tarihi:** 28 Eylül 2011

Introduction

Hemolysis, elevated liver enzymes, and low platelet count (HELLP) syndrome is regarded as a variant or complication of preeclampsia. The HELLP syndrome occurs in 0.5 to 0.9% of all pregnancies and in 10 to 20% of cases with severe preeclampsia (1). In the Tennessee Classification System diagnostic criteria, the presence of hemolysis (LDH>600U/L), elevated liver enzymes (AST>70U/L) and thrombocytopenia (<100.000/mm^3) were accepted as HELLP syndrome. The patients who met two of three criteria were accepted as partial HELLP syndrome (2).

Patients whose pregnancies are complicated by HELLP syndrome are at high risk for complications such as abruption

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placenta, disseminated intravascular coagulation, acute renal failure, adult respiratory distress syndrome, and multiple organ dysfunction syndrome (1, 2). The HELLP syndrome is associated with increased risk of adverse fetal outcome because of intrauterine growth restriction, and prematurity (2). Clinical characteristics and laboratory data could not provide prediction of clinical course and adverse outcomes (3).

In the present study, we analyzed the pregnancies complicated by HELLP syndrome and the effects of maternal clinical features and laboratory parameters on adverse maternal and fetal outcomes were investigated.

Material and Methods

The pregnancies complicated with HELLP syndrome who were delivered in the Department of Obstetrics and Gynecology, İzmir Atatürk Training and Research Hospital, Turkey from June 01, 2003 to June 01, 2010 were evaluated retrospectively. We recruited all the patients with HELLP syndrome in the study period. The presence of hemolysis (LDH>600U/L), elevated liver enzymes (AST>70U/L) and thrombocytopenia (<100.000/mm^3) were accepted as HELLP syndrome. We recorded the patients who had two of three criteria were as partial HELLP syndrome. The exclusion criteria were hypertension and proteinuria before the 20th gestational week and other medical conditions including renal, hepatic, hematologic and cardiovascular diseases.

The demographic and clinical data including maternal age, parity, past history of preeclampsia, and hypertension, presenting symptoms, clinical findings and gestational age at admission, systolic and diastolic blood pressures at its maximum during the management, duration of hospital stay were recorded for all patients. The results of liver function tests, complete blood cell counts, coagulation profile, and renal function tests were collected. The adverse maternal outcomes including eclampsia, abruption placenta, disseminated intravascular coagulation, postpartum hemorrhage, pulmonary edema, cerebral edema, visual loss and adverse fetal outcome including fetal growth restriction, neonatal intensive care admission and perinatal mortality were recorded.

Eclampsia is defined as tonic-clonic seizures occurring in a hypertensive pregnancy, with or without proteinuria. Postpartum hemorrhage is defined as blood loss greater than 500 mL during a vaginal delivery or greater than 1,000 mL with a cesarean delivery. Pulmonary edema was diagnosed based on clinical findings and chest radiograph. Cerebral edema was diagnosed based on neurologic complaints with clinical findings, and Magnetic Resonance Imaging. Disseminated intravascular coagulation was defined as the presence of three or more of the following criteria: low platelets (<100.000 per μl), low fibrinogen (<300 mg/dl), or prolonged prothrombin (>14 seconds) time.

Fetal growth restriction was defined as birth weight less than the fifth percentile. Fetal mortality was defined as intrauterine death of a fetus at any time during pregnancy. Perinatal mortality was defined as fetal death plus neonatal death within the first 7 days. Gestational age was determined according to the last menstrual period or first trimester ultrasonographic biometry. All patients received magnesium sulfate (Magnezyum sulfat 15% in 10 ml, Biofarma, Istanbul, Turkey) as a 4.5 g intravenous loading dose followed by a 2 g maintenance dose per hour. Continuous infusion of glycerol trinitrate (Perlinganit, 10 mg, Adeka, Samsun, Turkey) was administered to control severe hypertension. An aggressive management protocol was decided on. All cases over 34 weeks of gestation were delivered promptly. Pregnancies before 34 weeks of gestation were terminated within 48 hours following corticosteroid therapy. 12 mg betamethasone (Celestone amp, 3 mg, Eczacıbası, Istanbul, Turkey) was administered intramuscularly twice at a 24 hour interval. Induction of labor and delivery route were recorded. Apgar score, birth weight, requirement for neonatal intensive care unit and neonatal outcomes were evaluated. Developing complications were recorded.

Statistical analysis was carried out using SPSS 11.0 for Windows (SPSS Inc, Chicago, III, USA) statistical software. Categorical variables were described using frequency distribution and compared by Chi-square and Fisher's exact test. For continuous variables, descriptive statistics were calculated and reported as mean±standard deviation. Student-t test was used to compare mean scores of continued variables between two groups. $p<0.05$ was accepted as the level of significance.

Results

Between June 01, 2003 and June 01, 2010, of 8384 deliveries 44 cases were found to be complicated with HELLP syndrome. In this period, 232 deliveries were complicated with preeclampsia. The incidence of HELLP syndrome was 0.52 percent in our department. While 37 patients met the complete criteria for HELLP syndrome, 7 patients were classified as partial HELLP syndrome. Eighteen cases (40.9%) were nullipara and twenty-six cases (59.1%) multipara. The most common presenting clin-

Table 1. Histories and clinical features of HELLP syndrome cases

	Number	Percent
History		
Multiply pregnancy	2	4.5
Assisted Reproductive technology	2	4.5
Diabetes	2	4.5
Previous cesarean	4	9.1
Symptoms at presentation		
Headache	2	4.5
Edema	9	20.5
Epigastric discomfort	7	15.9
Nausea-vomiting	3	6.8
Hypertension	15	34.1
Convulsion	3	6.8
Confusion	1	2.3
Labor	4	9.1

ical feature was that the patient was referred for hypertension. Historical and clinical features of HELLP syndrome patients were showed in Table 1.

Five of the patients had arterial systolic blood pressures >160 mmHg or diastolic blood pressures >110 mmHg which are the criteria for severe preeclampsia. The platelet count was below 50,000 mm^3 in 12 patients, between 50,000 and 100,000 mm^3 in 28 patients, and between 100,000 and 150,000 mm^3 in four patients. Proteinuria was below 300 mg/24 hours in nine patients (20.5%), and over 5 g in one patient. Clinical and laboratory characteristics of the patients with HELLP syndrome were presented in Table 2.

Fetal growth restriction was determined in 16 patients. The presence of growth restricted fetuses did not predict either maternal (p=0.325) or fetal (p=0.864) adverse outcomes.

Gestational age at diagnosis among patients whose babies were growth retarded was significantly higher than that among patients without this complication (35.6±2.7 vs 32.6±4.8 weeks' gestation; p=0.03).

Preventive corticosteroid therapy was administered in 18 pregnancies before 34 weeks of gestation and all of them were terminated within 48 hours.

In forty cases, the pregnancies were terminated by cesarean section within 48 hours after diagnosing HELLP syndrome. General anesthesia was selected and performed for all cesarean sections. Indications for cesarean section included fetal distress, previous cesarean section, failed induction of labor, unfavorable cervix and persistent high arterial blood pressure (above 160/110 mmHg). The rate of cesarean section was 90.9% in this series.

Seven of the cases developed placental detachment and three of them had intrauterine fetal demise. Postpartum hemorrhage developed in four patients. While three of them could be managed by medical treatment, hysterectomy was performed in one patient. Acute renal failure, sepsis, or maternal death did not occur in the present series.

Twenty of the 41 live-born babies needed neonatal intensive care. In fifteen of them, birth weights were below 1500 g. Early

Table 2. Clinical and laboratory characteristics of HELLP syndrome cases

HELLP Syndrome (n:44)	Mean	Standard deviation	Range History
Maternal age (years)	28.93	7.90	17-45
Parity	1.60	1.51	0-5
Gestational weeks	33.68	4.41	24-40
Systolic blood pressure (mm Hg)	172.78	27.18	130-230
Diastolic blood pressure (mm Hg)	107.22	9.58	90-130
Alanine aminotransferase (U/L)	314.44	294.42	41-991
Aspartate aminotransferase (U/L)	466.94	374.02	69-1329
Lactic dehydrogenase (U/L)	2644	1852	600-9132
Total bilirubin (mg/dL)	2.01	3.21	0.20-13.60
Direct bilirubin (mg/dL)	1.05	1.76	0.05-7.53
Urea (mg/dL)	17.29	5.20	6-28
Creatinine (mg/dL)	0.86	0.22	0.50-1.41
Hemoglobin (g/dL) (%)	12.02	1.50	8.73-14.60
Hematocrit (g/dL) (%)	35.78	4.77	25-42
Platelet count ($X10^9L$)	68584.09	32810.57	29000-168000
Fibrinogen level (mg/dL)	328.81	139.64	129-534
Prothrombin time (sn)	11.44	2.11	9.10-16.30
INR (sn)	0.96	0.17	0.75-1.34
Fetal weight (grams)	2071.82	800.24	570-3180
Apgar score	7.36	2.01	3-10
Blood pressure recovery time (day)	3.65	2.49	1-9
ALT recovery time (day)	3.0	1.71	1-7
LDH recovery time (day)	2.95	1.67	1-7
Platelet count recovery time (day)	2.80	1.9	1-5
Postpartum stay in hospital (day)	7.66	3.29	4-21

ALT: Alanine aminotransferase, INR: International normalised ratio, LDH: Lactic dehydrogenase

neonatal death occurred in eleven infants during the first seven days of postnatal period. In all of the pregnancies complicated with perinatal mortality, birth weights were below 1500 g. The main indications for neonatal intensive care admission of twenty cases were respiratory distress syndrome due to prematurity. Mean gestational age was significantly lower in patients with adverse fetal outcomes than those without these complications (30.7±3.5 vs 37.2±2.3 weeks; p<0.001).

Arterial systolic blood pressure among the patients with adverse fetal outcome was significantly higher than among the patients without adverse fetal outcome (175.0±23.0 vs 163.2±12.8 mmHg; p=0.048). Similarly, diastolic blood pressure among the patients with adverse fetal outcome was significantly higher than among the patients without adverse fetal outcome (109.8±8.8 vs 101.±9.6 mmHg; p=0.005). There was no association between adverse maternal outcome and nadir platelet counts <50000 cells/mm^3. The patients with intrauterine growth retarded fetuses had significantly lower platelet counts than patients without intrauterine growth retarded fetuses (247173.3±142503.4 cells/mm^3 vs 134240.0±52930.0 cells/mm^3; p=0.019). There was no association between perinatal morbidity and low platelet count.

There was no difference in recovery time of the arterial blood pressure, platelet count, serum alanine aminotransferase, and lactic dehydrogenase levels between the patients treated with and without corticosteroid therapy for rescue surfactant. Recovery time for alanine aminotransferase was significantly longer in patients who developed eclampsia than patients did not (4.4±1.4 U/L vs 2.2±1.4 U/L; p=0.003). There was no significant association between recovery time for other laboratory parameters or blood pressure and adverse outcomes. In Table 3, it can be seen that complications were caused by HELLP syndrome.

Table 3. The complications of patients with HELLP syndrome

Complication	Number	Percent
Hematuria	16	36.4
Visual loss	5	11.4
Pulmonary edema	4	9.1
Cerebral edema	5	11.4
Eclampsia	18	40.9
Abruption placenta	7	15.9
Disseminated intravascular coagulation	1	2.3
Postpartum hemorrhage	3	6.8
Hysterectomy	1	2.3
Induction of labor	3	6.8
Cesarean section	40	90.9
Fetal growth restriction	16	36.4
Neonatal intensive care	20	45.5
Fetal mortality	3	6.8
Perinatal mortality	14	31.8

The clinical and laboratory data were studied for the relation with each adverse outcome variables, then for the relation with the presence of at least one of the outcome variables. The results were summarized in the Tables 4 and 5. Because the results were not found significant for prediction of adverse maternal and fetal outcomes, so that correlation analysis was not performed.

Discussion

We presented the findings of our complete and partial HELLP syndrome series. Our rate of HELLP syndrome was in 0.52% of deliveries and 18.96% of severe preeclampsia, consistent with the previously reported values (4, 5).

The peak frequency of the HELLP syndrome was between the 27th and 37th gestational weeks; 10% occured before the 27th week, and 20% beyond the 37th gestational week. Most women with HELLP syndrome were multiparous (1, 4). We found that mean gestational age was 33 weeks, 11% occurred before the 27th weeks, 18% after the 37th gestational week. 59.1% of our series were multiparous. In about 70% of cases, the HELLP syndrome developed between the 27th and 37th gestational weeks. Hypertension and proteinuria were the most common symptom at presentation in the majority of patients with the HELLP syndrome, which may be absent in 10-20% of the cases. Up to 30-60% of women have headache and about 20% visual symptoms (2). Excessive weight gain and generalized edema precede the syndrome in over 50% of the cases (1). Hypertension was the reason for referral in 34% of cases, but it was found that all of the cases had hypertension, proteinuria and edema. At presentation, headache was present in 4.5% and visual symptoms in 11% of cases.

The HELLP syndrome is associated with serious maternal and fetal complications (2, 5, 6). It is not yet established whether expectant management in preterm pregnancies with HELLP syndrome would improve perinatal outcome or not. Therefore prompt delivery is recommended if the HELLP syndrome occurs after the 34th week or the fetal and/or maternal conditions deteriorate. Abruption placenta, acute renal failure, DIC and subsequent postpartum bleeding were reported as common maternal complications (1). Abruption placenta was strongly correlated with the development of disseminated intravascular coagulation. Our rate of abruption placenta was consistent with respect to other studies (3, 5, 7-9). However, disseminated intravascular coagulation was encountered at a relatively lower rate than in other series (3, 7, 9, 10). Postpartum hemorrhage developed in four patients who had placental detachment, which was consistent with respect to other studies (11). Acute renal failure is encountered between 5 and 36 percent in patients with HELLP syndrome (3, 5, 7, 10, 12, 13). The patients with postpartum HELLP syndrome have significantly higher incidences of pulmonary edema and renal failure (3). In the present study, even if some of the patients had temporary oliguria, none of them developed acute renal failure. Furthermore, the HELLP syndrome occurred in none of them during the postpartum period. The absence of acute renal failure and maternal mortality may be relevant to this condition.

Eclampsia was reported between 5 and 52 percent in previous series (5, 7, 9, 11). We encountered much more eclampsia in our series than previously reported. We preferred to perform a cesarean section in patients complicated with eclampsia. Therefore our rate of cesarean delivery was higher than in previously reported studies.

Haddad et al. (7) found that women with nadir platelet counts <50000 cells/mm^3 had increased risk for adverse maternal outcomes when compared with nadir platelet counts between 50000 and 10000 cells/mm^3. They revealed that the incidence of disseminated intravascular coagulation increased among women with nadir platelet counts <50000 cells/mm^3. However, the risk did not increase for eclampsia, abruption placenta or other maternal adverse outcomes. Laboratory parameters such as LDH, ALT, bilirubin serum concentration had not been found associated with any adverse maternal outcomes. Ertan et al. found that patients with low platelet counts (<60,000 cells/mm^3) had a higher incidence of intrauterine growth retarded fetuses than patients with higher platelet counts (13). On the other hand, Cavkaytar et al. (11) suggested that clinical symptoms such as headache, visual changes, epigastric pain and nausea-vomiting were better predictors of adverse maternal outcome than laboratory parameters. In this study, eclampsia, placental abruption and other maternal adverse outcome were not associated with clinical symptoms such as arterial blood pressure, or the other laboratory parameters. The patients with intrauterine growth retarded fetuses had significantly lower platelet counts than patients without intrauterine growth retarded fetuses in our series. However, there was no association between perinatal morbidity and low platelet count.

Perinatal mortality rate related to the HELLP syndrome is between 8 and 40 percent (1, 6, 8-10, 12, 13). Most of the perinatal deaths were associated with abruption placenta, intrauterine asphyxia and extreme prematurity (8). In the present study,

Table 4. The association between the clinical symptoms and maternal and fetal adverse outcomes.

	Adverse maternal outcome				p value
	present		absent		
	Number	Percent	Number	Percent	
Multiple pregnancy	3	12.5	23	88.5	0.258*
Pregnancies after assisted reproduction treatment	2	7.7	24	92.3	0.505*
Diabetes mellitus	1	3.8	25	96.2	1.00*
Headache	1	3.8	25	96.2	1.00*
Edema	5	19.2	21	80.8	0.809
Epigastric discomfort	4	15.4	22	84.6	1.00*
Vomiting	1	3.8	25	96.2	0.558*
Convulsion	3	11.5	23	88.5	0.258*
Confusion	0	0	26	100	0.409*
Hypertension	8	30.8	18	69.2	0.576
Labor	3	11.5	23	88.5	0.258*
Adverse fetal outcome					
Multiple pregnancy	3	12.5	21	87.5	0.239*
Pregnancies after assisted reproduction treatment	2	8.3	22	91.7	0.493*
Diabetes mellitus	0	0	24	100	0.455*
Headache	1	4.2	23	95.8	1.00*
Edema	3	12.5	21	87.5	0.261*
Epigastric discomfort	4	16.7	20	80.3	1.00*
Vomiting	1	4.2	23	95.8	0.583*
Convulsion	1	4.2	23	95.8	0.583*
Confusion	1	4.2	23	95.8	1.00*
Hypertension	9	37.5	15	62.5	0.601
Labor	3	12.5	21	87.5	0.614*

* Fischer exact test

Table 5. The association between the maternal clinical features and laboratory parameters and adverse maternal and fetal outcomes

	Adverse maternal outcome		
	absent	present	p value
Maternal age (years)	29.4±7.2	28.2±8.9	0.626
Parity	1.5±1.5	1.7±1.7	0.679
Gestational week	34.0±4.6	33.2±4.3	0.572
Systolic blood pressure (mm Hg)	166.7±18.6	173.9±21.2	0.242
Diastolic blood pressure (mm Hg)	106.1±8.3	105.8±12.3	0.924
Alanine aminotransferase (U/L)	341.7±233.1	275.5±139.3	0.287
Aspartate aminotransferase (U/L)	381.2±311.1	325.2±169.5	0.492
Total bilirubin (mg/dL)	1.7±1.31	2.7±2.9	0.134
Direct bilirubin (mg/dL)	0.8±0.9	1.4±1.7	0.194
Urea (mg/dL)	16.8±4.6	18.0±6.0	0.459
Creatinine (mg/dL)	0.8±0.3	0.9±0.2	0.694
Hemoglobin (g/dL)	12.2±1.9	12.4±1.2	0.846
Hematocrit (%)	35.2±5.5	36.9±3.6	0.404
Platelet count ($X10^9$L)	74584.6±34725.4	59916.7±28549.5	0.147
Fibrinogen level (mg/dL)	357.5±109.4	430.0±139.7	0.082
Prothrombin time (sn)	11.0±1.3	11.4±2.1	0.678
INR	0.9±0.1	0.9±0.2	0.687
Adverse fetal outcome			
Maternal age	28.1±8.0	29.9±7.8	0.442
Parity	1.7±1.7	1.5±1.3	0.756
Gestational week	30.7±3.5	37.2±2.3	<0.001
Systolic blood pressure (mm Hg)	175.0±23.0	163.2±12.8	0.048
Diastolic blood pressure (mm Hg)	109.8±8.8	101.5±9.6	0.005
Alanine aminotransferase (U/L)	253.6±132.7	387.8±244.2	0.025
Aspartate aminotransferase (U/L)	308.5±196.4	418.0±318.1	0.169
Total bilirubin (mg/dL)	1.7±0.9	2.6±2.9	0.191
Direct bilirubin (mg/dL)	0.8±0.7	1.3±1.6	0.182
Urea (mg/dL)	16.6±5.4	18.1±4.9	0.359
Creatinine (mg/dL)	0.9±0.2	0.8±0.2	0.557
Hemoglobin (g/dL)	12.5±1.7	12.1±1.1	0.598
Hematocrit (%)	36.4±5.0	36.0±3.6	0.876
Platelet count ($X10^9$L)	67325.0±28150.1	70095.0±38375.8	0.784
Fibrinogen level (mg/dL)	407.6±143.6	362.2±101.0	0.272
Prothrombin time (sn)	11.8±1.3	10.8±2.1	0.255
INR	1.0±0.1	0.9±0.2	0.235

the rate of perinatal mortality was 31%. The main reasons of neonatal mortality were the complications attributed to prematurity. Also information about about neonatal long term survey was not possible due to the retrospective nature of the study.

Maternal mortality rate is 1.1-9.0% in pregnancies complicated with the HELLP syndrome (3, 6, 10, 12, 13). Although the most reported cause of death is cerebral hemorrhage, it was stated that multiple organ failure, disseminated intravascular coagulation and adult respiratory distress are contributing pathologies (1). Aggressive management of HELLP syndrome with expeditious

delivery prevented development of adult respiratory distress syndrome, acute renal failure, and maternal mortality in the present series, and also yielded higher perinatal mortality due to prematurity than previously reported series (14).

In their retrospective study, Meccacci et al. (15) stated that the use of dexamethasone in patients with HELLP syndrome was associated with faster regression and lower incidence of complications in comparison to heparin. In the current cohort, betamethasone, not dexamethasone, was used; no association was found between corticosteroid therapy and recovery time or maternal complications. Recovery time was longer in patients who developed eclampsia than patients without this complication.

Postpartum hospital stay is shorter than 6 days in 63% of Vigil-De Gracia's series and longer than eleven days in 8% of them (5). In the present cohort, 29 percent of the patients stayed in hospital shorter than 6 days and 13.6 percent of the patients longer than eleven days.

In the present study, we investigated the effect of clinical features and laboratory parameters related to HELLP syndrome. The patients in earlier weeks of gestation had higher arterial blood pressure, higher serum alanine aminotransferase levels, and consequently a more severe clinical picture. The development of the HELLP syndrome in early gestational weeks increased adverse fetal outcomes. However, no other association was found between clinical or laboratory features and adverse outcomes. Prompt intervention could have prevented maternal mortality. In view of the increased risk of morbidity and mortality, delivery is considered the ultimate therapeutic approach for the HELLP syndrome.

Conflict of interest

No conflict of interest was declared by the authors.

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HPV DNA and Pap smear test results in cases with and without cervical pathology

Servikal patolojisi olan ve olmayan olgularda HPV DNA ve Pap smear test sonuçları

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Abstract

Objective: The aim of the study was to determine the HPV prevalance and its relation to Pap smear, colposcopy and colposcopy directed biopsy in our region of Eskisehir, Turkey.

Material and Methods: A total of 615 women who applied to the outpatient clinic between December 2009 and December 2010 constituted our study population. All patients underwent pelvic examination and Pap smear sampling. Patients who had pathological cervical appearance or Pap smear results of ASCUS, AGUS, LSIL or HSIL were referred to colposcopy. Cervical samples for HPV DNA were taken from the patients before Pap smear sampling during the routine examination or before the colposcopic evaluation.

Results: Twenty six of 615 patients (4%) were HPV positive. Of these 26 patients, 12 were positive for HPV type 16, 3 for type 18, 3 for type 51, 2 for type 6, 1 for type 52, 1 for type 33, 1 for type 16 and type 31, 1 for type 6 and 52, 1 for type 56 and 90, 1 for type 39 and 66. In 4 patients with cervical cancer, and in 3 of 4 CIN III cases both HPV DNA and Pap smear were positive. In the Pap smear examination of 615 patients, cytology revealed 35 ASCUS (5.6%) 4 AGUS (0.6%), 2 CIN I (0.3%) results who were negative for HPV DNA. These patients with abnormal cytology (n=41) underwent colposcopy directed biopsy, there were 3 CIN I and 1 CIN III and all the other cervical biopsy results of these patients were benign (inflammation, chronic cervicitis).

Conclusion: HPV positivity in our hospital setting is low which is compatible with other studies in Turkey. In positive HPV cases there is a good correlation between HPV type and positive cervical biopsy results. (J Turkish-German Gynecol Assoc 2012; 13: 8-14)

Key words: HPV DNA, Pap smear, colposcopy directed biopsy

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Özet

Amaç: Çalışmanın amacı Türkiye'nin Eskişehir bölgesinde HPV prevalansı ve bunun Pap smear, kolposkopi ve kolposkopik biopsi ile ilişkisini belirlemektir.

Gereç ve Yöntemler: Aralık 2009 ile Aralık 2010 tarihleri arasında polikliniğimize başvuran 615 hasta çalışma grubumuzu oluşturdu. Bütün hastalara pelvik muayene yapılıp, hastaların hepsinden Pap smear alındı. Serviksinde patolojik görünüm olan hastalara veya Pap smear sonucu ASCUS, AGUS, LSIL veya HSIL olan hastalara kolposkopik inceleme yapıldı. Hastalardan ya rutin değerlendirme sırasında Pap smear alınmadan önce ya da kolposkopik muayene öncesinde HPV DNA için servikal örnek alındı.

Bulgular: Altıyüzonbeş hastanın 26'sı (%4) HPV pozitifti. Bu 26 hastanın 12'sinde HPV tip 16, 3 tanesinde tip 18, 3 tanesinde tip 51, 2 tanesinde tip 6, 1 tanesinde tip 52, 1 tanesinde tip 33, 1 tanesinde tip 16 ve tip 31, 1 tanesinde tip 6 ve 52, 1 tanesinde tip 56 ve 90, 1 tanesinde tip 39 ve 66 mevcuttu. Serviks kanseri olan 4 hasta ve CIN III olan 4 hastanın 3'ünde hem HPV DNA hem de Pap smear pozitifti. Altıyüzonbeş hastanın Pap smear sonuçlarına bakıldığında HPV DNA negatif olan 35 ASCUS (%5.6), 4 AGUS (%0.6), 2 CIN I (%0.3) olan hasta vardı. Anormal sitolojisi olan bu 41 hastaya kolposkopik biopsi yapıldı ve sonuçta sadece 3 hastada CIN I, 1 hastada CIN III varken kalan hastaların biopsi sonuçları benigndi (inflamasyon, kronik servisit).

Sonuç: Hastanemizde yapılan bu çalışmada elde edilen HPV oranları Türkiye'de yapılan diğer çalışmalarla uygun olarak düşük bulundu. HPV pozitif olan vakalarda HPV tipi ile pozitif servikal biopsi sonuçları arasında kuvvetli bir korelasyon mevcuttur.

(J Turkish-German Gynecol Assoc 2012; 13: 8-14)

Anahtar kelimeler: HPV DNA, Pap smear, kolposkopik biopsi

Geliş Tarihi: 14 Ağustos 2011 **Kabul Tarihi:** 11 Eylül 2011

Introduction

Human papilloma virus (HPV) is known as the most common venereal disease. More than 200 types of HPV are known and more than 100 of them cause diseases in humans in a wide spectrum, varying from simple warts to genital cancers. Viral infection in the genital region may result in tumour development, which may cause genital region cancers such as cervical cancer.

Diagnosis of HPV starts with clinical suspicion and certain diagnosis can be attained by histological, cytological examinations and molecular methods looking for HPV DNA. At present, there are various molecular methods for detecting HPV DNA. Expectation from these methods is to detect high grade lesions and risk of developing high grade lesions and also to detect women with low risk.

In our study, we aimed to find the HPV prevalence in the Eskişehir region and to find the relationship between

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Pap smear, colposcopically directed biopsy and HPV DNA positivity.

Material and Methods

Six hundred and fifteen women applied to ESOGU Medical Faculty Gynaecology outpatient clinic between December 2009 and December 2010 were included in this study. Patients were randomly selected as one from all ten patients.

All patients were informed about the study and all of them filled the form which was prepared for this study. Patients who were sexually active and who had uterus and cervix were included in the study. Ninety-seven of 712 patients who did not give informed consent to take part in the study were excluded. All patients underwent pelvic examination and Pap smear sampling. Patients who had pathological cervical appearance or Pap smear results of ASCUS, AGUS, LSIL or HSIL were referred to colposcopy. According to the above criteria one hundred and ninety four patients underwent colposcopic evaluation. Cervical samples for HPV DNA were taken from the patients before Pap smear sampling during the routine examination or before the colposcopic evaluation (Figure 1).

Cervical Pap smears and biopsies were examined by the pathologists unaware of the study.

Cervical samples of patients were taken and sent to the Microbiology Laboratory in Hybrid Capture Specimen Transport Medium (DIGENE). These specimens were stored at -70º until they were studied. HPV DNA was extracted from the samples by spin column method with Qiagen PureArt DNA Mini kit (Qiagen GmbH, Hilden/Germany). Then PCR was performed with each of prime Mix A and Mix B (Mix My 11/09 and Mix 125) provided by the LCD-Array HPV 3.5 kit (CHIPRON GmbH, Germany) on Gene Amp PCR system 9700.

Amplification was run as follows: Initial denaturation of 96ºC for 3 minutes, followed by 42 cycles of 94ºC/1min, 45ºC/1 min 30 sec, 72ºC/1 min 30 sec, and finally 3 minutes at 72ºC. Following this, amplification hybridization was performed; 22 μl of modulator per one patient were mixed in an Eppendorf tube. Then 10 μl of DNA was added. Thirty μl of this solution was added to each well of the LCD-Array. Eight patients were studied on one slide.

The slide was incubated for 30 minutes at 35ºC. Then the slide was rinsed 3 times in washing solutions and dried by centrifugation at 1000 rpm. "A 30" μl labelling solution mix was added to each well and slides were incubated 5 minutes at room temperature. Then washing steps were repeated and the slides were dried. 30 μl of stain solution were added on each well and incubated for 3 minutes at room temperature. The slides were rinsed and dried. After this step, hybridized sports were evaluated by automated analysis system (Computerized Kodak Prime Film 2700-Slide Reader Software). Paired spots on the wheel of the slides were reported as HPV types.

All data analyses were done by SPSS 15.0 and SigmaStat 3.5 package programs. Continuous quantitative data were

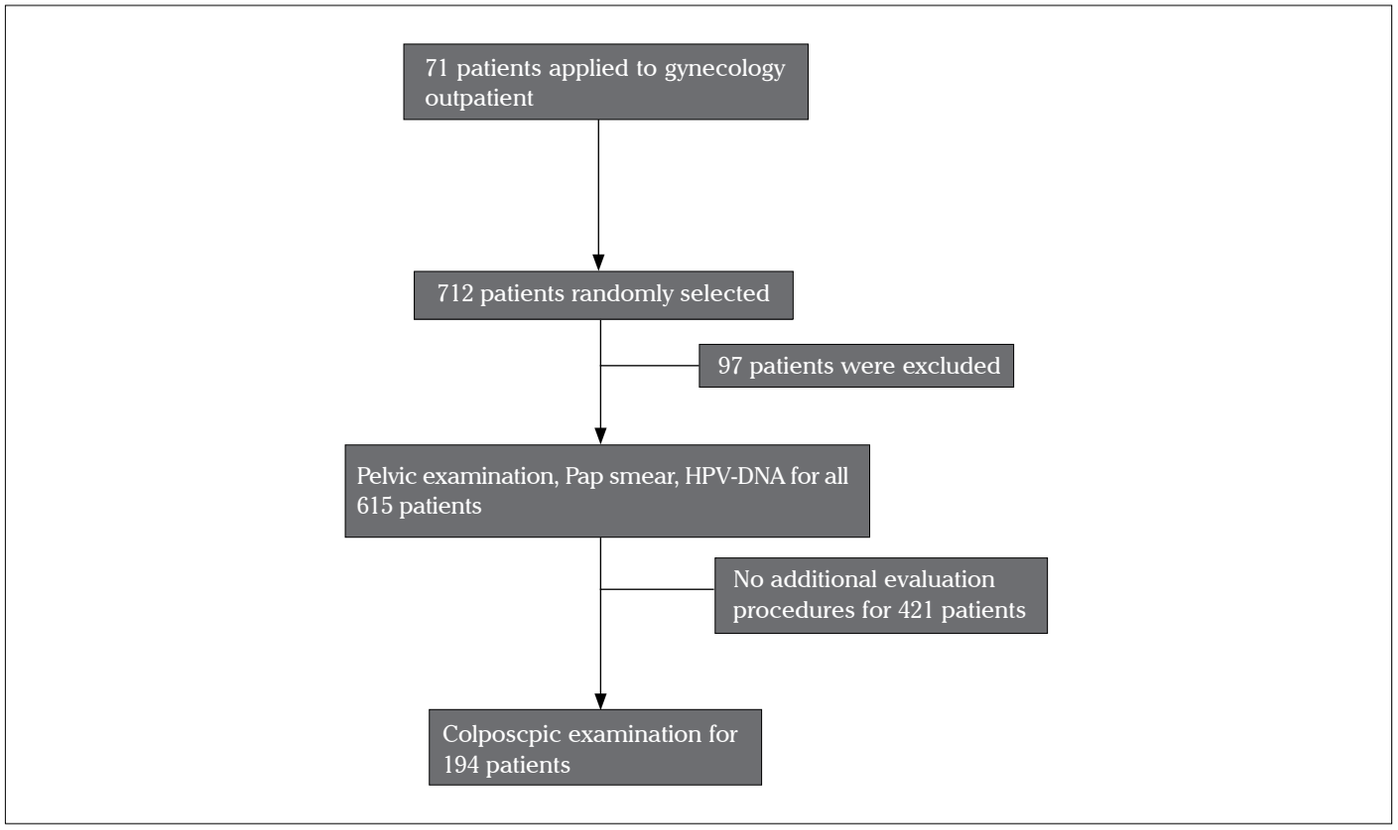

Figure 1. Patient selection and evaluation algorithm

denoted as; n, mean and standard deviation; qualitative data were denoted as n and percentage. One Way Anova and T-Test were used due to group number to analyse data consist with independent measurements showing normal distribution. Pearson Correlation Test was used to determine the relationship between the groups. To analyze the variations that do not distribute normally, Kruskal Wallis and Mann-Whitney U tests were used. Spearman Correlation Test was used to determine relationship between the groups. Data in a categorical structure were analyzed by Chi square test. A p value<0.05 was accepted as significant.

Results

Demographic characteristics of the patients included in the study are shown in Table 1.

Mean age of the women included in the study was 38.6±10.8 years. Mean age was 35.7±11.8 years in the HPV DNA positive group; and 38.7±10.7 years in HPV DNA negative group; and there was no significant difference between the two groups. 538 (87%) of the patients included in the study were married, 24 (3.9%) were single and 53 (8.6%) of them were widowed.

Table 1. Demographic characteristics

	HPV DNA positive	HPV DNA negative	p
Age (years)	35.7±11.8	38.7±10.7	>0.05
Gravidy (n)	2.3±2.2	2.6±1.9	>0.05
Parity (n)	1.4±1.2	1.7±1.2	>0.05
Abortus (n)	0.6±0.3	0.7±0.3	>0.05
Duration of Marriage (years)	14.4±12.6	17.1±11.6	>0.05
Age of Partner (years)	39.6±12.8	42.1±11.2	>0.05
Contraceptive Method			
None (n)	11	295	>0.05
Oral Contraceptive (n)	3	39	
IUD (n)	2	54	
Condom (n)	5	58	
Coitus Interruptus (n)	3	99	
Depo Progesterone (n)	0	1	
Tubal Ligation (n)	2	34	
Others (n)	0	9	
Duration of Contraception (years)	8.2±8.0	7.3±7.1	>0.05
Coitus per Week (n)	2.0±1.1	1.7±1.0	>0.05
Gynaecological Examination per life (mean) (n)	6.5±6.3	8.1±7.5	>0.05
Place of Birth			
Urban (n)	15	337	>0.05
Rural (n)	11	252	
Place of residence			
Urban (n)	20	406	>0.05
Rural (n)	6	183	
Cigarette smoking			**>0.05**
Positive (n)	9	139	>0.05
Negative (n)	17	450	

Table 2. The relationship between HPV positivity and cytology

		Normal Pap smear	Pathological Pap smear	p
HPV-DNA result	Negative	545	44	<0.05
	Positive	19	7	

HPV-DNA was positive in 26 (4.2%) of 615 patients, 14 (7.2%) of 194 patients who underwent colposcopy and 12 (2.8%) of 421 patients to whom colposcopy was not applied ($p<0.05$).

There was a significant difference between the two groups in terms of marital status and number of sexual partners.

Pap smear results were significantly different between the two groups (Table 2).

HPV-DNA types and Pap smear results of the HPV-DNA positive patients are shown in Table 3.

The relationship between HPV presence, type of the HPV and histology among patients examined by colposcopy is shown in Table 4. Colposcopy directed biopsy was not applied to 3 of 12 HPV type 16 positive cases, 1 of 3 HPV type 18 cases, 2 of 3 HPV type 51 cases, 1 of 2 type 6 cases and type 33, 56 and 90, 39 and 66, 52 cases (n:1) as pelvic examinations and Pap smears of these patients were normal.

One of 12 patients with Type 16 HPV DNA had LGSIL on Pap smear and the Pap smear of the patient who was positive for type 16 and type 31 HPV DNA was reported as HGSIL (Table 3). Three patients with Type 16 HPV DNA and the patient with Type 18 HPV DNA were diagnosed as cervical cancer; their Pap smears were positive for malignancy and the Pap smear of the one patient who had Type 51 HPV DNA positive was reported as HGSIL; her colposcopic biopsy result was found to be CIN 3 (Table 3, 4).

According to Pap smear results, all 35 patients that were detected as ASCUS were HPV DNA negative, whereas only 3 of those 35 patients' colposcopic biopsy results were CIN 1, 1 patient's biopsy result was CIN 3, the other 31 patients' biopsy results were reported as benign. The algorithm of patient evaluation is given in Figure 2. All AGUS, LSIL and HSIL patients at HPV-DNA negative group had benign pathology results. In the HPV-DNA positive group, 1 LGSIL patient's biopsy and 2 HGSIL patients' colposcopic biopsy revealed CIN 3.

Table 3. The relationship between HPV type and cytology in HPV-DNA positive patients

HPV Type	PAP SMEAR					
	Negative	ASCUS	AGUS	LSIL	HSIL	Cancer
16 (n=12)	8	-	-	1	-	3
18 (n=3)	2	-	-	-	-	1
51 (n=3)	2	-	-	-	1	
6 (n=2)	2	-	-	-	-	-
52.6 (n=1)	1	-	-	-	-	-
16.31 (n=1)	-	-	-	-	1	-
56.90 (n=1)	1	-	-	-	-	-
39.66 (n=1)	1	-	-	-	-	-
33 (n=1)	1	-	-	-	-	-
52 (n=1)	1	-	-	-	-	-
HPV DNA (-)	545	35	4	3	2	-

Table 4. The relationship between HPV presence, type and histology among the patients examined by colposcopy

	Colposcopy directed biopsy				
HPV Type	Negative	CIN I	CIN II	CIN III	Ca
16	5	-	-	1	3
18	1	-	-	-	1
16 and 31	-	-	-	1	-
51	-	-	-	1	-
33	-	-	-	-	-
52 and 6	1	-	-	-	-
56 and 90	-	-	-	-	-
52	-	-	-	-	-
39 and 66	-	-	-	-	-
6	1	-	-	-	-
Total	177	8	0	5	4

Discussion

Cervical cancer is a significant health problem especially in developing countries. For prevention, early detection and treatment of the preinvasive lesions that can progress to cervical cancer is very important.

In the United States (in 2009), the number of estimated new cases of invasive cervical cancer was 1270 and expected number of cancer related deaths was 4210. This represents approximately 1.5 percent of cancer deaths in women (1). Cervical cancer is the second most common cancer among women worldwide, with 83% of cases occurring in developing countries (2).

HPV infection is detected in 99.9% of the cervical cancer cases. Although the infection improves without any treatment in most women, the women with persisting HPV infection are at risk for CIN II, III and cervical cancer.

The Papanicolaou (Pap) smear screening test for cervical cancer was introduced in the United States in 1941, and led to the first systematic effort to detect early cancer. It has been associated with a sustained reduction in cervical cancer incidence and mortality.

The Pap smear has become a model for cancer screening. However, the effectiveness of Pap smear screening for cervical cancer has never been demonstrated in a randomized trial. Cervical cancer screening remains an evolving field with ongoing reevaluation of Pap smear screening practices and development of new screening technologies.

The Pap smear is designed as a screening test (to be administered to asymptomatic patients), rather than a diagnostic test (to confirm or refute the suspicion of disease). Reports of test sensitivity and specificity vary significantly. In a systematic review conducted by Nanda et al., sensitivity of Pap smear was found between 30%-87%, specificity was found between 86%-100% (3).

Although the Pap smear is the routinely used screening tool for cervical cancer, HPV-DNA screening in cervical samples is recommended as either an altenative or adjunvtive treatment to cervical cytology.

Considerable interobserver variability in smear interpretation is seen, although variability decreases as the grade of cervical intraepithelial neoplasia grade increases (4).

In a review by Cuzick et al. (5), more than 60,000 women were included. The sensitivity and specificity of HPV testing were

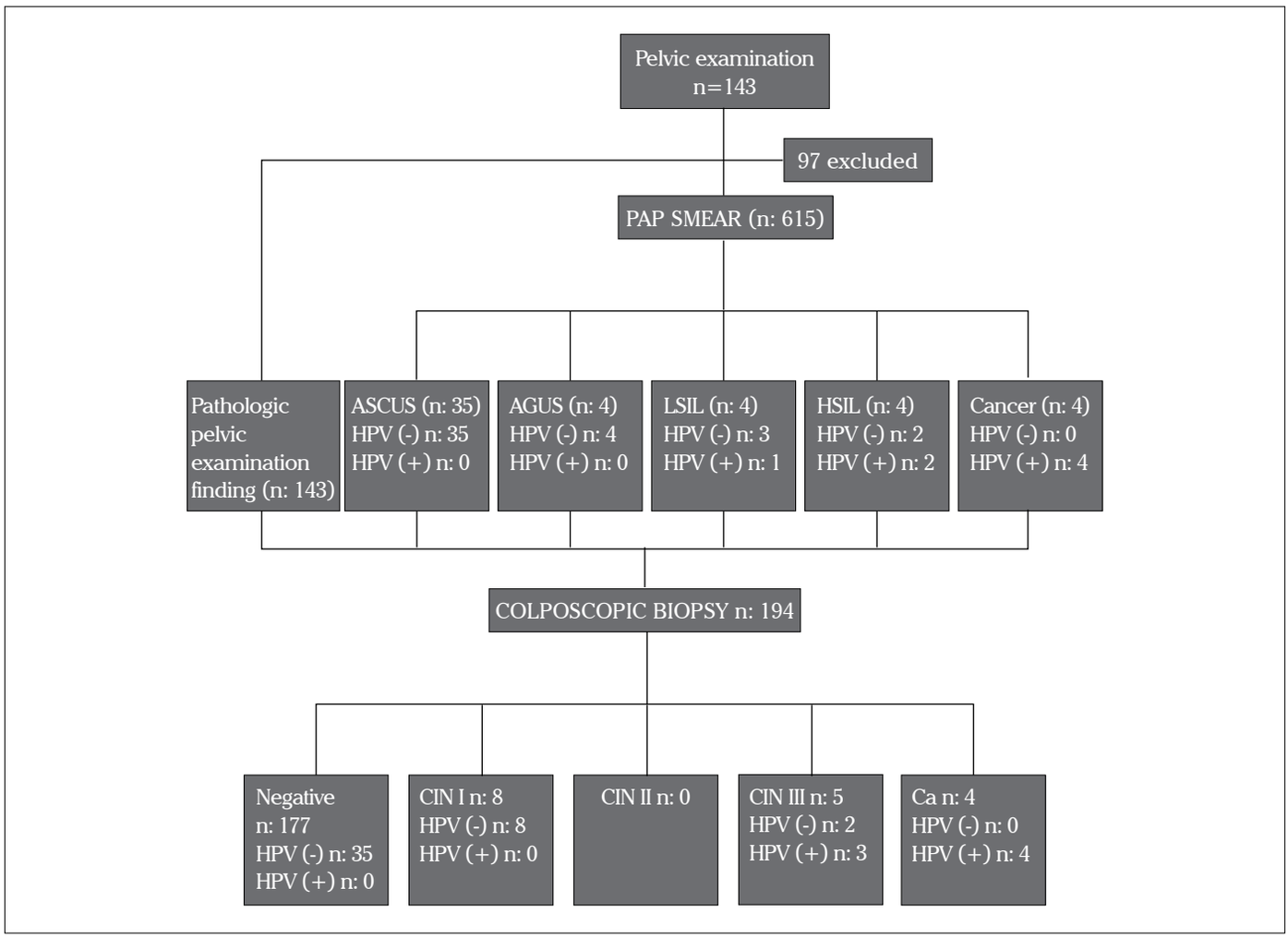

Figure 2. The algorithm of patient evaluation

compared with routine cytology. For lesions ≥CIN 2, HPV DNA was more sensitive (96.1% vs. 53.0%) but less specific (90.7% vs. 96.3%). According to that study, HPV sensitivity was uniformly high for all age groups, whereas the sensitivity of cytology was substantially better in women over the age of 50 than in younger women (79.3% vs. 59.6%). The specificity of both tests increased with age.

Kim et al. (6) showed that DNA testing had the potential to improve health benefits at a reasonable cost compared with current screening policies. According to Denny et al. (7), HPV DNA screening for the prevention of cervical cancer is particularly more usable for women older than 30 years, in the triage of women with equivocal cytology and for the post-treatment follow-up of women.

In the study that aimed to compare the sensitivity and specificities of HPV-DNA and Pap smear for 10154 women between the ages of 30-69, Koiopoulos et al. (8) found the sensitivity of HPV-DNA 94.6%, sensitivity of Pap smear 55.4%; specificity of HPV-DNA 94.2%, specificity of Pap-smear 96.8% for CIN II and III. When two tests are used together, sensitivity was 100% and specificity was 92.5%.

In the current study, in the HPV-DNA negative group, the Pap smear results of 35 patients were ASCUS, 3 patients were LGSIL, 2 patients were HGSIL. Only 3 of those 35 patients' colposcopic biopsy results were CIN 1, 1 patient's biopsy result was CIN 3, the other 31 patients' biopsy results were reported as benign. All AGUS, LSIL and HSIL patients at HPV-DNA negative group had benign pathology results. In the HPV-DNA positive group, 1 LGSIL patient's biopsy and 2 HGSIL patients' colposcopic biopsy revealed CIN 3 patients positive HPV-DNA and tumour cells in their Pap smear were diagnosed as cervical cancer. HPV-DNA was found positive in 19 patients who had normal Pap smears. Based on this information, it can be said that the HPV DNA test is very useful in early detection of cervical lesions and for the follow up of the patients with ASCUS Pap smears. Several studies showed that HPV-DNA is as cost-effective as recurrent Pap smears. In cases with ASCUS, if HPV DNA is evaluated, 50% of colposcopies will be unnecessary (9).

In our study, 3 of the 4 cervical cancer patients were positive for HPV type 16, and 1 of the cervical cancer patient was positive for HPV type 18. HPV type 16 and 18 are the most common HPVtypes found in cervical cancer cases. HPV type 16 is the most oncogenic type and type 18 is the second common. Munoz et al. classified 15 HPV types as high risk (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82), 3 types as probably high risk (26, 53, and 66) and 12 types as low risk (6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, and CP6108) (10).

In this study, HPV was positive in 4.2% of the study population and its incidence was 7.2% in the high risk group. In the studies about HPV from Turkey, the incidence was reported between 2.1% and 16.4% (11-16). In the study by de Sanjose et al. (17), overall HPV prevalence in 157879 women with normal cervical cytology was estimated to be 10.4% (95% CI 10.2-10.7). Corresponding estimates by region were Africa 22.1% (20.9-23.4), Central America and Mexico 20.4% (19.3-21.4), northern America 11.3% (10.6-12.1), Europe 8.1% (7.8-8.4), and Asia 8.0% (7.5-8.4). In all world regions, HPV prevalence was highest in women younger than 35 years of age, decreasing in women of older age. In Africa, the Americas, and Europe, a clear second peak of HPV prevalence was observed in women aged 45 years or older. Similar to our study, Monsonego et al. (18) found that the HPV incidence was significantly higher in the patients who underwent colposcopic biopsy because of abnormal Pap smears compared to the control group. As a result, it can be concluded that in this region, HPV-DNA incidence is lower. It may be because of traditions and customs, perspective of sexual life, and form of sexual behaviours.

Because of the definite causal relationship between HPV and perinvasive or invasive cervical pathologies, it will continue to exist as one of the most emphasized microorganisms. Several studies have shown the usefulness of HPV-DNA testing in the triage of patients with abnormal cytology or as a primary test. If HPV-DNA tests have a reasonable cost, it will be possible to use it commonly for the socially based screening programmes.

Conflict of interest

No conflict of interest was declared by the authors.

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Evaluation of risk factors in cesarean delivery among multiparous women with a history of vaginal delivery

Önceden vajinal doğum yapmış olan multipar kadınlarda sezaryen doğum risk faktörlerinin değerlendirilmesi

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Abstract

Objective: To predict the risk of cesarean delivery (CS) for multiparous women who have undergone previous vaginal delivery.

Material and Methods: A prospective observational study was performed, among multiparous pregnancies that were between 38 and 41 gestational weeks and had a singleton, vertex presentation fetus. Women's physical activity score, obstetric history, intrapartum and postpartum events were assessed. Multivariable logistic regression was used to explore risk factors associated with CS.

Results: Of the 245 total 83.7% had spontaneous labor and 16.3% were induced. Seventy-five percent of the induced women required CS, whereas only 19.5% of those with spontaneous labor required CS ($p<0.001$). The logistic regression analysis model included maternal weight gain, physical activity score, cervical dilatation, and fetal weight as the predictors of CS. We detected 7 (10%) maternal complications in women who underwent intrapartum CS.

Conclusion: Labor induction is significantly associated with increased risk of cesarean delivery among previously vaginally delivered women and maternal weight gain, physical activity score, cervical dilatation, and fetal weight are most accurate parameters in the prediction of the risk of CS delivery. Intrapartum CS has an increased risk of maternal morbidity.

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Key words: Labor induction, spontaneous labor, intrapartum cesarean, vaginal delivery, multiparity

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Özet

Amaç: Çalışmamızın amacı önceden vajinal doğum yapmış olan multipar kadınlarda sezaryen doğum riskini belirlemektir.

Gereç ve Yöntemler: Tekil, 38-41 haftada, verteks geliş gebeliği olan multipar kadınlarda yapılmış prospektif gözlemsel bir çalışmadır. Kadınların fiziksel aktivite skorlaması, obstetrik öyküleri, intrapartum ve postpartum olaylar değerlendirilmiştir. Çok değişkenli lojistik regresyon analizi yapılarek sezaryen ile ilgili risk faktörleri araştırılmıştır.

Bulgular: İkiyüz kırkbeş olgunun %83.7'si spontan eylem ve %16.3'ü indüksiyondu. İndüksiyon yapılan kadınların %75'i sezaryen gerektirirken spontan eylemi olanların sadece %19.5'ine sezaryen gerekti. Lojistik regresyon için seçilen model anne kilo artışı, fiziksel aktivite skoru, servikal dilatasyon ve bebek kilosunu sezaryenin belirteçleri olarak içermektedir. İntrapartum sezaryene giden 7 (%10) kadında maternal komplikasyon saptadık.

Sonuç: Daha önce normal doğum yapmış kadınlarda doğum indüksiyonu önemli ölçüde artmış sezaryen riski ile birliktedir; gebelikte alınan kilo, fiziksel aktivite skoru, servikal açıklık ve fetal kilo sezaryen riskinin belirlenmesinde en doğru parametrelerdir. İntrapartum sezaryen maternal morbidite riskini artırmaktadır.

(J Turkish-German Gynecol Assoc 2012; 13: 15-20)

Anahtar kelimeler: Doğum indüksiyonu, spontan eylem, intrapartum sezaryen, vajinal doğum, multiparite

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Introduction

High and rising rates of cesarean section delivery (CS) constitute an important health problem in Turkey. According to a report from the Turkish Ministry of Health, these operations represent 42.7% of deliveries in all hospitals nationwide in 2009 (1). Over the past two decades, a large proportion of the female population in Turkey has begun to enjoy a more urbanized lifestyle. The majority of the women we see in our daily obstetric practice are in this sedentary patient group. Advanced maternal age, decreased physical activity due to changing lifestyle, chronic health risks, such as obesity, diabetes, and hypertension, fetal macrosomia, extensive use of continuous fetal monitoring, and changes in women's preferences are the factors responsible for the elevated CS rates (2, 3). Today, women demand more involvement and control in decisions regarding their health, and many who are advised to have induced labor will ask their obstetrician whether this increases the likelihood of CS.

Previous studies indicated that emergency or intrapartum CS is associated with higher risk of maternal and fetal complications than vaginal and elective caesarean deliveries (2, 4, 5). Maternal mortality has also been shown to be higher in emergency caesarean deliveries (6).

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Early prediction of caesarean risk is important in order to decrease the maternal and fetal morbidity related to emergency CS. The objective of this study was to evaluate the risk factors for prediction of CS.

Materials and Methods

We conducted a prospective observational study between July 2010 and October 2010 at Fatih University Hospital, Ankara and at the State Hospital in Denizli, Turkey. The study protocol was approved by the review boards of both institutions. Each participant gave written informed consent before she was enrolled. A health professional at each institution (an obstetrics resident at Fatih University Hospital and a trained nurse at Denizli State Hospital) identified women who were admitted for delivery and who had a history of term vaginal delivery (VD). Multiparous pregnant women in this group who were between 38 and 41 gestational weeks and had a singleton, vertex presentation fetus were recruited for the study. Women with scheduled CS and those who had undergone CS in a previous pregnancy were excluded. Women who were induced because of pregnancy complications (such as pregnancy-induced hypertension, pre-gestational or gestational diabetes mellitus, intrauterine growth restriction oligohydramnios, polyhydramnios) and those who underwent elective induction between 38 and 41 gestational weeks were included.

Information regarding gestational age, maternal demographic characteristics, obstetric history, and intrapartum and postpartum events were obtained during interviews with the mothers. Gestational age was determined based on the last menstrual period and ultrasound findings in the first trimester. A total of 245 subjects were enrolled. Sixty-eight percent of the deliveries took place at Denizli State Hospital and 32% took place at Fatih University Hospital.

We assessed the women's physical activity using the Modified Grimby Scale (7). The categories were (1) moving only for necessary chores; (2) walking or other outdoor activities 1-2 times a week; (3) walking or other outdoor activities several times a week; (4) exercising 1-2 times a week to the point of perspiring and heavy breathing; (5) exercising more than 1-2 times a week to the point of perspiring and heavy breathing; (6) exercising for fitness several times a week to the point of perspiring and heavy breathing. Women in categories 1 and 2 were classified as sedentary and those in categories 3 and higher were classified as active. There is no Turkish validation of the Grimby scale.

Labor was diagnosed as regular uterine contractions combined with effacement of the cervix and dilatation of 2 cm or more and/or spontaneous rupture of the membranes. Induction was defined as initiation of uterine contractions to promote delivery before spontaneous onset of labor. Women who presented with contractions and required augmentation were not included in the induction group. Induction was carried out using oxytocin infusion and examinations were performed every 0.5 to 1 hours. Preinduction cervical ripening was not used at either hospital during the study period.

In our study, the term CS was used to refer to intrapartum CS. Indications of CS delivery were; failure to progress in labor, fetal distress, and failed induction. Failed induction was defined as CS performed before the cervix was dilated to 4 cm and the absence of non-reassuring fetal status. A patient was considered to have exhibited failure to progress if arrest of dilatation occurred after 4 cm or if arrest of descent was recorded in her medical record. Caesarean deliveries performed on maternal request during labor were excluded from the study.

The primary outcome was mode of delivery. We also examined the distribution of reasons for CS, and maternal and fetal complications arising from the type of the birth.

Statistical analyses were performed using SPSS software, version 17.0 (SPSS, Chicago, IL, USA). A power calculation was made using a computer program to determine the minimum sample size assuming alpha 0.05 and 95% power (G*Power Ver. 3.1.2, Franz FAUL, Universität Kiel, Germany, http://www.psycho.uni-duesseldorf.de/aap/projects/gpower/). A retrospective pilot study we conducted on 50 pregnancies in women with history of vaginal delivery revealed that 11% of these women had undergone CS in their most recent pregnancy. For the current study, we identified a sample size of 240 as sufficiently large to detect differences between vaginally delivered and CS required group in women with history of vaginal delivery.

The Shapiro-Wilk test was used to evaluate the distribution of variables. Because the data were not normally distributed, nonparametric tests were used for analyses. Categorical variables were compared using the chi-square test or Fisher's exact test. The Mann-Whitney U test was used to compare continuous variables. A p value of less than 0.05 was regarded as significant. A multiple logistic regression model (backward: likelihood ratio binary logistic regression) was used to assess independent risk factors for mode of delivery while controlling for potential confounders. Maternal age and gestational age were used as independent scale variables. Body mass index (BMI), weight gain during pregnancy, physical activity score, cervical dilatation upon admission, fetal weight at delivery, and type of labor (spontaneous or induced) were used as independent categorical variables. The probabilities of entry and removal were regarded as 0.05 and 0.10, respectively. All variables were included in stepwise multivariate logistic regression analysis using a backward elimination procedure. At each stage, the variable with the largest p value was eliminated and the procedure was then repeated until all remaining variables were statistically significant ($p<0.05$). Odds ratios (OR) and 95% confidence intervals (CI) were also calculated using multivariate logistic regression analysis.

Results

Two hundred and forty-five multiparous women with singleton, vertex pregnancies at 38-41 gestational weeks' were recruited to the study. Seventy (28.6%) of the 245 women underwent CS and 175 (71.4%) underwent VD. Of the 245 total, 205 (83.7%) had spontaneous labor and 40 (16.3%) were induced. Of the study population, 94.3% of vaginally delivered (VD) patients, and 57.1% of patients undergoing CS were admitted hospital with spontaneous labour, whereas 5.7% in the VD group and 42.9% in the CS group were admitted for induction of labor

(p<0.001). Thirty (75%) of the 40 women who were induced required CS, whereas only 19.5% (40) of those with spontaneous labor required CS (p<0.001).

Table 1 presents baseline characteristics, with the subjects grouped according to mode of delivery. There were no significant differences between the VD and CS groups with respect to maternal age, gravida, parity, maternal height or gestational age at delivery (p>0.05 for all). There was also no significant difference between these groups' median physical activity scores. Of the 245 pregnant women, 88.6% were sedentary and only 28 women were active. All the active women were in category 3 or 4; that is, none had a score above 4. Larger proportions of the sedentary women underwent induced labor and CS, respectively, compared to the active group; however, these differences were not statistically significant (Tables 1 and 2). The sedentary subgroup had a higher median pre-pregnancy BMI than the active subgroup (22.1 vs. 20.2, respectively; p>0.05) and also exhibited heavier fetal weights at delivery (3560 g vs. 3390, respectively; p>0.05), these differences also were not significant. Sedentary group gained more weight during pregnancy (13 kg vs. 11 kg, respectively; p=0.015).

Comparisons of the VD and CS groups showed that the median interval from most recent delivery to the index delivery was significantly shorter in the VD group than in the CS group (5 years vs. 7 years, respectively; p=0.023). Women in the VD group were, on average, of lighter weight than those in the CS group (BMI: 23.7 vs. 25.1, respectively; p=0.006), and gained less weight during pregnancy than the CS group (11.0 kg vs. 12.0 kg, respectively; p=0.037). Cervical dilatation on initial examination was significantly greater in the VD group than in the CS group (3.8 cm vs. 1.8 cm, respectively; p<0.001). The VD group had a lower median estimated fetal weight at the last examination, lighter fetal weight at delivery than in the CS group (p<0.05 for both).

Compared with the 165 women who had spontaneous labor and VD, the 40 who underwent spontaneous labor but ulti-

Table 1. Characteristics of women grouped by mode of delivery

	Vaginal deliveries (n=175)	Cesarean deliveries (n=70)	*p
Mean age (yrs)±SD	29.7±4.6	30.7±5.0	0.217
Gravida	2 (1)	3 (1)	0.655
Parity	2 (1)	2 (1)	0.808
Maternal height (cm)	160 (7)	162 (7)	0.250
BMI kg/m^2	23.7 (4)	25.1 (8)	**0.006**
Weight gain (kg)	11.0 (6)	12.0 (6)	0.037
Occupation (%)			0.223
Housewife	111 (63.4)	50 (71.4)	
Working women	64 (36.6)	20 (28.6)	
Physical activity score	1 (1)	1 (1)	
Sedentary	154 (88.0)	63 (90.0)	
Active	21 (12.0)	7 (10.0)	0.657
EFW (g)	3300 (600)	3500 (700)	**0.020**
Interval	5 (4)	7 (5)	**0.023**
1-5 years	117 (66.9)	31 (44.2)	
6-10 years	58 (33.1)	39 (55.8)	
Week of delivery	39.3 (2.0)	39.2 (2.1)	0.894
Cx dilatation (cm)	3.8 (2.0)	1.8 (1.9)	**<0.001**
Cx effacement (%)	40 (50)	30 (40)	0.113
Labor type (%)			**<0.001**
Spontaneous	165 (94.3)	40 (57.1)	
Induction	10 (5.7)	30 (42.9)	
Birth weight (g)	3300 (578)	3440 (660)	**0.025**

Values are noted as median (IQR: interquartile range), or mean±SD (standard deviation), *p<0.05 is significant, BMI: Body mass index at first prenatal visit, Weight gain: Weight gain during pregnancy, EFW: Estimated weight of fetus at the last perinatal sonography, Interval: time from previous delivery to index delivery, CX: cervix

Table 2. Characteristics of women grouped by type of labor

	Spontaneous labor group (n=205)	Induction group (n=40)	*p
Mean age (yrs)±SD	29.6±4.7	30.4±5.6	0.634
Week of delivery	38.5 (2)	39.2 (2)	0.169
Dilatation (cm)	4.0 (2.0)	2.0 (0.2)	0.029
EFW (g)	3350 (600)	3400 (937)	0.915
Interval (yrs)	5.0 (4.0)	5.5 (4.0)	0.231
Weight gain (kg)	12.0 (5.0)	11.0 (8.0)	0.711
Physical activity:			0.230
Sedentary	183 (87.6)	34 (94.4)	
Active	26 (12.4)	2 (5.6)	
Perinatal problem (%)	20 (9.7)	10 (25)	0.022
Birth weight (g)	3365 (595)	3400 (690)	0.558
CS (%)	40 (19.5)	30 (75.0)	<0.001
			OR=12.3;95%CI 5.5-27.3
Indications for CS			
Failure to progress	22 (55)	7 (23.3)	0.007
Fetal distress	8 (20)	6 (20.0)	0.987
Failed induction	-	12(40.0)	
Prolonged 2nd stage	10 (25)	5 (16.6)	0.620
Complications of CS	6 (15)	1 (3.3)	0.096
Complications of VD	14 (8.2)	2 (33.3)	0.082
Admission to NICU	3 (1.4)	3 (8.3)	0.018

Values are noted as medians (IQR: interquartile range) or mean±SD (standard deviation), *p<0.05 is significant, EFW: Estimated weight of fetus at the last perinatal sonography, CS: Cesarean delivery, VD: Vaginal delivery, NICU: Neonatal intensive care unit

mately delivered via CS had a longer interval from prior delivery to index delivery (Z=1743; p=0.081) and delivered heavier infants (Z=2853; p=0.004). The subgroup that was induced but required CS had a similar rate of CS-related complications than the subgroup with spontaneous labor that required CS (3.3% and 15% respectively, p=0.096). Seven of the 70 pregnant women that required intrapartum CS, developed complications: ureter injury (n=1), bladder injury (n=1), uterine artery rupture (n=1), uterine atony (n=4).

Table 3 shows the results of the logistic regression modeling. Maternal BMI was not significantly correlated with mode of delivery (p>0.05). In the initial χ^2 analysis, labor induction was associated with twelve-fold greater risk of CS delivery compared to spontaneous labor (OR 12.37, 95% CI 5.59-27.39; p<0.001); however, this risk was attenuated after adjustment for confounders (OR 3.32, 95% CI 1.44-10.44; p=0.007), (Table 3). The model identified maternal weight gain (OR 1.51, 95% CI 1.01-1.98, p=0.042), physical activity score (OR 0.12, 95% CI 0.10-0.72, p=0.028), cervical dilatation upon admission (OR 0.22, 95% CI 0.07-0.64, p=0.005), and fetal weight at delivery (OR 0.99, 95% CI 0.99-1.00, p=0.033) as predictors of CS.

Discussion

This study evaluated the factors for the prediction of cesarean delivery and the effect of physical activity on the ease of labor in multiparous women. We found that, of 245 women with a history of vaginal birth, 84% experienced spontaneous labor and 16% required labor induction. The induced multiparous women had a three-fold greater risk of CS than those who experienced spontaneous labor. This finding is consistent with reports of several authors (3, 8, 9) that showed a two- to threefold higher risk of CS among women who received labor induction. However, there are some important differences between previous studies and ours. First, most prior investigations have focused on nulliparous women, thus the rates of labor induction for this patient group were higher than the rate we observed. Second, most previous investigators applied cervical ripening methods before oxytocin infusion, whereas we did not. The proportion of women requiring labor induction in our study was small, but a much larger proportion of these women had pregnancy complications and than the group with spontaneous labor. Also, they had less favorable cervix and their infants were much more likely to require admission to the neonatal intensive care unit.

Table 3. Results for variables in logistic regression step 4*

Parameters	Wald	p	OR	95% CI for OR	
				Lower	Upper
BMI	3.194	0.059	1.307	1.079	1.589
Weight gain	4.205	0.042	1.512	1.010	1.981
Activity	4.857	0.028	0.127	0.104	0.726
Dilatation	7.255	0.005	0.225	0.079	0.643
Type of labor	6.037	0.007	3.320	1.447	10.440
Fetal weight	4.569	0.033	1.004	1.000	1.016

*Variables entered in step 1: Maternal age, gestational age at delivery, BMI, weight gain during pregnancy, physical activity score, type of labor, cervical dilatation upon admission, estimated fetal weight, and fetal weight at delivery, CI: confidence interval, OR: odds ratio, BMI: body mass index at the beginning of pregnancy

We prospectively studied clinical parameters to identify strong predictors of mode of delivery in women with a history of VD. Several authors (9-11) have reported, the risk of CS was influenced by labor induction, maternal weight gain during pregnancy, cervical dilatation at enrollment, and physical activity score of women. Results of our study showed that greater maternal BMI was statistically associated with CS delivery in the bivariate correlation analysis, but this correlation was not significant after adjustment for confounders. A new study has also identified excessive weight gain during pregnancy and medically induced labor as independent risk factors for intrapartum CS (12). Another study by Uyar et al. (13) showed that the BMI of women at the end of pregnancy and transvaginal cervical length were better predictors in determining the success of labor. However, our parameters were; BMI at the beginning of pregnancy and weight gain during pregnancy. Weight gain during pregnancy is an important determinant of the BMI at the end of pregnancy. We consider that this methodological difference leads to various results in this issue.

Regular physical activity has been proven to result in marked benefits for mother and fetus (14). Few studies that have directly examined the effects of physical activity on labour and delivery indicate that, for women with normal pregnancies, physical activity is accompanied with shorter labour and decreased incidence of operative delivery (15). We prospectively administered a modified version of the Grimby scale (which is extremely easy to use and interpret) to the patients in our study. After adjusting for maternal demographic factors, we also found that decreased physical activity of pregnant multiparous women was associated with increased risk of CS. The sedentary women in our sample were more likely to require labor induction than the active women, and pre-pregnancy BMI and fetal weight at delivery were also greater in the sedentary subgroup, but none of these differences was statistically significant. Many women in Turkey tend to live in city high-rises and have minimal physical activity. Our earlier observations led us to believe that women with low levels of physical activity would experience more problems at initiation of labor and throughout the labor process. Our initial expectation from this study was to find supporting evidence for this. Nearly 90% of the 245 subjects were sedentary and only 28 women were active with scores of 3 or 4, none higher. Melzer et al. (14) reported that the prevalence of a sedentary life style in the Swiss population is lower than 39%. We believe that the reason we did not find a significant statistically relationship between physical activity and type of labor was an insufficient number of physically active patients in the sample, which may led to Type II error. Further studies enrolling more women with higher levels of physical activity are required to confirm the association between physical activity and labor.

Intrapartum CS carries a higher risk of maternal morbidity than elective CS, hence, prediction of the former is important for optimal patient management (8). In our sample, we observed 10% morbidity in the subgroup that required intrapartum CS. The complication rate for intrapartum CS in multiparous women who had induced labor was not significantly different from the rate among those with spontaneous labor.

Nonetheless, the findings of this study must be interpreted together with recognition of its limitations. First, we were unable to record Bishops scores. Second, our study group was mostly composed of women with a sedentary life style, as well as the borderline statistical significance regarding the difference in incidence of labor induction and CS between active and sedentary women, which does not allow us to draw firm conclusions as to the effects of the recommended level of physical activity on the type of delivery.

This study prospectively evaluated the risk of CS in multiparous women with a history of VD. In conclusion, cervical dilatation at enrollment and labor induction are important predictors of CS. Increased weight gain during pregnancy, and decreased physical activity score are statistically associated with increased risk of CS. Roughly 10% of women who undergo intrapartum CS develop serious complications. In order to optimally manage labor, it is important to be able to predict which women are more likely to require CS. Accurate information should be provided to expectant mothers regarding their risk of CS and the higher risk of maternal morbidity after intrapartum CS. These are important elements in the decision-making process regarding type of delivery.

Conflict of interest

No conflict of interest was declared by the authors.

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Serum and follicular fluid Anti-Mullerian hormone concentrations at the time of follicle puncture and reproductive outcome

Follikül aspirasyon zamanı serum ve follikül sıvısında Anti-Mullerian hormon konsantrasyonu ve reprodüktif sonuçlar

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Abstract

Objective: The objective of the study is to determine and compare the levels of Anti-Mullerian hormone (AMH) and estradiol (E2) in serum and follicular fluid (FF) on the day of oocyte pick up (OPU) with the cycle parameters and the outcome of in vitro fertilization (IVF) treatment.

Material and Methods: The long stimulation protocol was used in 37 (86%) women; the microdose flare-up protocol was used in 6 (14%) women. Concentrations of AMH and E2 were measured in serum and FF of 43 women undergoing IVF treatment on the day of OPU.

Results: Significant positive associations were observed between serum AMH concentrations and the total number of oocytes retrieved (r=0.343, p=0.024). Serum AMH and FF AMH levels on the day of OPU were significantly increased in the group of women who achieved clinical pregnancy (p=0.017, p=0.028). For serum AMH, a cut-off level of 1.64 ng/ml was used for the prediction of clinical pregnancy; for FF AMH, a cut-off level of 3.8 ng/ml was used for the prediction of clinical pregnancy. Serum AMH and FF AMH levels were significantly and positively correlated with implantation rate (r=0.401, p=0.008; r=0.317, p=0.039). No significant correlation was found between serum and FF AMH concentrations and fertilization rate.

Conclusion: Serum AMH and FF AMH concentrations are positively correlated with implantation and clinical pregnancy rates.

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Key words: AMH, E2, follicular fluid, IVF

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Özet

Amaç: Çalışmanın amacı oosit aspirasyon günü serum ve follikül sıvısında Anti-Mullerian hormon (AMH) ve estradiol seviyelerini saptamak ve siklus parametreleri ve in vitro fertilizasyon tedavisi sonuçları ile karşılaştırmaktır.

Gereç ve Yöntemler: Otuz yedi (%86) hastada long stimulasyon, 6 (%14) hastada mikrodoz flare-up protokol uygulanmıştır. Oosit aspirasyon günü in vitro fertilizasyon tedavisi alan 43 hastanın serum ve follikül sıvısında AMH ve estradiol konsantrasyonları saptanmıştır.

Bulgular: Serum AMH konsantrasyonları ve toplanan oosit sayıları arasında anlamlı pozitif ilişki saptanmıştır (r=0.343, p=0.024). Oosit aspirasyon günü serum AMH ve follikül sıvısı AMH seviyeleri klinik gebelik saptanan hastalarda anlamlı olarak yüksek bulunmuştur (p=0.017, p=0.028). Serum AMH için 1.64 ng/ml, follikül sıvısı için 3.8 ng/ml klinik gebeliğin öngörülmesinde cut-off değeri olarak saptanmıştır. Serum AMH ve follikül sıvısı AMH seviyeleri ile implantasyon oranı arasında anlamlı pozitif korelasyon saptanmıştır (r=0.401, p=0.008; r=0.317, p=0.039). Serum ve follikül sıvısı AMH konsantrasyonları ve fertilizasyon oranları arasında anlamlı ilişki bulunmamıştır.

Sonuç: Serum ve follikül sıvısı AMH konsantrasyonları implantasyon oranları ve klinik gebelik oranları ile ilişkilidir.

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Anahtar kelimeler: AMH, E2, follikül sıvısı, IVF

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Introduction

Anti-Mullerian hormone (AMH), a member of the transforming growth factor-β (TGF-β) superfamily, is known to be derived from the granulosa cells of growing follicles (from the primary to the large antral follicle stage) (1). The TGF-β superfamily members inhibin A, inhibin B, and activin are related to ovarian follicle development as well as AMH, which is emerging as an important regulator of ovarian function, especially follicle selection and development (1, 2). Serum basal AMH levels correlate with the number of antral follicles and age (3). Serum basal levels of AMH significantly decrease over time in young normoovulatory women, whereas other markers associated with ovarian aging do not change (4). AMH has been shown to inhibit FSH-induced follicle growth in female mice (5). The period of AMH production coincides with the period of oocyte meiotic arrest (6). Within the human ovary, AMH inhibits follicle recruitment and FSH-dependent follicle growth as well as selection (2, 7), and prevents depletion of the primordial follicle pool (8).

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These inhibitory actions of AMH on early follicle development are overcome by the FSH treatment during *in vitro* fertilization (IVF). After gonadotropin treatment during IVF, the AMH concentrations in small and large antral follicles positively correlate with granulosa cell responsiveness to FSH (9).

Serum basal AMH concentrations are useful for predicting ovarian response in women undergoing IVF treatment (10); however, concerning the relationship between serum AMH levels and the pregnancy rates after assisted reproduction therapies, there are conflicting data in the literature. Some investigators (11-14) could not find a correlation between basal AMH levels and pregnancy, whereas others (15-18) observed a better prognostic value for clinical pregnancy than other available markers. The putative correlation of AMH in the follicular fluid (FF) and reproductive outcome has been investigated in several studies.

The aim of the present study was to investigate and compare the relationship of AMH and estradiol (E2) levels in serum and FF on the day of oocyte pick up (OPU) to the cycle parameters, prognostic parameters and the outcome of IVF treatment.

Materials and Methods

Patient characteristics and IVF procedure

Forty-three women aged between 18-40 years and undergoing IVF treatment were studied prospectively. Inclusion criteria included the presence of both ovaries, normal pretreatment hormonal status, and the absence of other endocrine pathologies, such as thyroid and adrenal disorders or hyperprolactinemia. Women with polycystic ovary syndrome were not included in the study. Informed consent was obtained from all women. The study was approved by the institutional review board of the hospital.

The long stimulation protocol was used in 37 (86%) women; the microdose flare-up protocol was used in 6 (14%) women. For the patients treated using the long protocol, pituitary down-regulation was achieved by gonadotropin-releasing hormone (GnRH) agonist, leuprolide acetate (Lucrin daily injection, Abbott, Turkey). Gonadotropin stimulation was initiated on the third day of the subsequent withdrawal bleeding. For the patients treated using the microdose flare-up protocol, a GnRH agonist was administered starting on the 24th day of the menstrual cycle, with the addition of gonadotropins on the 26th day. Dose adjustments were performed on the 4th day of stimulation and thereafter according to the sonographic findings and serum E2 levels. The women were administered human chorionic gonadotropin (HCG, Ovitrelle 250 mcg, Serono, Turkey) when at least 3 follicles had reached a diameter of >18 mm. Transvaginal oocyte retrieval was performed 36 hours later. All patients received luteal phase support of progesterone (Crinone 8% vaginal gel, Serono, Turkey) daily starting from the day of the OPU. Intracytoplasmic sperm injection (ICSI) was performed for all patients. Fertilization was assessed using an established pronuclei (PN) scoring system. Embryos were transferred on day 3 or 5 after OPU. One or 2 embryos were transferred depending on the woman's age and embryo quality. Embryo transfers were performed under transabdominal ultrasonographic guidance. Biochemical pregnancy was established when serum BHCG level >20 IU/l was found on day 12 after embryo transfer. Clinical pregnancy was defined as a positive serum BHCG result with ultrasound evidence of a gestational sac and fetal heart beat at 6 weeks from the date of embryo transfer.

Collection of serum and follicular fluid

Blood samples were obtained on the day of OPU immediately before the procedure. Sera were obtained after centrifugation and stored at -80°C. Follicular fluid was obtained from the first retrieved follicle to avoid contamination of blood and flush medium. Care was taken to avoid contaminated samples. Follicular fluid samples were centrifuged for 15 minutes at 3000 rpm and stored at -80°C before the analysis.

Determination of AMH and E2 in serum and follicular fluid

Serum and FF AMH levels were determined by using an ultrasensitive ELISA (AMH ELISA kit; Diagnostic System Laboratories, Texas, USA). Results were expressed as ng/ml. Serum and FF E2 concentrations were determined by an automated chemiluminescence technique (ADVIA Centaur CP, Tarrytown, USA).

Statistical analysis

Data analysis was performed using SPSS for Windows, version 11.5 (SPSS Inc., Chicago, IL, United States). Whether the distributions of continuous variables were normal or not was determined using the Shapiro-Wilk test. Continuous variables are expressed as mean±standard deviation or median (minimum-maximum), where applicable. The mean differences between groups were compared using Student's t-test. Otherwise, the Mann-Whitney U-test was applied for the evaluation of the median values. Degrees of associations between continuous variables were tested using Spearman's correlation analysis. Whether the effects of both serum and FF AMH on clinical pregnancy were statistically significant was evaluated by multiple logistic regression analysis adjustment for age and AFC. A p-value less than 0.05 was considered statistically significant.

Results

The study group consisted of 43 women with a mean age of 30.1±4.2 years. Thirty-one patients (72%) had unexplained infertility, and 12 patients (28%) had male factor infertility. Clinical and cycle parameters and FF and serum concentrations of AMH and E2 of the patients with respect to the pregnancy outcome are shown in Table 1. No difference was observed between the groups that did or did not achieve clinical pregnancy with respect to age, BMI, baseline E2, FSH, AFC, amount of gonadotropin used, number of MII oocytes or fertilization rate. Serum AMH and FF AMH levels on the day of OPU were significantly increased in the group of women who achieved clinical pregnancy (p=0.017, p=0.028). To investigate the correlation of

Table 1. Clinical and cycle parameters, FF and serum concentrations

Variables	Clinical Pregnancy (n=14)	No pregnancy (n=29)	p value
Clinical and cycle parameters			
Age (years, mean±SD)	29.3±4.4	30.5±4.2	0.249
BMI (kg/m^2, mean±SD)	24.4±3.7	26.0±4.9	0.299
Basal E2 (pg/ml,range)	52.5 (4-119)	52 (8-79)	0.816
Basal FSH (ıu/l, mean±SD)	6.2±1.59	6.6±2.65	0.657
AFC (n, range)	13 (6-24)	11 (0-24)	0.126
Dose of gonadotropins (IU, range)	1225 (900-4113)	1872 (600-7500)	**0.020**
MII oocytes (n, range)	7 (2-15)	7 (1-16)	0.516
Fertilization rate (%, range)	63.3 (9.1-100.0)	50.0 (12.5-100.0)	0.567
FF hormone levels			
AMH (ng/ml, range)	4.1 (0.35-13)	1.87 (0.32-16)	**0.028**
E2 (pg/ml, range)	544.6 (171.7-849.8)	422.2 (16,6-1385.0)	0.162
Serum hormone levels on the day of OPU			
AMH (ng/ml, range)	1.87 (0.04-4.15)	0.35 (0.03-14)	**0.017**
E2 (pg/ml, range)	1118.5 (59.1-2015.2)	712.4 (66.1-1984.1)	0.058

BMI: body mass index, AFC: antral follicle count, AMH: Anti-Mullerian hormone, FF: follicular fluid. Statistically significant p values are shown in bold

serum and FF AMH and clinical pregnancy, cut-off values were calculated using ROC analysis. For serum AMH, a cut-off level of 1.64 ng/ml was used to predict clinical pregnancy (sensitivity 71.4%, specificity 69%, positive predictive value 52.6%, negative predictive value 83.3%). For FF AMH, a cut-off level of 3.8 ng/ml (sensitivity 64.3%, specificity 79.3%, positive predictive value 60%, negative predictive value 82.1%) was used.

When multivariant logistic regression analysis was used for the prediction of clinical pregnancy, serum and FF AMH concentrations lost their statistical significance when adjusted for age and AFC. Serum and FF AMH were not the only independent factors for the prediction of clinical pregnancy.

Correlations between serum and FF AMH and E2 concentrations and clinical and hormonal parameters are shown in Table 2. Serum AMH concentrations were positively and significantly correlated with FF AMH concentrations (r=0.638, p<0.001). There was a significant positive correlation between serum AMH and E2 concentrations (r=0.429, p=0.004). Significant positive associations were observed between serum AMH concentrations and AFC, total number of oocytes retrieved, MII oocytes and implantation rate (r=0.476, p<0.001; r=0.343, p=0.024; r=0.389, p=0.01; and r=0.401, p=0.008, respectively). However, negative correlations were observed between serum AMH levels, age and the amount of gonadotropins used (r=-0.311, p=0.043; r=-0.596, p<0.001). FF AMH levels were significantly and positively correlated with AFC and implantation rate (r=0.343, p=0.025; r=0.317, p=0.039) and negatively correlated with baseline FSH levels (r=-0.333, p=0.029). No significant correlation was found between serum and FF AMH concentrations and fertilization rate.

Discussion

The assessment of the ovarian reserve before an IVF treatment is important to identify both poor- and high-responder patients. Poor ovarian reserve also predicts decreased fertility rates, although the relationship between declining ovarian reserve and decreased fertility rates remains incompletely understood (19). Several parameters have been proposed for the estimation of ovarian reserve including basal or clomiphene citrate-stimulated FSH levels, follicular phase inhibin B levels, ovarian volume, AFC and ovarian stromal blood flow. However, the associated predictive values remain controversial. In addition, it remains a challenge to identify young women with normo-ovulatory cycles but with a low ovarian reserve. Gnoth et al. demonstrated that AMH was an important screening test for reduced ovarian reserve in women. They proposed that, by using AMH levels ≤1.26 ng/ml, it was possible to identify 97% of women with reduced ovarian reserve and predict low response to gonadotropin stimulation in 88% of cases in groups of comparable age (20). Consistent with the study by Wunder et al. a significant correlation of serum AMH levels with ovarian response as expressed by the number of oocytes retrieved is observed in the present study (21). In a study by Elgindy et al. that investigated longitudinal changes in AMH levels during different phases of the menstrual cycle in patients undergoing IVF treatment, no significant differences were found between AMH levels taken on day 3, on the day of ovulation and 7-8 days later (midluteal phase). The number of oocytes retrieved was found to be significantly correlated with midluteal AMH, day 3 AMH and ovulatory AMH (22). In addition, Fanchin et al. observed

Table 2. Correlations between serum and FF AMH and E2 levels and clinical and hormonal parameters

	FF AMH		Serum AMH		FF E2		Serum E2	
	r	p	r	p	r	p	r	p
FF AMH	1.000	.	0.638	<0.001	0.218	0.161	0.062	0.694
Serum AMH on day of OPU	0.638	<0.001	1.000	.	0.170	0.275	0.429	**0.004**
FF E2	0.218	0.161	0.170	0.275	1.000	.	0.221	0.154
Serum E2 on day of OPU	0.062	0.694	0.429	0.004	0.221	0.154	1.000	.
Age	-0.174	0.264	-0.311	0.043	0.031	0.845	-0.277	0.073
AFC	0.343	0.025	0.476	<0.001	-0.171	0.273	0.165	0.291
Basal FSH	-0.333	0.029	-0.281	0.068	0.184	0.236	-0.065	0.679
Basal E2	-0.062	0.695	0.056	0.723	0.142	0.363	0.214	0.168
Dose of gonadotropins	-0.264	0.087	-0.596	<0.001	-0.087	0.579	-0.376	**0.013**
Total oocytes retrieved	0.072	0.647	0.343	0.024	0.072	0.648	0.568	**<0.001**
MII oocytes	0.143	0.361	0.389	0.010	0.055	0.725	0.586	**<0.001**
Fertilization rate	-0.148	0.344	0.029	0.854	0.047	0.767	0.125	0.424
Implantation rate	0.317	0.039	0.401	0.008	0.136	0.386	0.239	0.123

Statistically significant p values are shown in bold. Positive correlations (r>0), negative correlations (r<0), FF: follicular fluid, AMH: Anti-Mullarian hormone, OPU: oocyte pick-up, E2: Estradiol, FSH: Follicule stimulating hormone

a remarkable lack of variation of serum AMH levels between two distinct points in the menstrual cycle (day 3 and the day of OPU), showing that AMH levels remain stable throughout the cycle independent of FSH (9, 23). Our results, together with those of other studies in the literature, support the assumption that serum AMH levels can predict the number of oocytes that will be yielded by an IVF cycle. We also found a positive correlation between serum AMH and serum E2: the expected serum E2 concentrations are associated with the number of oocytes retrieved.

With respect to fertilization, we did not find a significant association between fertilization rate and serum or FF AMH levels. This finding is consistent with some, but not all, studies in the literature. Takahashi et al. (24) reported that the FF AMH levels of fertilized patients were 3.42 times higher than those of non-fertilized patients. However, they found no correlation between serum AMH and high-quality embryos. These results indicate that serum AMH levels did not reflect high-quality fertilization. In a study by Silberstein et al. (25), which included 257 patients, the authors found that AMH levels at the time of HCG administration reflect both ovarian reserve and better embryo morphology. However, Fanchin et al. and Wunder et al. (9, 21) found similar fertilization rates regardless of the AMH concentrations in serum or FF. Due to contradictory reports regarding fertilization, oocyte quality and embryo morphology in the literature, these issues deserve further investigation.

The results of this study indicate that serum and FF AMH levels are good predictors of implantation and clinical pregnancy. Cut-off values for both serum and FF AMH were calculated for the prediction of clinical pregnancy. Cut-off concentrations for serum AMH and FF AMH were found to be 1.64 ng/ml (71.4% sensitivity, specificity 69%) and 3.8 ng/ml (64.3% sensitivity, 79.3% specificity), respectively. High FF AMH levels constitute a useful marker for implantation. FF AMH concentrations reflect granulosa cell functioning. It is postulated that granulosa cell metabolism and embryogenic competence of the oocyte are interrelated (9). FF AMH concentrations on the day of OPU may contribute to embryo quality, which in turn yields competent embryos with high implantation potential, and thus results in a high probability of pregnancy. Our results are consistent with the results of a study by Fanchin et al. (9) They found that high clinical pregnancy and implantation rates correlated with FF AMH levels and concluded that FF AMH measurements could help to identify the embryos that are most likely to achieve implantation in IVF cycles. Silberstein et al. (25) found that AMH levels at the time of HCG administration (≥2.7 ng/ml) portended improved oocyte quality as reflected by higher implantation rates and a trend toward improved clinical pregnancy rate. In the study by Nelson et al. (26), which investigated the value of serum AMH in the prediction of live birth and ovarian response to stimulation, it was found that plasma AMH is an accurate predictor of live birth and strongly correlated to the risk of excessive response to ovarian stimulation.

There are several clinical implications of these findings. Serum AMH seems to be a predictor of ovarian reserve as represented by oocyte yield in IVF cycles. This may allow development of individualized and optimized treatment strategies prior to the first cycle of ovarian stimulation. It is also important for the counseling of couples for making decisions after a failed IVF cycle, especially in patients with low ovarian reserves.

Recent studies have shown that a low response to ovulation induction is associated with early menopause (20, 27, 28). AMH seems to be a promising parameter for the occurrence of menopausal transition (20, 29). Thus, AMH may serve as a screening test for diminished ovarian reserve, especially in patients with regular cycles (3).

The results of our study, which were confirmed by the study of Wunder et al. (21), demonstrated a positive correlation between serum and FF AMH concentrations. A finer determination of AMH in FF is not necessary because serum concentrations of AMH yield similar data.

Because FF AMH concentrations are positively correlated with implantation and clinical pregnancy rates, FF AMH measurement during embryo transfer may be an additional parameter aside from embryo morphology with which to distinguish the embryos that are most likely to achieve implantation and thus clinical pregnancy.

In conclusion, the results of the study indicate that serum AMH and FF AMH concentrations are positively correlated with implantation and clinical pregnancy rates. In addition, serum AMH concentrations are associated with the number of oocytes and the number of mature oocytes retrieved.

Conflict of interest

No conflict of interest was declared by the authors.

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Plasma apelin levels in patients with polycystic ovary syndrome

Polikistik over sendromlu hastalarda plazma apelin düzeyleri

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Abstract

Objective: The aim of the study was to evaluate plasma apelin levels in patients with polycystic ovary syndrome (PCOS) and healthy controls.

Material and Methods: Plasma apelin levels, serum lipid levels, serum hormone levels, and homeostasis model assessment-insulin resistance (HOMA-IR) values of 32 patients with PCOS and 31 healthy women forming the control group were checked.

Results: Plasma apelin levels of the PCOS group (0.350±0.083 ng/ml) were significantly higher than those of the control group (0.246±0.045 ng/ml) ($p<0.001$). No significant correlation was detected between apelin levels and biochemical or clinical data in PCOS group.

Conclusion: Plasma apelin levels were significantly higher in PCOS patients. (J Turkish-German Gynecol Assoc 2012; 13: 27-31)

Key words: Apelin, polycystic ovary syndrome, Homeostatic Model Assessment insulin resistance (HOMA-IR), Ferriman Gallwey score (FGS), waist to hip ratio

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Özet

Amaç: Bu çalışmanın amacı, polikistik over sendromu (PKOS) hastaları ve sağlıklı kontrol grubundaki hastalarda, plazma apelin düzeylerini değerlendirmektir.

Gereç ve Yöntemler: Plazma apelin düzeyleri, serum lipid düzeyleri, serum hormon düzeyleri ve homeostasis model assessment-insulin rezistans (HOMA-IR) değerleri 32 PCOS'lu hasta ve sağlıklı kadın kontrol grubunu oluşturan 31 hastada ölçüldü.

Bulgular: Plazma apelin düzeyleri PKOS grubunda (0.350±0.083 ng/ml), kontrol grubuna göre (0.246±0.045 ng/ml) ($p<0.001$) anlamlı olarak daha yüksek bulundu. PKOS grubundaki apelin düzeyleri ile biyokimyasal veya klinik veriler arasında anlamlı bir ilişki tespit edilmedi.

Sonuç: Plazma apelin düzeyleri PCOS'lu hastalarda anlamlı olarak daha yüksektir.(J Turkish-German Gynecol Assoc 2012; 13: 27-31)

Anahtar kelimeler: Apelin, polikistik over sendromu, Homeostatic Model Assessment insülin rezistansı (HOMA-IR), Ferriman Gallwey skoru (FGS), bel kalça oranı

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Introduction

Polycystic ovary syndrome (PCOS) is the most frequent reproductive endocrinopathy in fertility age women and, although varying among different diagnostic criteria, its frequency is about 6-8% in the reproductive period (1). PCOS is a syndrome characterized by menstrual dysfunction, infertility, hyperandrogenism and insulin resistance (2). Patients with PCOS have long term risks such as cardiovascular diseases, type 2 diabetes mellitus (DM), dyslipidemia and endometrial cancer (3). In addition, more than 50% of patients have android type obesity and increased waist to hip ratio (WHR), which are related with cardiovascular disease and increased diabetes mellitus risk (4).

After the discovery of adipokines, which are adipose tissue derived peptides, authors have recently become interested in the relationship of PCOS with adipokines. Adipokines are biologically active peptides secreted from adipose tissue (5), and it has been shown that tumor necrosis factor α (TNF-α), interleukin 6 (IL-6), C reactive protein (CRP), insulin like growth factor 1 (IGF-1), sex steroids, resistin, visfatin, adiponectin and apelin had connection with insulin resistance (6). Apelin is a recently discovered peptide that was designated as an endogenous receptor ligand and is found in several organs like heart, brain, kidneys and lungs (7, 8). In recent studies, various levels of apelin and apelin receptor (APJ) expression have been observed in different stages of cattle ovarian follicles (9, 10).

Apelin was found to be related with obesity and insulin resistance (11, 12). It has been shown that this adipokine had effects on water intake and hypothalamo-hypophyseal axis (13). Apelin has also been found to effect cardiovascular system in

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terms of hypotension (14), positive inotrophy and angiogenesis (15, 16). It has also been reported that apelin and apelinergic system were effective on mammalian ovarian development, follicular atresia and thecal tissue angiogenesis (9, 10).
The aim of our study was to compare plasma apelin levels in patients with PCOS and healthy control subjects and to determine the correlations between apelin and hormonal and biochemical data.

Materials and Methods

We enrolled 32 women with PCOS and 31 healthy controls, between ages 18 to 35 years. All subjects were given informed consent prior to the study. Local ethical committee approval was obtained before the study was undertaken.
The inclusion criteria for both groups were being between ages 18-35 years not having systemic diseases like atherosclerosis, diabetes mellitus and hypertension, not using oral contraceptives during or 6 months before the research period and not having undiagnosed uterine bleeding or a diagnosis of neoplasia before being included into the study. Diagnosis of PCOS was made according to the diagnostic criteria of European Society for Human Reproduction and Embryology and The American Society for Reproductive Medicine (ESHRE/ASRM) guidelines (17). According to these guidelines, it was possible to reach a diagnosis of PCOS when at least 2 of these 3 elements are present: hyperandrogenism, chronic anovulation and polycystic ovaries.

Biochemical evaluation and Ultrasonography
Age, weight and height of all subjects included in the study were noted and body mass indices were calculated as kg/m^2. Hirsutism was evaluated with Ferriman Gallwey score (FGS) (17) and the waist to hip ratio (WHR) values was obtained by taking waist measurement (narrowest point between the ribs and iliac crest) and hip measurement (at the point of the greatest protrusion of the buttocks) and finding the ratio of the two measurements (18). Luteinizing hormone (LH), follicle stimulating hormone (FSH), estradiol, free testosterone, total testosterone, androstenedione, sex hormone binding globulin (SHBG), dehydroepiandrostenedione sulphate (DHEA-S), 17-0H progesterone, prolactin, free T3 (fT3), free T4 (fT4), thyroid stimulating hormone (TSH), aspartate amino transferase (AST), alanine amino transferase (ALT), low density lipoprotein (LDL), high density lipoprotein (HDL), triglyceride, total cholesterol, fasting glucose and insulin levels were measured from the blood specimen taken between 08:00-10:00 am. on the 3rd day of the menstrual cycle, after 10 hours of fasting.
Serum glucose was measured with commercial kits (Roche Hitachi, Roche Diagnostics, Mannheim, Germany). Insulin resistance is defined as reduced glucose response to a given amount of insulin. The estimate of insulin resistance by the homeostasis model assessment-insulin resistance (HOMA-IR) was calculated in all patients with the following formula: HOMA-IR= Fasting insulin (mU/L) x fasting glucose (mmol/L)/ 22.5 (19). Of the hormonal parameters, LH, FSH, estradiol, total testosterone, DHEA-S, insulin, prolactin, fT3, fT4 and TSH were analyzed by immune chemiluminesance method (Roche-Hitachi Modular Analytics E-170, Indianapolis/USA), androstenedione, SHBG, 17-0H progesterone and free testosterone by micro ELISA method (Bio-tek instruments inc. Miroquant Cal/USA) and the biochemical parameters were studied by enzymatic colometric methods (Olympus AU 600 Tokyo/Japan).
Four cc blood specimen was centrifuged at 3500 revolution per minute (rpm) for 7 minutes in lavender tubes with EDTA, and the obtained plasma specimen were saved in -70°C to be further analyzed for plasma apelin levels. Plasma apelin levels were analyzed by enzyme immunoassay (EIA) method by using Phoenix Pharmaceuticals Apelin-36 (Human) EIA Cal./USA. kit, and Bio-tek instruments inc. Miroquant Cal/USA, Bio-tek instruments inc. ELX 50 auto strip washer Cal./USA and IKA schutter MTS-2 shaker Staufen/Germany devices were used.
Pelvic (Siemens Acuson CH6-2 5.71 MHz. abdominal probe, USA) or transvaginal (Siemens Acuson EC9-4 6.15 MHz. transvaginal probe, USA) ultrasonography was performed to all women (Siemens Acuson Antares™, USA). The ultrasonographic finding of 10-15 follicles with a diameter of 2-8 mm, forming the appearance of a pearl necklace and an increase in stroma and ovarian volume was considered as polycystic ovary appearance (1).

Statistical analysis
Data obtained from the study was analyzed by using 'Statistical Package for the Social Science' (Version 15.0; Inc, Chicago, IL, USA). The results were expressed as means±SD. The distribution of each variable was assessed by the Kolmogorov-Smirnov one sample test. When the data was in accordance with the normal distribution, Student's t-test was used, otherwise Mann Whitney U test was used to compare the groups. The relationship between continuous variables were evaluated by Pearson correlation coefficients. $p<0.05$ was considered statistically significant. Power analysis was performed by Power and Sample Size Calculator V.3.0 for apelin.

Results

There was no significant difference between the ages of women with PCOS and the control group ($p>0.05$). Apelin levels ($p<0.001$), waist to hip ratios ($p=0.002$), Ferriman Gallwey scores, free testosterone levels ($p=0.034$), triglyceride levels ($p=0.021$), 17-OH progesterone levels ($p=0.048$) and LH/FSH ratio ($p=0.011$) of the PCOS group were significantly higher than the control group and HDL and SHBG levels of the PCOS group were lower than the control group ($p=0.005$ and $p=0.01$ respectively). No significant difference was found between the other parameters. The comparison of clinical and biochemical parameters were shown in Tables 1 and 2.
No significant correlation was detected between apelin levels and biochemical or clinical data in patients with PCOS and the control group except for the negative correlation between apelin and SHBG levels in the control group ($r=-0.492$, $p=0.004$). Besides, significant positive correlations were detected between BMI and waist to hip ratio and BMI and HOMA-IR

values in the PCOS group (r=0.459, p=0.008 and r=0.365, p=0.040 respectively).

Discussion

In our study, apelin levels were found to be higher in patients with PCOS compared to the controls. No correlation was found between apelin and the other parameters in either groups except for the negative correlation between apelin and SHBG in the control group. We have also found positive correlations between BMI and waist to hip ratio and BMI and HOMA-IR values in patients with PCOS. Both groups consisted of subjects with normal weights and there was no significant difference between the groups in terms of BMI. Obesity is observed about 50-70% in patients with PCOS and mostly in the form of android obesity where waist to hip ratio increases. Android type obesity and increase in intraabdominal adiposity were also shown in non-obese PCOS patients (18). In this study, waist to hip ratios were found to be significantly higher in the PCOS group and this complies with the literature verifying an increase in waist to hip ratios in normal weighted PCOS patients. In recent studies it has been found that BMI and apelin levels were positively correlated (11, 12). In the present study although apelin levels and WHR were higher in the PCOS group, there was no correlation between WHR and apelin levels. In this context, higher levels of apelin in patients with PCOS may be associated with the other mechanisms besides android obesity.

Android type obesity seen in polycystic ovary syndrome is related with hyperinsulinemia, glucose tolerance impairment, diabetes and increase in androgen production (20). In our study, no statistically significant correlation was established between apelin and HOMA-IR levels. However, previous studies demostrated the relationship between apelin and insulin resistance in humans and rats (6, 11). In our study, mean HOMA-IR

Table 1. Demographic characteristics of the PCOS and the control groups

	PCOS Mean±SD	CONTROL Mean±SD	p
Age (Years)	23.81±4.38	23.55±4.13	0.807
BMI (kg/m^2)	22.51±3.20	21.94±1.63	0.376
WHR	0.75±0.06	0.71±0.03	0.002
FGS	14.31±3.89	4.32±1.68	<0.001

BMI: body mass index, WHR: waist to hip ratio, FGS: Ferriman Gallwey score

Table 2. The comparison of PCOS and the control groups in respect of biochemical and hormonal parameters

	PCOS Mean±SD	CONTROL Mean±SD	p
Apelin (ng/ml)	0.350±0.083	0.246±0.045	<0.001
LH/FSH	1.91±0.85	1.38±0.74	0.011
E2 (pg/ml)	54.24±27.65	52.97±23.58	0.846
Prolactin (ng/ml)	15.87±4.52	13.96±6.22	0.166
TSH (μU/ml)	1.94±1.02	1.98±0.83	0.857
FT3 (pg/ml)	3.32±0.43	4.24±5.53	0.351
FT4 (pg/ml)	16.59±2.11	16.76±1.82	0.734
DHEA-S (μg/dl)	285.36±119.22	256.01±122.33	0.339
Androstenedione (ng/ml)	3.02±1.43	2.85±1.19	0.597
Free testosterone (pg/ml)	2.40±1.67	1.59±1.29	0.034
Total testosterone (ng/ml)	0.639±0.356	0.605±0.488	0.752
SHBG (nmol/L)	47.26±28.83	68.48±34.45	0.010
17-OHP (ng/ml)	1.40±1.10	1.00±0.50	0.048
Insulin (μU/ml)	11.52±6.91	10.02±5.02	0.331
Glucose (mg/dl)	89.56±8.85	87.74±9.64	0.438
HOMA-IR	2.58±1.65	2.17±1.05	0.245
TG (mg/dl)	86.97±35.01	69.48±22.93	0.021
HDL (mg/dl)	54.29±10.74	63.16±13.31	0.005
LDL (mg/dl)	80.67±24.37	81.54±21.70	0.881
Total cholesterol (mg/dl)	154.13±26.96	158.16±25.22	0.542

LH: luteinizing hormone, FSH: follicle stimulating hormone, E2: estradiol, SHBG: sex hormone binding globulin, DHEA-S: dehydroepiandrostenedione sulphate, 17-OHP: 17-0H progesterone, FT3: free T3, FT4: free T4, TSH: thyroid stimulating hormone, TG: triglyceride LDL: low density lipoprotein, HDL: high density lipoprotein, HOMA-IR: homeostatic model assessment insülin resistance

values in the PCOS group were higher than that of the controls, although the difference was not statistically significant. In the literature, many studies demostrating insulin resistance in non-obese PCOS patients exist (21-23), as well as the other studies stating that insulin resistance was not different than the normal population as in our study (24, 25).

Although it is difficult to evaluate, apelin levels in patients with PCOS due to lack of sufficient studies, other studies assessing different adipokines may be illuminating. For example, TNF-α levels were found to be higher in patients with PCOS than healthy women in some studies (26), and similar in others (27). Also, TNF-α was shown to contribute to insulin resistance in non-obese PCOS patients (28). It was shown that TNF-α increased apelin levels in human adipose tissue (29). There are also studies emphasizing visfatin, which is an adipokine that has similar properties like apelin, increased in patients with PCOS (30). Adiponectin levels were found to be lower in PCOS patients than healthy women (31). We have observed higher levels of apelin in patients with PCOS but the mechanisms underlying were not investigated in this study. In cause-effect relationship, higher levels of plasma apelin in PCOS might be related to androgenic obesity, increased waist to hip ratio, increased adiposity, impairment in LH/FSH interaction, hypothalamo-hypophyseal axis effects and local paracrine and endocrinological attitudes deriving from the nature of the polycystic ovaries and also the compensatory mechanisms due to the metabolic changes in PCOS.

Recent studies have shown that apelin had potential therapeutic effects. Centrally (ICV) injected apelin-13, decreased food intake in both fed and hungry mice; this effect was not seen in peripheral usage of apelin and this shows that apelin induces anorectic effects (32). Apelin increased core body temperature independent of food intake, increased locomotor activity and contributed to negative energy balance (33). Also apelin inhibited glucose induced insulin secretion (34). In a recent study, it has been shown that 14 days of apelin application to lean and obese mice, decreased body fattening independent of food intake, decreased insulin, leptin and triglyceride levels and increased the expression of non-binding proteins and adiponectin levels (35). Although apelin is a potential treatment agent for cardiovascular diseases, insulin resistance, obesity and diabetes mellitus co-occur with PCOS, this subject is still disputable and broad comprehensive studies are required.

In our study, TG levels were found to be significantly higher and HDL levels were significantly lower in patients with PCOS than the control group, consistent with the literature (36). As in other studies, we have found that 17-OH progesterone and free testosterone levels were significantly higher and SHBG levels significantly lower in patients with PCOS (37). Also LH/FSH levels were significantly higher in the PCOS group, which is consistent with the literature (38).

In conclusion, we have found that the plasma apelin levels were higher in patients with PCOS compared to health controls. This high levels might be independent of serum androjen levels and BMI or IR. Further studies are required for clearly enlightening the mechanisms and physiopathology.

Conflict of interest

No conflict of interest was declared by the authors.

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Assessment of anxiety and depression levels of pregnant women with hyperemesis gravidarum in a case-control study

Hiperemezis gravidarum'lu gebelerde anksiyete ve depresyon sıklığının bir olgu-kontrol çalışması ile değerlendirilmesi

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Abstract

Objective: The aim of this study was to determine the depression and anxiety levels of pregnant women with hyperemesis gravidarum by using the Beck depression and anxiety inventory scoring system in a Turkish population.

Material and Methods: To ascertain this relationship, a case-control study was conducted involving 86 pregnant women in their first trimester of pregnancy. Forty-one subjects had hyperemesis gravidarum, and 45 were healthy pregnant women who served as control subjects. The groups were adjusted for age, parity, and body mass index. All included women were subjected to baseline laboratory investigations including serum TSH and total hCG levels.

Results: There were no statistically significant differences between the groups with respect to the demographic and obstetric parameters and baseline laboratory investigations except the mean serum potassium level, which was significantly lower in patients with hyperemesis gravidarum than in the control group (p=0.039). Patients with hyperemesis gravidarum had significantly higher depression and anxiety scores than control cases (p=0.0001 and p=0.049, respectively).

Conclusion: Our results suggest that increased anxiety and depression levels may be involved in the pathogenesis of hyperemesis gravidarum and extra psychological support may be necessary during the treatment and follow-up of these patients.

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Key words: Hyperemesis gravidarum, depression, anxiety, pregnancy, psychological tests

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Özet

Amaç: Bu yazıda Beck anksiyete ve depresyon skorlama sistemi kullanılarak hiperemezisli gebelerde anksiyete ve depresyon düzeylerinin araştırılması amaçlandı.

Gereç ve Yöntemler: Çalışma için 1. trimesterdeki 86 gebeyi içeren bir olgu-kontrol çalışması yapıldı. Hiperemezis gravidarumlu 41 hasta ve sağlıklı kontrol grubu olarak 45 gebe dahil edildi. Gruplar yaş, parite ve vücut kitle indeksi değerleri açısından eşleştirildi. Tüm hastalardan serum TSH ve hCG düzeylerini de içeren bazal laboratuvar tetkikleri istendi.

Bulgular: Gruplar arasında serum potasyum düzeyinin hiperemezisli olgularda anlamlı olarak düşük olması (p=0.039) dışında demografik, obstetrik ve laboratuvar değerleri açısından farklılık yoktu. Hiperemezisli hastaların depresyon ve anksiyete skorları kontrol grubuna göre anlamlı olarak daha yüksekti (Sırasıyla p=0.0001 ve p=0.049).

Sonuç: Artmış anksiyete ve depresyon düzeyleri hiperemezis gravidarumun patogenezinde rol oynayabilir. Bu hastaların takip ve tedavilerinde ekstra psikolojik destek gerekebilir.

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Anahtar kelimeler: Hiperemezis gravidarum, depresyon, anksiyete, gebelik, psikolojik testler

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Introduction

Hyperemesis gravidarum (HG), a severe form of morning sickness, is one of the most common pregnancy-related complications. Considerable variations in the occurrence of HG, both between and within countries, have been reported. According to Eliakim, the prevalence of HG varies between 0.3% and 2% (1). HG is characterized by dehydration, electrolyte imbalance, nutritional depletion and the loss of at least 5% of body weight. HG is considered to be one of the most important pregnancy-related complications that begins in the first trimester and can last throughout pregnancy, although the symptoms usually resolve by week 20 (2). The condition generally requires frequent visits to the emergency room and sometimes repeated hospitalization for intravenous hydration.

Recently published studies have found that the point prevalence of depression ranges from 8.5% to 39.0% at different times during pregnancy and from 6.5% to 12.9% at different times during the first year postpartum, which are both

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slightly higher than the depression frequency in non-pregnant women (3-5).

HG is currently conceptualized as a biological illness with an unknown pathophysiological cause. Theories have suggested the influence of human chorionic gonadotropin, the pituitary axis, transient adrenal hyperthyroidism and psychogenic factors (6-8). It is well known that women of childbearing age are at an increased risk for depression and anxiety (9) and that pregnancy may increase the risk of depressive episodes (10). However, there is no data in the current literature to support the possibility that HG is a psychologically mediated process.

On the basis of this background, the present study used the Beck inventory to determine a possible relationship between the HG and increased level of depression and anxiety in these women.

Material and Methods

A case-control study was conducted involving 86 pregnant women in their first trimester of pregnancy. The women were selected from the outpatient obstetrics clinic of our Department of Obstetrics and Gynaecology between September 2010 and April 2011. The patients were divided into two groups. The first group included patients with a diagnosis of HG (study group) and the second group was composed of healthy pregnant women who served as controls. The groups were adjusted for age, parity, and BMI.

All participants were informed about the study and agreed to participate in the research. The study protocol was approved by the local ethics committee of the institution. The study was conducted in accordance with the basic principles of the Declaration of Helsinki.

Patient selection

The inclusion criteria for study group were as follows: (1) diagnosis of HG in a singleton pregnancy documented by the presence of severe vomiting (more than 3 times per day without any other obvious cause), an inability to maintain oral nutrition, weight loss of more than 3 kilograms and at least one positive ketonuria test (1, 2); (2) ability to speak Turkish and no physical or psychological disabilities that would prevent participating in the interventions; (3) no evidence of antenatal bleeding; (4) no pre-existing medical or psychiatric comorbid condition; (5) no antibiotic treatment, H2 blockers or proton pump inhibitors in the preceding month.

A comprehensive medical history was obtained from all pregnants, including a history of medical disorders (e.g., peptic ulcer) and chronic medication intake (e.g., non-steroidal anti-inflammatory drugs) and exclusion of hyperthyroidism, psychological disorders, hepatic disorders, urinary tract infections or intracranial disorders.

An ultrasound scan was performed for all cases, including foetal biometry, placental site, amount of amniotic fluid and exclusion of any relevant obstetric condition (e.g., twin pregnancy, molar pregnancy or missed abortion).

In all patients, blood samples were taken for biochemical tests and hemogram during admission. Urine analysis for ketones was carried out for the detection of starvation ketosis.

Pregnant patients with HG were given a standard initial treatment of intravenous fluids with saline (with the addition of potassium chloride as required if patient was hypokalemic), oral thiamine (10 mg daily) and an intravenous antiemetic.

Data collection

Data were collected at the time of admission using a series of forms completed during face-to-face interviews by trained interviewers to determine the psychological status of the patients. After obtaining written informed consent, one of the co-authors (who was blinded to the study groups) carried out the interviews. The first form consisted of questions regarding the demographic characteristics of the patients. The second form included the Turkish versions of the Beck Depression Inventory (BDI) and Beck Anxiety Inventory (BAI). The BDI and BAI are 21-item self-report instruments used to assess the severity of symptoms of depression and anxiety, respectively (11, 12). Each response is assigned a score, ranging from 0 (not at all bothered) to 3 (severely bothered), indicating the severity of the symptoms.

Individual questions of the BDI assess mood, pessimism, sense of failure, self-dissatisfaction, guilt, punishment, self-dislike, self-accusation, suicidal ideas, crying, irritability, social withdrawal, body image, work difficulties, insomnia, fatigue, appetite, weight loss, bodily preoccupation and loss of libido. It was translated into Turkish, and its reliability was recalculated by Hisli (13). For the Turkish population, a score of 17 or above represents depression, according to Hisli (13). We used these cut-off scores to determine the levels of depression.

The BAI is designed to measure anxiety levels. It is scored by summing the responses, with higher total scores indicating higher levels of anxiety. The validity and reliability of the BAI in the Turkish population have been shown by Ulusoy et al. (14).

Sample size

The sample size of 41 patients per arm provides approximately 80% power at the two-sided significance level of 5% to detect at least a twofold increase in the frequency of anxiety and depression in patients with HG, assuming that the incidence of anxiety and depression is 10% in uncomplicated pregnancies (10).

Statistical analysis

Statistical analyses were performed using the SPSS for the Windows version 13.0 program. Continuous variables were reported as mean±standard deviation (SD). Categorical variables were reported as number and percent. Normality for continuous variables in groups was determined by the Shapiro Wilk test. The variables showed a normal distribution ($p>0.05$), so an unpaired t test and a Pearson's chi-square test were used to compare the continuous and categorical variables between the groups. A value of $p<0.05$ was considered statistically significant.

Results

Obstetric characteristics of the groups in terms of age, parity, gestational age and weight at admission were similar and are

shown in Table 1 (p>0.05). The distribution of educational level and monthly income in the study and control groups is given in Table 2.

There were no statistically significant differences between the groups with respect to the baseline laboratory investigations (Table 3) except the mean serum potassium level, which was significantly lower in patients with HG than in the control group (p=0.039).

Mean BDI and BAI scores for the two groups are given in Table 4. Patients with HG had significantly higher BDI and BAI scores than control cases (p=0.0001 and p=0.049, respectively). Furthermore, 63.4% (n=26) of the patients in the HG group and 28.8% (n=13) of the control cases had a BDI score higher than 17. The difference was statistically significant (p=0.0001).

The mean number of vomiting attacks in the HG group was 5.7, ranging from 1-10 per day. All patients in the study group were

Table 1. Demographic data of the HG and control groups

	HG (n=41) (mean±SD)	Control (n=45) (mean±SD)	p
Maternal age (years)	27.5±6.0	29.6±6.5	0.141
Parity	1.14±1.20	1.10±1.17	0.815
Gestational age (weeks)	9.5±2.3	10.5±2.8	0.07
Pre-preg. weight	64.5±9.0	62.4±10.2	0.336
Pre-preg. BMI	24.0±2.4	23.5±3.4	0.24
Weight at admission	62.6±8.3	64.3±9.3	0.518
BMI at admission	23.1±2.4	24.1±3.2	0.09

Table 2. Distribution of educational level and monthly income of the HG and control groups

Parameter	HG (n=41)		Control (n=45)		p
Education	**N**	**%**	**N**	**%**	
Primary-Middle school	26	64	22	48.9	0.127
High school	9	22	19	42.2	
College	6	14.6	4	8.9	
Monthly income					
*TL <500	13	31.7	18	40	0.844
*TL 500-1000	15	36.6	13	28.9	
*TL 1000-2000	8	19.5	9	20	
*TL >2000	5	12.2	5	11.1	
* Turkish liras					

Table 3. Laboratory values of the HG and control groups

	HG (n=41) (mean±SD)	Control (n=45) (mean±SD)	p
Hb (g/dl)	12.8±1.03	15.1±17.3	0.398
White blood cell (10^3/ml)	8.8±2.4	9.3±1.9	0.276
Platelet (10^3/ml)	241±49.9	248±59.2	0.532
Na (mmol/l)	135.2±2.36	133.9±18.9	0.661
K (mmol/l)	3.8±0.39	4.01±0.48	0.039
ALT (U/l)	17.4±13.6	16.2±10.9	0.644
AST (U/l)	15.9±8.6	14.2±4.20	0.254
Urea (mg/dl)	8.4±2.2	8.07±2.79	0.533
Creatinine (mg/dl)	0.67±0.71	0.54±0.10	0.228
TSH (μIU/ml)	1.02±0.83	1.22±0.93	0.336
Total hCG (10^3) IU/ml)	105±47.14	89±42.11	0.107

Table 4. Comparison of BDI and BAI scores of the HG and control groups

	HG (n=41) (mean±SD)	Control (n=45) (mean±SD)	p
BDI	20.9±10.4	11.8±10.24	0.0001
BAI	18.5±11.5	13.6±11.24	0.049

admitted to the inpatient department for the treatment of hyperemesis. The mean length of hospital stay was 1.4 days, ranging from 1-7 days. None of the patients required parenteral nutrition.

Discussion

This study has shown that women with severe vomiting during their pregnancy had considerably more anxiety and depression than a well-matched control group of healthy antenatal women. Hisli defined the depression limit point in the Beck depression scale as 17 and above for the Turkish population (13). According to this cut-off value, it was determined that more than half of our patients were depressive. This considerably higher level of depression in pregnant patients with HG may be due to inadequate food intake, lack of energy and severe fatigue, lack of socialization, loss of hope that nausea and vomiting will cease before birth and fear of not being able to feed the developing baby.

It is unquestionable that pregnancy itself is a major life stressor that can precipitate or exacerbate depressive tendencies. In a Swedish population-based study, Andersson et al. reported a 14-percent point prevalence of psychiatric disorders during pregnancy (15, 16).

Increased stress, depression or anxiety related with pregnancy may be more marked in women suffering from hyperemesis. However, these women are often excluded from studies of the emotions of women during pregnancy, and not enough is known about their psychological state. It is important to recognise and treat maternal anxiety and depression in pregnant patients with hyperemesis, both for the sake of the women themselves and their foetuses. It has been shown that women with prenatal anxiety or stress have higher rates of spontaneous abortion (17, 18) and are more likely to deliver premature infants (19). There is also evidence that if a mother is significantly stressed while pregnant, her child is substantially more likely to have emotional or cognitive problems, including an increased risk of attention deficit/hyperactivity disorder, anxiety and language delay (20).

A few previous studies have examined the relationship between anxiety and/or depression and HG, and the results have been inconclusive. Swallow et al. showed that nausea and vomiting in early pregnancy was associated with psychiatric morbidity (21). They reported that the severity of nausea and vomiting correlated independently with the level of anxiety/insomnia and depression. Similarly, Poursharif et al. showed that in a large cohort of women with HG, over 80% reported a negative psychosocial impact, including anxiety and depression, some of which continued after the pregnancy (22). In their review, Kim et al. suggested that the quality of life of women with HG is severely disrupted and normalizing the patient's sense of demoralization should always be considered during the treatment of these cases (23). Other studies, however, contradict these findings and found no increase in psychiatric illness in women with HG during pregnancy (24, 25). In the present investigation, we also found that mean BDI and BAI scores were significantly higher in hyperemetic pregnant patients than in the control antenatal women ($p<0.05$). In the HG group, 26 women (63.4%) had a BDI score of higher than 17, signifying depression for the Turkish population (13), compared with only 13 (28.8%) of the controls.

It is unclear whether the psychological or psychiatric morbidity is a cause or consequence of HG. In the past, severe vomiting during pregnancy was often perceived as an expression of maternal resentment towards her unwanted pregnancy. Various psychological stresses have been linked with hyperemesis, including emotional immaturity, strong mother dependence and anxiety and tension related to the pregnancy. More recently, however, investigators have argued that the psychological symptoms are a result of stress arising from the physical burden of HG rather than a cause (21). All the patients in the present study knew they had hyperemesis of pregnancy. Interestingly, Sikkema et al. found anxiety levels to be lower in women with preeclampsia who were unaware of their condition (26).

The present study has clear limitations. The major limitation to this study design was the fact that only a small number of hyperemetic pregnant patients were surveyed and that the results were obtained from a single institution. Other limitations of our study included its subjective nature and data collection method, which created difficulties in ascertaining a 'cause and effect' relationship between the higher anxiety and depression and HG. In conclusion, this study adds to the evidence that those suffering with HG during pregnancy may be more anxious and depressed than women with uncomplicated pregnancies. Depression and anxiety during pregnancy are treatable but can be devastating for maternal and foetal health. Therefore, health professionals need to be aware that extra psychological support may be necessary during the treatment and follow-up of hyperemetic pregnant women.

Conflict of interest

No conflict of interest was declared by the authors.

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The investigation of tumoral angiogenesis with HIF-1 alpha and microvessel density in women with endometrium cancer

Endometrium kanserli olgularda tümör anjiogenezinin HIF-1 alfa ve mikrodamar dansitesi ile değerlendirilmesi

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Abstract

Objective: Hypoxia inducible factor 1 alpha (HIF-1α) is a nuclear protein upregulated in response to reduced cellular oxygen concentration which therefore acts as a marker for hypoxia. The aim of this study was to determine tumoral angiogenesis with immunohistochemical markers in endometrium cancer and its relation with stage, grade, survival rates and other prognostic factors.

Material and Methods: Using the database in our Gynecologic Oncology clinic, we selected 94 patients who were diagnosed with endometrial cancer and underwent primary surgery at our institution between 2001 and 2010. Tissue microarrays believed to demonstrate the optimum part of the tumor were reprepared from the paraffin blocks. Angiogenesis and microvessel density (MVD) were investigated with the aid of HIF-1α and CD34 antibodies.

Results: High expression of HIF-1α was significantly more frequent in advanced grade endometrial cancers (p=0.044). HIF-1α expression was highly correlated with CD34 expression in the tumor cells (p<0.001). However lack of relation among stage, overall survival rates and histological types were analyzed with HIF-1α. When we compared HIF-1α positive and negative cases with cervical, adnexial, lymphovascular and myometrial invasion, there was no difference between these groups. MVD was evaluated with CD34 and it was remarkable and significantly different on advanced grade tumors (r=0.268; p=0.009). A similar significant difference was observed between the high expression of CD34 and type II endometrial cancer histology (p<0.001). However, there was no relationship between the MVD and stage or survival rates.

Conclusion: High expression of HIF-1α is associated with tumoral angiogenesis in endometrial adenocarcinomas. Further studies targeting HIF-1α for disrupting mechanisms essential for tumor growth in endometrium cancer will be significant investigations in the future.

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Key words: Endometrium cancer, HIF-1α, hypoxia, angiogenesis

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Özet

Amaç: Hipoksi ile indüklenen faktör-1α (HIF-1 α) bir nükleer proteindir ve azalan hücre içi oksijen konsantrasyonuna cevap olarak regüle edilir. Bu nedenle bir hipoksi belirteci gibi görev yapar. Bu çalışmanın amacı endometrium kanserli olgularda tümör anjiogenezini immunohistokimyasal belirteçler ile incelemek ve evre, derece ve sağkalım gibi prognostik faktörlerle olan bağlantısını aydınlatmaya çalışmaktır.

Gereç ve Yöntemler: 2001 ile 2010 tarihleri arasında Jinekolojik Onkoloji Bölümü'nde endometrium kanseri nedeniyle tanı alan ve ameliyat edilen 94 olgu çalışmaya dahil edildi. Olguların tümörü en iyi gösterdiğine inanılan kesitlerinin parafin blokları tekrar oluşturulup, HIF-1α ve CD34 antikorları kullanılarak anjiogenez ve mikrodamar dansitesi (MDD) değerlendirildi.

Bulgular: HIF-1α pozitifliği ile yüksek grada sahip endometrium kanserli olgular arasındaki fark istatistiksel olarak anlamlı idi (p=0.044). Kullanılan immunohistokimyasal belirleyicilerin boyanma sonuçları birbiri ile karşılaştırıldığında HIF-1α pozitifliği izlenen olgularda CD34 ekspresyonunun daha fazla olduğu görüldü (p<0.001). HIF-1α ile evre, toplam sağkalım, histolojik tip arasında anlamlı ilişki saptanmadı. HIF-1α pozitif ve negatif olguların servikal, adneksiyal, lenfovasküler ve myometrial invazyon özellikleri kıyaslandığında iki grup arasında fark gözlenmedi. CD34 ile değerlendirilen mikrodamar dansitesinin derece (grad) ve histolojik tip ile yapılan analizinde ise ileri grad tümörlerde ve tip II histolojik tip endometrium kanserlerinde mikrodamar dansitesi yüksek olarak belirlendi (r=0.268; p=0.009, p<0.001). Mikrodamar dansitesi ile evre ve sağkalım üzerinde ise anlamlı bir ilişki görülmedi.

Sonuç: HIF-1α ekspresyonu ile endometrium adenokanserleri tümör anjiogenezi arasında bir ilişki mevcuttur. Endometrium kanserlerinde tümör gelişimi için gerekli yolakları engelleyebilecek HIF-1α odaklı çalışmalar ilerleyen zamanların ilgi çekici araştırma konularından olacaktır. (J Turkish-German Gynecol Assoc 2012; 13: 37-44)

Anahtar kelimeler: Endometrium kanseri, HIF-1α, hipoksi, anjiogenez

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Introduction

Endometrial cancer is the most commonly identified invasive neoplasm of the female genital tract and the fifth most common cancer in the female population. New cases diagnosed each year in the world number approximately 150,000 (1). According to the clinicopathological features and genetic basis, endometrium cancers have been classified into two major types: type I and type II. Endometrioid and mucinous tumors also referred to as type I are well differentiated neoplasms and usually related with estrogen stimulation. Serous and clear cell carcinomas known as type II tumors, on the other hand, fail to produce regular patterns, demonstrating nuclear atypia, high mitotic activity and poor prognosis (2).

Angiogenesis is essential for tumor growth, invasion, and metastasis. If neovascularization is absent, tumors cannot grow because oxygen lack in the centre of the tumor results in apoptosis and necrosis. Therefore, cancer cells have to adapt themselves to hypoxia by several mechanisms (3).

Hypoxia inducible factor-1 (HIF-1), that is a major regulator of cell adaptation to hypoxic stress, plays a critical role in angiogenesis. HIF-1 is composed of the two subunits HIF-1α and HIF-1β. A common subunit, HIF-1β, is constitutively expressed in both normoxia and hypoxia. The oxygen-regulated subunit is HIF-1α and it determines HIF-1 activity. Under nonhypoxic conditions, HIF-1α is subject to ubiquitination and proteasomal degradation. However in hypoxia, its activity is stabilized (4). HIF-1α controls the expression of more than 40 target genes whose protein products play crucial roles in cellular pathways including angiogenesis, tumoral growth, erythropoiesis and biological events associated with aggressive tumor behavior (5). HIF-1α expression is increased in many cancers including bladder, ovary, lung, pancreas and gastrointestinal stromal tumors (6). HIF-1α upregulation has been demonstrated to be absent in the inactive endometrium but present in hyperplasia and endometrioid type adenocarcinoma, with increasing expression (7). However, the role of HIF-1α expression in other types of endometrial carcinoma and its association with grade, stage, invasion depth, survival and prognosis have not been well documented in the literature.

The aim of this study was to evaluate the tumoral angiogenesis and the expression of HIF-1α protein in relation to microvessel density (MVD) and to investigate relationships with the other clinicopathologic features in type I and type II endometrial adenocarcinomas.

Materials and Methods

The materials for this study were retrieved from the database of the Department of Obstetrics and Gynecology and Division of Pathology, Pathology, Trakya University, Faculty of Medicine, between 2001 and 2010. We identified 100 formalin-fixed, paraffin-embedded tumor samples from women with endometrial adenocarcinoma who underwent surgery at our institution. After obtaining approval from the Ethical committee of our institution, we performed a retrospective chart review of the patients' demographic, pathological and clinical data.

The histological type of endometrial cancer was determined using the World Health Organization (WHO) criteria (8). The tumors were classified as type I (endometrioid and mucinous) and type II (serous and clear cell). Mucinous tumors were excluded in the study and all patients with type II histology (clear and serous cell), diagnosed and operated in our center throughout the study period were included. Additionally, all advanced-staged and high grade type I endometrial adenocarcinomas were also included in this study. For obtaining homogenous distribution, we carefully selected cases in which equalisation of the grade I, II and III in each group was attempted. Surgical staging was determined using the revised criteria recommended by the International Federation of Gynecology and Obstetrics, 2009 (FIGO) (9). All patients underwent total abdominal hysterectomy with pelvic and paraaortic lymphadenectomy with or without salphingoophorrectomy. If the patient had Type II tumor, an omentectomy and appendectomy were also performed. Microscopic grading was based on the FIGO grading system. All type II cancers were accepted as grade III tumors. The specimens were evaluated for the stage and grade of the tumor, adnexial and lymphovascular involvement, cervical and myometrial invasion.

After the initial evaluation, of 100 cases, 6 patients were excluded from the study due to the unavaliability of their paraffin block for immunohistochemical staining. Therefore, ninety-four hysterectomy specimens from patients operated on for endometrium cancer were analyzed and the paraffin-embedded blocks believed to demonstrate the optimum part of the tumor were reprepared. Angiogenesis and microvessel density were investigated with the aid of HIF-1α and CD34 antibodies.

Expression of HIF-1α and CD 34 was determined immunohistochemically, using the avidin-biotin-peroxidase technique. 4-5μ thick slides were deparaffinized in xylol. Slides were heated in 0.01 M citrate buffer for 20 min in a microwave oven. For HIF-1α, sections were antigen retrieved by using an EDTA buffer. After cooling for 20 min and washing in phosphate-buffered saline solution (PBS), endogenous peroxidase was blocked with 3% H2O2 for 10 min, followed by cooling for 20 min and washing in PBS. The slides were then incubated for overnight at 4°C temperature with primary antibodies against HIF-1α (1:300, Clone H1alpha67) and CD 34 (1:100, Clone QBEND/10) (Table 1). Positive controls were used as follows: Cervical squamous cell cancer - HIF-1α and endometrium- CD34.

Table 1. Immunohistochemical methods

Antibody	Code	Incubation period
HIF-1α Ab-4 / MCL- mouse (Clone H1alpha67)	Thermo-Scientific / MS-1164-P1	One night
CD34 / MCL- mouse (Clone QBEND-10)	Seytek / A00070	30 minute

Assessment of immunohistochemistry

Expression of HIF-1α is seen in both nucleus and cytoplasm (10). However, only nuclear staining was assessed in this study. The extent and intensity of the nuclear staining were measured separately. For HIF-1α assessment, staining intensity was scored as 0 (negative), 1 (weak), 2 (medium), and 3 (strong). Extent of staining was scored as 0 (0%), 1 (1-25%), 2 (26-50%), 3 (51-75%), and 4 (76-100%) according to the percentages of the positive staining areas in relation to the whole carcinoma area. The sum of the intensity and extent score was used as the final staining score (0-7) for HIF-1α. Tumors having a final staining score of >2 were considered to be positive (10).

For MVD assessment, microvessels were evaluated immunohistochemically by CD34. A modification of the technique described by Weidner et al. (11) was used in determining MVD. Sections were first scanned at low power (×40 and ×100). Then the highest vascularization areas, called 'hot spots', were chosen at a magnification of ×100 and microvessel counting was performed at a magnification of ×200 in 5 different 'hot spot' areas. The final microvessel score was defined as the average of the vessel counts from five high vascularization areas.

Statistical analysis

Statistical analyses and graphs were performed by using Statistical Software Package for the Social Sciences, (SPSS). Correlations between HIF expression and different clinicopathological parameters was performed by the Student *t* test, Fisher's exact test or the Mann-Whitney U test as appropriate. Correlation between two continuous and ordinal variables were determined by the Pearson correlation and Spearman's rank test, respectively. Survival times were estimated in months from the date of diagnosis to the date of death or last follow-up and survival curves were plotted using the Kaplan-Meier method. Statistical significance was defined as a probability value less than 0.05.

Table 2. Stages of cases with type I&II histology

Stage	Type I Cases (%)	Type II Cases (%)
I	50 (53.2%)	3 (3.2%)
II	10 (10.6%)	1 (1.1%)
III	14 (14.9%)	13 (13.8%)
IV	2 (2.1%)	1 (1.1%)

Table 3. Clinicopathological features of cases with endometrial cancer

Clinico-histopathological characteristics		Cases (%)
Type	I	76 (80.9%)
	II	18 (19.1%)
Stage	IA	45 (47.9%)
	IB	8 (8.5%)
	II	11 (11.7%)
	IIIA	7 (7.4%)
	IIIB	1 (1.1%)
	IIIC1	12 (12.8%)
	IIIC2	7 (7.4%)
	IVB	3 (3.2%)
Grade	1	36 (38.3%)
	2	30 (31.9%)
	3	28 (29.8%)
Histological Type	Endometrioid	76 (80.9%)
	Clear cell	9 (9.6%)
	Serous	9 (9.6%)
Recurrence	(-)	85 (90.4%)
	(+)	9 (5.6%)

Results

To study the tumoral angiogenesis with HIF-1α and CD34 in type I and II endometrial carcinoma, we analysed 94 tumor tissue sections for HIF-1α and CD34 expression. 76 type I and 18 type II endometrial adenocarcinomas were retrieved from these slides. The mean age at the time of diagnosis was 59.96±9 years (range: 31-80). Using the revised FIGO staging system (9): 53 cases of Stage I (56.4%), 11 cases of Stage II (11.7%), 27 cases of Stage III (28.7%), and 3 cases of Stage IV (3.2%) were determined. Table 2 also demonstrates stage according to the histological type. Based on the FIGO grading system, 36 cases were classified as Grade I (38.3%), 30 cases were Grade II (31.9%) and 28 cases were Grade III (29.8%). The clinicopathological data of the patients are listed in Table 3 and 4.

Median follow-up was 31±10.6 months (range, 1-95 months). During follow-up, 9 patients developed distant recurrences (9.6%). Recurrence sites were the intestine and colon in 3 cases, lumbar vertebra in 5 cases and multiple localization in the body including pelvis in 1 case. Thus, the total number of women with pelvic recurrences was 4. The median follow-up in

Table 4. Histopathological features

Myometrial invasion		Number of cases (%)
Myometrial invasion	No	10 (10.6%)
	Yes	84 (89.4%)
Cervical invasion	No	63 (67%)
	Yes	31 (33%)
Lymphovascular invasion	No	60 (63.8%)
	Yes	34 (36.2%)
Lymph node metastasis	No	67 (71.2%)
	Yes	27 (28.7%)
Adnexal involvement	No	74 (78.7%)
	Yes	20 (21.3%)

patients with recurrence was 44.8 months. Of 9 recurrent cases, clear cell histology was defined in 4 cases, serous cell type in 3 and endometrioid type in 2 cases. Also, to compare according to the stage, 7 recurrent cases were in stage III, 1 case in stage II and 1 case in stage I. During follow-up of these 9 cases, 4 patients died.

In the study, the impact of cervical, myometrial, adnexal and lymphovascular invasion on overall survival (OS) was also investigated. Only the positivity of the adnexal involvement and lymphovascular invasion were observed to be significant statistically ($p<0.001$, $p<0.001$).

The overall survival, disease free survival (DFS), progression free survival (PFS) and survival rates of all cases were analyzed (Table 5). In the comparison of survival parameters between type I and type II patients, statistical significance was found in all of these four survival times (Figure 1). Looking at the relationship between the survival rates and the stage, we found that survival rates declined in advanced-staged cancers.

Immunohistochemical findings

Ninety-four hysterectomy specimens from patients operated on for endometrium cancer were evaluated by immunohistochemical analysis. The positive nuclear reaction with HIF-1α was observed in 28 (29.7%) patients. As mentioned earlier, only the nuclear reaction was accepted as positive for HIF-1α expression (Figure 2, 3). According to the histological type, no significant difference in expression of HIF-1α between type I and type II ndometrial adenocarcinoma (24 of type I vs 4 of type II; $p=0.318$) was observed (Table 6).

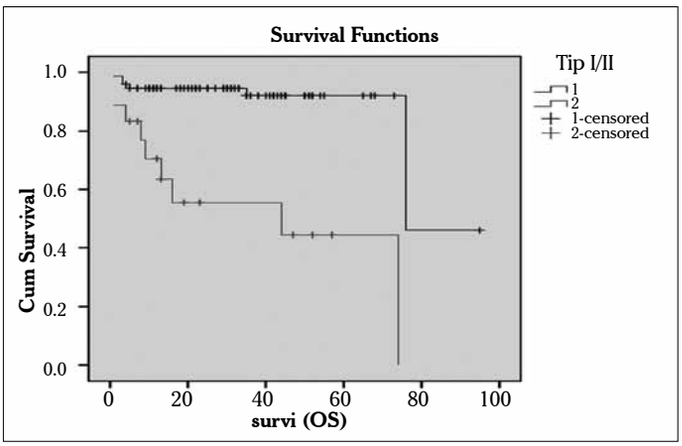

Figure 1. Overall survival of patients with type I and II cancer
Cum Survival: Overall survival, Survi (OS): Survival in months
1: Type I cancers 2: Type II cancers, Kaplan-Meier method)

High expression of HIF-1α was seen in 19.4% of grade I, 46.7% of grade II and 25% of grade III tumors of all the study population ($p=0.044$). We also found that there was still a significant difference between grade and HIF-1α expression by exluding type II tumors from the analysis ($p=0.008$). Since all type II tumors were accepted as grade III, we investigated the relationship between grade and HIF-1α expression in type I patients by generating a homogenous group (Table 7). According to the stage, HIF-1α positive and negative cases were listed in Table 8. No significance was found between the stage and HIF-1α expression.

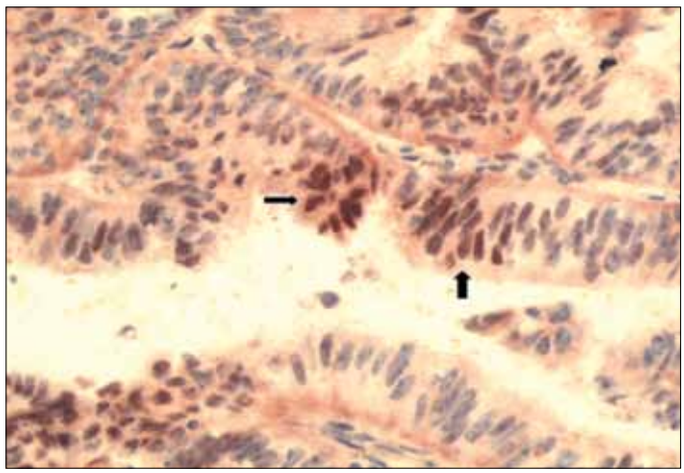

Figure 2. HIF-1α positivity in endometrioid type adenocarcinoma (HIF-1α, X200)

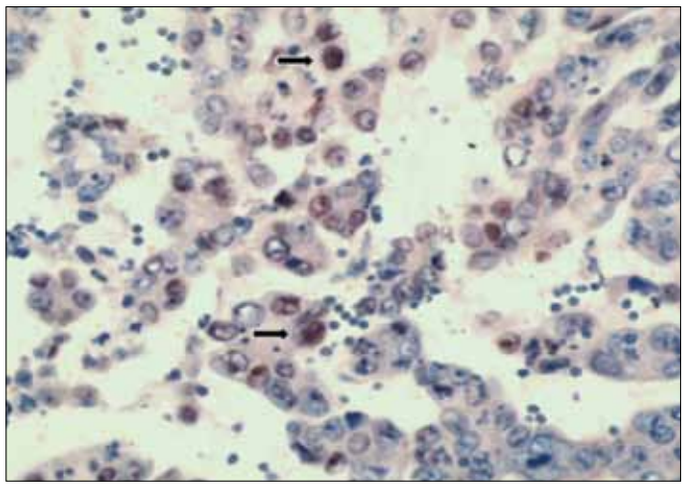

Figure 3. HIF-1α positivity in serous type adenocarcinoma (HIF-1α, X200)

Table 5. Survival times and rates of patients

	All cases (n=94)	Type I (n=76)	Type II (n=18)	p
Overall survival in months	31.61	79.7	41.3	<0.001
Disease free survival (DFS) in months	29.47	87.2	24.7	<0.001
Progression free survival (PFS), in months	28.56	87.0	19.6	<0.001
Survival rate (%)	83.9	92.1	55.5	<0.001

HIF-1α expression in type I and II endometrial adenocarcinoma and its correlation with clinicopathological characteristics were analyzed (Table 9). The positivity of HIF-1α expression showed no significant correlation with parameters of tumor features including cervical, myometrial and lymphovascular invasion and adnexal metastasis. High expression of HIF-1α was observed in 1 of 9 recurrent endometrial adenocarcinoma case. The overall survival rate of patients with high HIF-1α expression (n=28) was 78.6% and of patients with negative HIF-1α expression (n=66) was 86.2%. The median survival of patients with positive HIF-1α expression was 61.2 months compared to 74.6 months for those with negative HIF-1α expression. Although both overall survival rate and survival time were shorter in cases with high HIF-1α expression, this relationship was not statistically different (p=0.222) (Figure 4).

Disease free survival (DFS) and progression free survival (PFS) of patients with positive and negative HIF-1α expression followed a trend similar to overall survival. Longer durations were observed in patients with negative HIF-1α expression but this was not found to be significant.

Microvessels were evaluated by immunohistochemical studies using CD34 antibody and average MVD was found as 46.7±6.9 (Figure 5, 6). A statistically significant difference was also noted in MVD between histological types (p<0.001). Average MVD was 45.24±6.61 in type I tumors, whereas this was 52.98±4.55 in type II tumors. Between grades, another significant difference was reported in MVD. Spearman's correlation test showed a significant positive correlation between MVD and grade (p=0.009, r=0.268) (Figure 7). However, no correlation was seen between tumor stage, survival rates and MVD.

On analyzing the expression of immunohistochemical markers with each other, we found a significant correlation of HIF-1α with MVD. High HIF-1α expression showed higher MVD (Table 10), (p<0.001).

Table 6. Expression of HIF-1α in type I and II endometrial adenocarcinoma

Histological type		HIF-1α (+) (n=94, %)
Type I	Endometrioid type (n=76)	24 (31.6%)
Type II	Serous type (n=9)	3 (33.3%)
	Clear-cell (n=9)	1 (11.1%)
Total		28

Table 7. HIF-1α expression according to the grades in type I endometrioid tumors

	Grade I (n, %)	Grade II (n, %)	Grade III (n, %)
HIF-1α (+)	7/36 (19.4%)	14/30 (46.7%)	3/10 (30%)

Discussion

Angiogenesis is vital in the development and progression of cancer. Malignant cells continuously proliferate and consume oxygen rapidly, resulting in a hypoxic microenvironment. This critical pathway is believed to be the primary regulator of angiogenesis. Recent studies showed regions of significant hypoxia

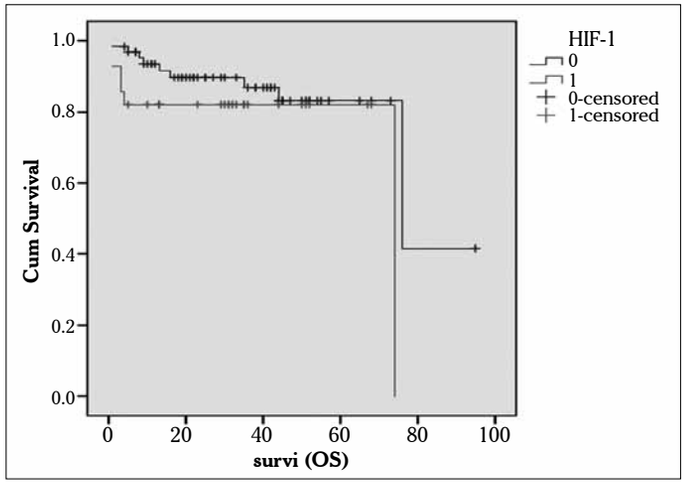

Figure 4. Overall survival of patients with HIF-1α expression
Cum Survival: Overall survival, Survi (OS): Survival in months
1: HIF-1α positive 0: HIF-1α negative, Kaplan-Meier method)

Table 8. HIF-1α expression according to the stages

	Stage I (n, %)	Stage II (n, %)	Stage III (n, %)	Stage IV (n, %)	Total
HIF-1α (+)	15/53 (28.3%)	4/11 (36.4%)	7/27 (25.9%)	2/3 (66.7%)	28
HIF-1α (-)	38/53 (57.6%)	7/11 (63.6%)	20/27 (74.1%)	1/3 (33.3%)	66

Table 9. HIF-1α expression according to the clinicopathological features

	HIF-1α (+) (n)	HIF-1α (-) (n)	p
Recurrence (n=9)	1	8	0.555
Cervical invasion (+) (n=31)	10	21	0.811
Myometrial invasion (+) (n=84)	25	59	0.622
Adnexal involvement (+) (n=20)	5	15	0.784
Lymphovascular invasion (+) (n=34)	10	24	0.573

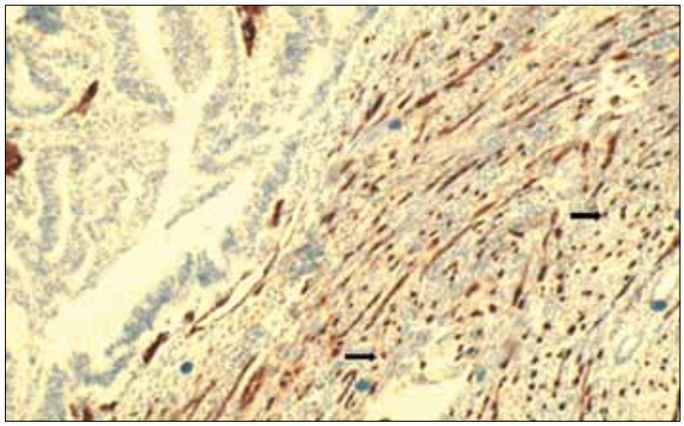

Figure 5. CD34 positivity in endometrioid type adenocarcinoma (CD34, X200)

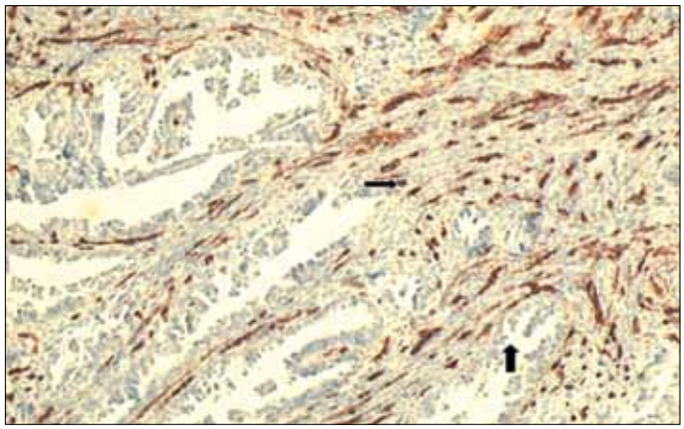

Figure 6. CD34 positivity in serous type adenocarcinoma (CD34, X200)

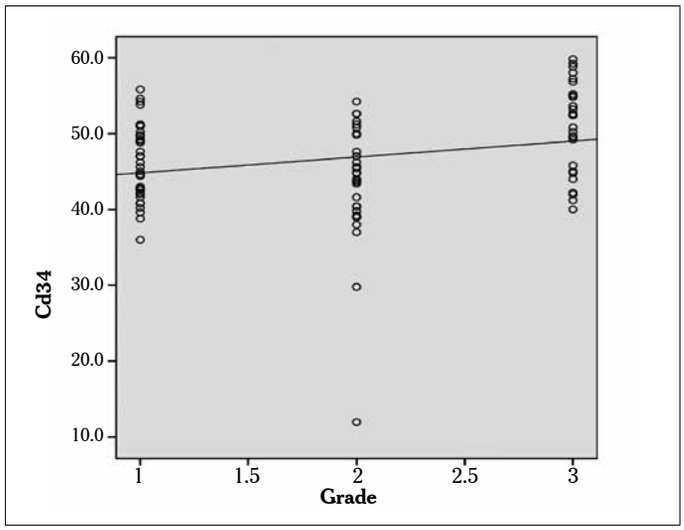

Figure 7. CD34 was correlated with grade
(Spearman's correlation test)

in many tumors (12). HIF-1, a heterodimeric transcription factor, is the key survival gene for cells in a hypoxic environment. HIF-1 is a nuclear factor that is induced in hypoxic cells, composed of HIF-1α and HIF-1β subunits. HIF-1 activates the expression

Table 10. HIF-1α expression and MVD

	CD34 (MVD)
HIF-1α (+)	48.27±8.04
HIF-1α (-)	42.61±5.50
p	<0.001

of over 40 genes at the transcriptional level. In this study, we investigated the immunohistochemical expressions of HIF-1α and CD34 in type I and type II endometrial adenocarcinomas.

HIF-1α is over-expressed in various tumors such as cervical cancer, gastrointestinal stromal tumors, lung cancers, bladder tumors (13-15). It has been shown that HIF-1α is released by two mechanisms in tumor cells. In solid tumors like glioblastoma multiforme, chronic hypoxia is the leading cause of HIF-1α expression. Another way is by a genetic pathway. Tumors with high vascularization such as colon cancers, hemangioblastoma and renal cell carcinomas exhibit HIF-1α expression in this manner. In endometirum cancers, chronic hypoxia-associated HIF-1α expression has been reported in the literarute (16, 17).

HIF-1α overexpression has been described in endometrial cancers (18, 19). Sivridis et al. (18) found overexpression of HIF-1α in 49% of women with Stage I endometrioid adenocarcinomas. However, in this study, other histological types of endometrial carcinomas and different stages have not been included. Pansare et al. (19) also investigated HIF-1α expression in endometrial cancers and only endometrioid and serous cell cancers were defined in their study. However, unlike the previous ones, our study evaluated both type I and type II tumors which included clear cell adenocarcinomas of the endometrium additionally and all stages of endometrium cancer cases.

The findings of our study showed that HIF-1α expression was observed in 31.6% of endometrioid, 33.3% of serous cell and 11.1% of clear cell tumors. There was no significant difference between these three histological types. To the best of our knowledge, this is the first study comparing the expression of HIF-1α in three types of endometrial adenocarcinomas. Our result did not support those of previous studies according to the histological types. Pansare et al. showed that HIF-1α expression reported in 80% of type II and 26% of type I tumors. Lee et al. also studied HIF-1α expression in ovarian cancers and they found that, in clear cell tumors HIF-1α *wa* expressed more than in endometrioid and serous cell cancers (20). On the other hand, Osada et al. found that there was no difference in HIF-1α expression of various histological types of ovarian cancers (21).

Stage is known as the most important prognostic factor in endometrium cancer. In advanced stages, survival of patients declines. In the present study, the positivity of HIF-1α expression was seen in 15 (53.5%) of stage I, 4 (14.2%) of stage II, 7 (25%) of stage III and 2 (7.1%) of stage IV cases. Our study showed no significant difference in HIF-1α expression between various stages of endometrial adenocarcinoma. In the literature, the association of stage and HIF-1α expression was investigated (19). In type I endometrial cancer, high expression of HIF-1α showed a significant correlation with advanced stages. However this correlation was not observed in type II tumors. Likewise, Ozbudak et al. showed increased rates of HIF-1α

high expression in advanced stages of endometrioid adenocarcinoma (22).

Increased HIF-1α expression is associated with aggressive tumor behaviour and ability to metastasize. As a result of this, cervical, myometrial and lymphovascular invasion and adnexial involvement were seen more frequently. In one study, myometrial and adnexial involvement were revealed in a group of patients with high HIF-1α expression (19). However, Sivridis et al. showed that there was no relation between myometrial and lymphovascular invasion and HIF-1α expression (18). In this study, we also found no correlation between tissue invasion and HIF-1α expression.

In the present study, the HIF-1α *staining pattern* was shown to be statistically different in various grades of the tumors. High expression of HIF-1α was significantly different in advanced grades. After exluding all type II grade III tumors from the analysis, we still found a similar correlation with grade and HIF-1α expression ($p=0.008$). Contradictory results have been described in the comparison of grade with the HIF-1α overexpression in endometrial carcinoma. Increased HIF-1α expression has been reported in low grade tumors in endometrial cancers (18). In ovarian tumors, high HIF-1α expression was correlated with grade of the tumors. However, in pancreatic ductal adenocarcinoma, no association was observed between grade and HIF-1α expression (20, 23).

Although endometrium cancer is the most common female genital tract cancer, the majority are diagnosed at an early stage and they are associated with a favorable prognosis. Recurrence develops in 10-15% of patients and is limited to the pelvic cavity in half of the cases. During follow-up, 9 cases developed recurrence and in 5 of them, a vertebra was the recurrence site. Recurrence is usually reported in two years after the initial surgery. Likewise, the average recurrence time in our study was 21.5 months.

In this study, MVD was measured by immunohistochemistry using CD34. In predicting prognosis by calculating microvessels, CD34 was shown to have better results than other antibodies (24). Our findings revealed that MVD was significantly higher in type II tumors than type I tumors (52.98 ± 4.55 vs 45.24 ± 6.61) ($p<0.001$). Since type II tumors are more aggressive and are related to high grades, these results with MVD are consistent with the literature. Our results further demonstrated that a significant correlation has been reported in advanced grades with MVD. We found that values of MVD increased gradually through early to advanced grades of endometrial adenocarcinoma ($p=0.009$, $r=0.268$). Our findings also showed that the relationship between the HIF-1α expression and MVD was statistically significant ($p<0.001$). This result confirmed the relationship between hypoxia and angiogenesis.

Studies evaluating HIF-1α in cancers other than endometrial cancer have revealed that patients with tumors that express high levels of HIF-1α have a poor outcome (25). Birner et al. (26) demonstrated shorter survival rates of patients with high HIF-1α expression in early stage invasive cervical cancers. However, contradictory results have been described as to the prognostic value of HIF-1α overexpression in endometrial carcinoma. Pansare et al. (19) reported that HIF-1α expression did not show a correlation with survival in endometrial cancer. On the other hand, Sivridis et al. (18) showed that overexpression of HIF-1α is associated with a poor prognosis. In our investigation, we found no relationship between survival rates and HIF-1α expression. As a result, correlation of HIF-1α with survival and prognosis is still under discussion. On the other hand, our data is limited in terms of evaluating survival rates. Since our median follow-up time was 31 ± 10.6 months, the range (1-95 months) of follow-up was wide and inconclusive.

HIF-1α staining is detected in both the nucleus and cytoplasm of the cell. However HIF-1α *is only activated in* the nucleus. In some studies, both nuclear and cytoplasmic staining was scored. In our study, we took into account nuclear staining only. Nonetheless, immunohistochemical studies are difficult to evaluate and conflicting data in the literature about HIF-1α staining is still under investigation.

In conclusion, high expression of HIF-1α is associated with tumoral angiogenesis in endometrial adenocarcinomas. Further studies targeting HIF-1α for disrupting mechanisms essential for tumor growth in endometrial cancer will be significant a investigations in the future.

Conflict of interest

No conflict of interest was declared by the authors.

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The effects of immersion in water on labor, birth and newborn and comparison with epidural analgesia and conventional vaginal delivery

Suda doğumun, travay, doğum ve yenidoğan üzerine etkileri ve epidural analjezi ile normal doğum ve konvansiyonel vajinal doğum ile karşılaştırılması

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Abstract

Objective: To document the practice of labour in water, to assess the effects of water immersion during labor and/or birth (labour stages 1, 2 and 3) on maternal, fetal and neonatal wellbeing and to compare the outcomes and safety with conventional vaginal deliveries and deliveries with epidural analgesia.

Material and Methods: Two-hundred and seven women electing for waterbirth (n=207) were compared with women having conventional vaginal deliveries (n=204) and vaginal deliveries with epidural analgesia (n=191). Demographic data, length of 1st, 2nd and 3rd stage of labor, induction and episiotomy requirements, perineal trauma, apgar scores, NICU requirements and VAS scores were noted.

Results: The 1st stage of labor was shorter in waterbirths compared with vaginal delivery with epidural analgesia but the 2nd and 3rd stage of labor were shortest in patients having waterbirth compared with conventional vaginal delivery and vaginal delivery with epidural analgesia. Patients having waterbirth had less requirement for induction and episiotomy but had more perineal laceration. All women having waterbirths had reduced analgesia requirements and had lower scores on VAS. There was no difference in terms of NICU admission between the groups. Apgar scores were comparable in both groups. There were no neonatal deaths or neonatal infections during the study.

Conclusion: The study demonstrates the advantages of labor in water in terms of reduction in 2nd and 3rd stage of labor, reduction in pain and obstetric intervention such as induction or amniotomy. (J Turkish-German Gynecol Assoc 2012; 13: 45-9)

Key words: Water birth, analgesia, neonatal outcome

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Özet

Amaç: Suda doğum pratiğinin dökümentasyonu ve doğum travayı ve/veya doğum eylemi (doğumun birinci, ikinci ve üçüncü evresi) sırasında maternal, fetal ve yenidoğan iyilik hali üzerine etkilerini değerlendirerek, sonuçları ve güvenirlilik açısından epidural analjezi ile vajinal doğum ve konvansiyonel vajinal doğum sonuçları ile kıyaslanması.

Gereç ve Yöntemler: Suda doğum yapan ikiyüzyedi (n=207) kadın, konvansiyonel vajinal doğum yapan (n=204) ve epidural analjezi ile vajinal doğum yapan (n=191) kadınlarla kıyaslandı. Hastaların demografik verileri, doğum eyleminin 1., 2. ve 3. evresinin uzunluğu, indüksiyon ve epizyotomi ihtiyacı, perine travması, yenidoğanın apgar skorlaması, yoğun bakım ihtiyacı ve VAS skorları değerlendirildi.

Bulgular: Konvansiyonel vajinal doğum ve epidural analjezi ile vajinal doğum yapanlarla kıyaslandığında suda doğuranlarda, doğumun 1. evresi kısalmakla birlikte özellikle 2. ve 3. evrelerin çok kısalmış olduğu görüldü. Suda doğum yapan hastalarda oksitosin ihtiyacı belirgin olarak daha azdı, epizyotomi oranı da daha düşüktü fakat daha fazla perineal laserasyon saptandı. Suda doğum yapan kadınların hepsinde analjezi ihtiyacı azalmıştı ve VAS skorları düşüktü. Gruplar arasında yenidoğan yoğun bakım ihtiyacı açısından fark yoktu. Apgar skorları benzerdi. Çalışma dönemi boyunca hiç yenidoğan ölümü veya yenidoğan enfeksiyonu saptanmadı.

Sonuçlar: Çalışma, suda doğumun, doğumun ikinci ve üçüncü evre sürelerini kısaltmak, doğum ağrısını azaltmak ve indüksiyon ve amniyotomi gibi obstetrik yaklaşım ihtiyacını azaltıcı avantajları olduğunu göstermiştir. (J Turkish-German Gynecol Assoc 2012; 13: 45-9)

Anahtar kelimeler: Suda doğum, analjezi, yenidoğan sonuçları

Geliş Tarihi: 15 Eylül 2011 **Kabul Tarihi:** 22 Kasım 2011

Introduction

In 1983, Odent published the results of the first hundred water births in The Lancet (1). It was postulated that anxiety and pain may trigger a stress response during labour (2) leading to reduced uterine activity and dystocia (3). Labouring in water may overcome this stress response by aiding relaxation and relief of pain (4). Zanetti-Daellenbach et al. revealed that water deliveries performed in a selected low risk collective needed less analgesia had a shorter duration of first and second stages of labour, a lower episiotomy rate and were not associated with any adverse maternal

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or fetal outcome (5). The advantages of immersion in water during labour and/or birth include reduced pain, increased functional diameter of the true pelvis, increased quality of contractions, increased release of endorphins, decreased need for opiates, increased movement for the mother as well as improved positioning in different stages of labour (6). There are also studies that have reported the disadvantages associated with water birth which include maternal and neonatal infections, as well as the possibility of respiratory problems for the newborn (7, 8). Cluett and Burns in a review of 11 trials concluded that water immersion during the first stage of labour reduced the use of epidural/spinal analgesia, but there was limited data for other outcomes related to water use during the first and second stages of labour (9). They also stated that there was no evidence of increased adverse effects on the fetus/neonate or woman from labouring in water (9).
The aim of this study is to document the practice of labour in water, to assess the effects of water immersion during labour and/or birth (labour stages 1, 2 and 3) on maternal, fetal and neonatal wellbeing and to compare the outcomes and safety with conventional vaginal deliveries and deliveries with epidural analgesia.

Materials and Methods

In a prospective clinical trial, the interview and observation techniques were used to study 610 pregnant women who were admitted to Zekai Tahir Burak Women's Health Education and Research Hospital, between June 2007 and September 2008. Women electing for water birth (Study Group 1, n=207) were compared with vaginal deliveries with epidural analgesia (Study Group 2, n=191) and women having conventional vaginal deliveries (Control, n=204). The pregnant women were given comprehensive information on water birth before they were asked to participate in the study. Ethical approval was obtained from the local research ethics committee prior to the study, and written informed consent obtained from all patients. This study was conducted in accordance with the basic principles of the Helsinki Declaration. The inclusion criteria were gestational age between 37-42 weeks, no previous history of cesarean section, intact membranes, absence of placental abruption or placenta previa, no malpresentation, normal sized single fetus, and normal results of fetal wellbeing tests. The pregnant women with medical or obstetric risk factors were excluded (n=8). Women presenting on the delivery suite with painful uterine contractions had an initial cervical assessment. This was taken as the onset of the active phase of labour in all groups. They were assigned to control and two study groups. The women were put in a standardized warm water pool which is large enough to allow the pregnant women move freely. At the time of delivery the water temperature was set to between 37 and 37.5°C so that the baby was not stimulated to breathe underwater by the cooler temperature of the pool. Fetal heart monitoring was performed at regular intervals with Doppler or NST. In the second stage of labour, care was taken to ensure the controlled delivery of the head of the fetus. The newborn was placed gently in the mother's arms within seconds but without rushing and then the cord clamped and cut. Delivery of the placenta and the membranes was completed outside the pool. After the delivery, the pool was emptied and cleaned with antiseptic solution. Cultures were taken for the determination of pathogenic bacteria. Demographic data, length of 1st, 2nd and 3rd stages of labor, requirement for induction and episiotomy, perineal trauma, apgar scores, neonatal intensive care unit (NICU) requirements and visual analog scale (VAS) scores were noted on a questionnaire. The women evaluated their birth experience with the VAS (10 cm long VAS from 1 to 10 corresponding to the amount of pain felt by the woman with number 1 for no pain and with number 10 for dreadful pain).

Statistical analysis

Data were evaluated by SPSS for Windows release 15.0 (Chicago Inc.). To compare groups, we used the Chi-square test for categorical variables, Oneway ANOVA and Bonferroni tests for continuous variables that have normal distribution, Kruskall-Wallis oneway ANOVA for continuous variables that have no normal distribution. As described, variables, frequencies and percentages were given for categorical variables, Mean±standard deviations and median were given for continuous variables. Alpha=0.05 was accepted as a statistically significant value. In order to detect±2 percentage point difference in VAS scores between groups, for having alpha=0.05, power=0.97, it was predicted that approximately 200 subjects for each group should be taken (NCSS-Pass Pocket Program was used) (Chow SC et al.) (10).

Results

The study groups consisted of 207 water births (Group 1) and 191 vaginal deliveries with epidural analgesia (Group 2), the control group (Group 3) of 204 patients gave birth by the conventional vaginal delivery method at the hospital. The women in the three groups were matched with respect to age, BMI and gestational age (Table 1). There were 276 primigravidae and 326 multiparous women having water births (Table 2). The mean age of the women were 26.2±5.1, 26.1±4.5 and 25.5±5.1 respectively (Table 1). The mean cervical dilatation at admission in both group 1 (5.3 cm) and group 2 (4.6 cm) was not significantly different from group 3 (4.7 cm). The duration of the 1st stage of labour was shortest in the conventional vaginal delivery group whereas the duration of the 2nd and the 3rd stages of labour were shortest in the water birth group (Table 1). There was a highly significant reduction in the induction and episiotomy requirements in the water birth group (Table 1). VAS scores were the lowest in the water birth group, so there was less analgesia requirement (Table 1). Conversely, the perineal laceration rate was higher in the water birth group, however most of these lacerations were minimal. Systolic and diastolic blood pressures seem to be lower in the water birth group, however the differences were not clinically significant. The decrease in hemoglobin levels as an indication of blood loss during labour were not statistically significant. The birthweight of the infants were highest, however Apgar scores were slightly lower in the water birth group. There was no difference in the

Table 1. Demographic data, labour characteristics and neonatal outcomes of the three groups

	Labour in water (n=207)	Vaginal delivery with epidural analgesia (n=191)	Conventional vaginal delivery (n=204)	p
Age (years)	26.2±5.1	26.1±4.5	25.5±5.1	NS
BMI (kg/m^2)	29.2±5.3	28.2±6.3	27.6±3.6	NS
Gestational week (weeks)	39.1±1.1	39.1±1.3	38.8±1.3	0.0001
Antenatal care (n, %)	170 (82.1%)	179 (33.7%)	159 (77.9%)	0.0001
1st stage of labour (min)	265.6±546.6	268.7±177.4	240.1±190.8	0.0001
2nd stage of labour (min)	10.9±5.02	28.3±13.3	23.9±14	0.0001
3rd stage of labour (min)	3.8±1.5	5.3±4.4	8.02±3.3	0.0001
Induction (n,%)	11 (5.3%)	58 (30.4%)	57 (27.9%)	0.0001
Episiotomy (n,%)	56 (27.1%)	132 (69.1%)	182 (89.2%)	0.0001
Perineal laceration (n,%)	43 (20.8%)	13 (6.8%)	3 (1.5%)	0.0001
VAS*	4.7±1.3	5.8±0.9	5.6±1.1	0.0001
Systolic blood pressure (mmHg)	110±11	111±10	113±9	0.016
Diastolic blood pressure (mmHg)	69±8	70±9	72±8	0.013
Pulse (n)	85±5	85±6	86±4	0.063
Decrease in hemoglobin level (g/dl)	0.22±1.1	0.51±1.05	-0.12±5.89	NS
Birthweight (g)	3364.5±412.9	3228.1±370.2	3275.3±377.5	0.002
1st min Apgar scores				0.001
<7	26 (12.6%)	0 (0.0%)	3 (1.5%)	
≥7	181 (87.4%)	191 (100%)	201 (98.5%)	
5th min Apgar scores				-----
<7	0 (0.0%)	0 (0.0%)	0 (0.0%)	
≥7	207 (100%)	191 (100%)	201 (100%)	
*NICU (n,%)	5 (2.4%)	7 (3.7%)	2 (1%)	NS

Mean±standart deviations and median were given for all variables, p value <0.05 was accepted as statistically significant, VAS*: Visual analog scale, *NICU: Neonatal intensive care unit

rates of admissions to the NICU between the groups (Table 1). There were no documented neonatal infections. There was no adverse perinatal outcome or neonatal deaths. All the babies were born in good condition.

When primigravidas and multiparous women having water birth are considered (Table 2), the 1st stage of labour is longer in both groups compared with controls, however the 2nd and 3rd stages of labour were the shortest in both primigravidas and multiparous women labouring in water compared with controls. The need for induction and episiotomy for both primigravidas and multiparous women was lowest in water birth group compared with others. Perineal laceration rates were higher in both groups compared with controls. VAS scores were lowest in the water birth group in both primigravidas and multiparous women. Apgar scores were slightly lower in the water birth group, however NICU admission rates were not statistically different in the water birth group for both primigravidas and multigravidas (Table 2).

Discussion

Water births have rapidly become one of the most popular birth methods. There is evidence of use of water immersion as a therapeutic medium for physical and psychological illnesses by the Chinese, Egyptians, Japanese and Assyrians, as well as Greeks and Romans (9). Water immersion during labour, including birth, used for relaxation and pain relief, has a long history. In 1995, the first international water birth conference was held in London, followed by many researches and conferences.

The positive physiological effects of hydrotherapy can facilitate the neurohormonal interactions of labour, reducing pain, and potentially facilitates the progress of labour (11, 12). Water immersion may be associated with improved uterine perfusion, less painful contractions and a shorter labour with fewer interventions (13-15). Several reports have shown that water immer-

Table 2. Comparison of water birth, epidural analgesia and conventional vaginal delivery in primiparous and multiparous women

	Primiparous (n=276)				Multiparous (n=326)			
	Group 1 (Labor in water)	Group 2 (vaginal delivery with Epidural analgesia)	Controls (Conventional vaginal delivery)	p	Group 1 (Labor in water) with Epidural analgesia)	Group 2 (vaginal delivery	Controls (Conventional vaginal delivery)	p
	(n=52)	(n=156)	(n=68)		(n=155)	(n=35)	(n=136)	
1st stage (min)	331.3±832.5	282.7±184.0	300±151.9	0.002	245.3±423.7	204.6±126.1	210.0±201.3	0.012
2nd stage (min)	11±5	28.6±13.7	27.2±16.7	0.0001	10.9±5.01	26.8±11.6	22.2±12.1	0.0001
3rd stage (min)	3.9±2.3	5.5±4.6	7.6±3.2	0.0001	3.7±1.2	4.5±3.3	8.1±3.3	0.0001
Induction (n, %)	3 (5.8%)	50 (32.1%)	30 (44.1%)	0.0001	8 (5.2%)	8 (22.9%)	27 (19.9%)	0.0001
Episiotomy (n, %)	27 (51.9 %)	105 (67.3%)	66 (97.1%)	0.0001	29 (18.7%)	27 (77.1%)	116 (85.3%)	0.0001
Perineal laseration (n, %)	14 (26.9%)	10 (6.4%)	1 (1.5%)	0.0001	29 (18.7%)	3 (8.6%)	2 (1.5%)	0.0001
VAS*	4.6±1.2	5.8±1.04	5.7±0.97	0.0001	4.7±1.3	5.8±0.7	5.6±1.18	0.0001
Apgar 1st min >=7	40 (76.9%)	156 (100%)	67 (98.5%)	0,001	141 (91.0%)	35 (100%)	134 (98.5%)	0.004
<7	12 (23.1%)	0 (0.0%)	1 (1.5%)		14 (9.0%)	0 (0.0%)	2 (1.5%)	
Apgar 5th min>=7	52 (100)	156 (100%)	68 (100%)	----	155 (100)	35 (100%)	136 (100%)	----
NICU*	4 (7.7%)	7 (4.5%)	0	NS	1 (0.6%)	0	2 (1.5%)	NS

VAS*: Visual analog scale, NICU*: Neonatal intensive care unit

sion shortens the process of labour (1, 15), however some others found no significant difference for the duration of the 1st stages of labour (13, 16-20). The present study also demonstrates that the 1st stage of labour is not shortened by immersion in water in either primigravidas or multigravidas. Cammu et al., Eckert et al., Rush et al. and Woodward et al. provided data on analgesia and anesthesia use in their studies and found that there was a significant reduction in the incidence of analgesia and anesthesia use among women placed in water during the first stage of labour (16, 17, 19, 20). In the present study, we have shown that VAS scores indicating the pain felt by the women were lowest among women having water birth, even lower than the women labouring with epidural analgesia. In agreement with these studies, we observed that immersion in water greatly reduces the pain and need for additional analgesia.

Labouring in water has been found to reduce stress hormones and cathecolamines which inhibit oxytocin and labour progress (9). In our study, the duration of the second stage of labour was found to be shorter in water births, consistent with the results of studies of Chaichian et al. and Otigbah et al. (6, 21). The fetus may be more likely to adopt a more relaxed and flexed position, because the mother can easily explore different positions to maximise her pelvic diameters (22). The duration of the 3rd stage of labour, which is the delivery of the placenta, is also significantly reduced after water births. This minimizes amount of blood loss during this period. The lower blood loss in water births can also be explained by the hydrostatic pressure in the pool, by the less severe lacerations or possibly by a facilitated control of the third stage of labour.

In the study, patients having waterbirth had less requirement for obstetric interventions such as induction and episiotomy but had more perineal lacerations. However, the lacerations in water birth group were less severe than those in epidural analgesia and conventional delivery group. Otigbah et al. found that primigravidas having water births had less perineal trauma and the overall episiotomy rate was 5 times greater in the control group, but overall, more women having water births had perineal tears (21). On the other hand, there are studies which show no difference in perineal trauma (23, 24). The reason why women having water births had more tears may be explained by the difficulty in accessing the women's perineum during birth, resulting in more perineal trauma. However, the widespread belief that with episiotomies 3rd and 4th degree lacerations are avoided is open to question, because in our study episiotomy rates were lowest after water births, and the lacerations were minimal.

There are some concerns about water birth for the fetus. These are thermoregulation during labour, infection and onset of respiration at birth. As the water temperature of the pool does not exceed the maternal body temperature, fetal hyperthermia and associated cardiovascular and metabolic disturbances will not occur (25). None of the newborns in our study took its first breath in water. This has been explained by the diving reflex which shows that, when the face or especially the glottis comes in contact with fluid, respiration movements are inhibited. Aspiration will occur only when the diving reflex fails, because of anesthesia or severe asphyxia or because of the inappropriate pool temperature. When we consider neonatal infection, several reported comparative studies, cohort studies and audits report no increased risk of infection to the newborn (5, 19, 21). This is also confirmed by the study that there were no documented neonatal infections. Overall, water birth does not cause an increased risk of adverse effects to the fetus/newborn.

In conclusion, the study demonstrates the advantages of water birth in terms of reduction in the duration of the 2nd and 3rd stages of labor, reduction in pain and obstetric intervention such as induction or episiotomy. Labouring in water significantly reduces pain and the requirement of epidural/spinal analgesia. It is a management approach which contributes positively to maternal physiological and psychological health by reducing the augmentation which is known to increase the risk of uterine hyperstimulation and fetal hypoxia and by reducing the obstetric interventions which are associated with lower maternal satisfaction. There is no increased adverse effects to the fetus or labouring women. Water birth may be an alternative birth method that can be offered in selected patients.

Conflict of interest

No conflict of interest was declared by the authors.

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Preimplantation genetic diagnosis (PGD) according to medical ethics and medical law

Tıp etiği ve tıp hukuku açısından preimplantasyon genetik tanı (PGT)

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Abstract

Assisted reproductive techniques not only nourish great and sometimes illusive hopes of couples who yearn for babies, but also spark new debates by reversing opinions, beliefs and values. Applications made to infertility clinics are increasing due to the influences such as broadcasts made by the media concerning assisted reproductive techniques and other infertility treatments, increase in the knowledge that people have about these problems, late marriages and postponement of childbearing age owing to sociological changes. Pre-implantation genetic diagnosis (PGD) is a technique applied to couples who are known to carry genetic diseases or who have children with genetic diseases. This technique is conducted by doctors in Turkey for its important contribution to decreasing the risk of genetic diseases and in order to raise healthy generations. In this paper, the general ethical debates and the legal situation in Turkey will be discussed.

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Key words: Preimplantation genetic diagnosis, artificial implantation, genetic diseases, medical ethics, medical law

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Özet

Yardımcı üreme teknikleri, çocuk arzusu duyan çiftlerde büyük ve bazen aldatıcı da olabilen umutları beslemenin yanı sıra, düşünceleri, inançları ve değerleri alt üst ederek yeni tartışmalar yaratmıştır. Medyanın yardımcı üreme teknikleri ve diğer infertilite tedavileri ile ilgili yayınlar yapması, insanların bu sorunlarla ilgili bilgilerinin artması, sosyolojik değişiklikler nedeniyle geç evlenme ve çocuk doğurma yaşının ertelenmesi gibi nedenlerle infertilite kliniklerine başvurular artmıştır. Preimplantasyon genetik tanı (PGT), daha önce genetik bir hastalık taşıdığı bilinen çiftlerde, genetik hastalıklı çocuğu olan çiftlerde uygulanan bir tekniktir. Türkiye'de genetik hastalık riskinin düşürülmesi yönündeki çok önemli katkısı ve sağlıklı nesiller yetiştirilmesi amacıyla bu tekniğe izin verilmektedir. Bu yazıda konuyla ilgili etik tartışmalara ve tıp hukuku açısından Türkiye'deki yasal duruma değinilmiştir. (J Turkish-German Gynecol Assoc 2012; 13: 50-5)

Anahtar kelimeler: Preimplantasyon genetik tanı, yapay döllenme, genetik hastalıklar, tıp etiği, tıp hukuku

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Introduction

Assisted reproductive techniques not only nourish great and sometimes illusive hopes of couples who yearn for babies, but also spark new debates by reversing opinions, beliefs and values. Applications made to infertility clinics are increasing due to influences such as broadcasts made by the media concerning assisted reproductive techniques and other infertility treatments, increase in the knowledge that people have about these problems, late marriages and postponement of childbearing age owing to sociological changes.

Many new problems have arisen and await solutions in this field in the context of donors, recipients, babies to be born and healthcare professionals.

While debates in this regard focus especially on the fundamental rights of individuals in European countries; this subject has also begun to become a current issue in our country after the inclusion of in vitro fertilization treatment in the scope of compulsory health expenditures.

Although in vitro fertilisation (IVF) with the husband's sperm is considered reasonable for married couples in many societies and is included in the scope of compulsory insurance; in vitro fertilisation by a donor and its various methods give rise to many arguments. Fertilisation with medical assistance created different types of motherhood and fatherhood, and children who are the products of science, by separating sexuality from reproduction, selecting the embryos without any genetic diseases, conception from bloodline and biological affinity from emotions and nourishment. In addition to questions such as, "Who are the mother and father? What does parenthood mean?", problems arising from the lack of commercial and ethical standards, which require large amounts of money and significant inequalities, have sparked great debates in academies, courts, the media and public (1).

When we look at the development of assisted reproductive techniques, we realize that there is a substantial historical accumulation process in this field. Throughout the history, scientists looked for treatments and lenitives for infertility, which was a major fear, meanwhile quack doctors exploited the distress and desires of childless couples and earned a considerable amount of money. However in 1978, a miraculous development occurred; the first test tube baby, Louise Brown, was

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born in Manchester, England and brought fame to Dr. Edward. The method was successful for the first time, although considerable experimental accumulation on assisted reproduction treatment was behind it. The first experiment was conducted in 1791 by an English doctor named Hunter, and later a similar experiment was carried out in 1804 in France. The idea of donor artificial insemination (DAI) caused negative reactions from the very beginning and was disapproved by the Vatican. Nevertheless this method was conducted in 1884 for the first time in the USA by Dr. Pancost. Later, in 1940, Dr. Parker tried sperm cryopreservation techniques (2).

After this process of accumulation produced results in 1978, the first test tube baby Amandine was born in France and up to today thousands of babies were born through in vitro fertilization techniques. Dr. Carl Wood supervised the birth of the first cryopreserved embryo baby, Zoe, in 1984 in Melbourne. Doctors in the Netherlands and Britain followed his method and the rate of medical and biological development increased (3).

Reproductive technology and major problematical areas

With every new medical or biological development, new problems arise. Concerning the arguments on reproductive technology, the status of the child and a specific family structure are the vital elements. In the Western world, in addition to the subjects of debate such as 'the right to have a child' or 'rights of the child'; subjects such as 'surrogate motherhood', 'insemination after death' or 'impregnation with sperm donation' have also gained importance today and legal arrangements have been made in many European countries and in the USA in this respect. Within a few years, tens of thousands of pregnancies have taken place through donor in vitro fertilisation, Egg and Sperm Research and Protection centers have been established and laws and regulations have been passed (4).

These techniques have been particular concerns not only to the persons that wished to take advantage of them but also to the child and the society in which the child would grow up. Problems regarding this matter have been not only technical but also extremely conceptual; legal and ethical aspects of this matter have been discussed under several titles, such as the naturality/artificiality of assisted reproductive techniques, moral and ethical position of human pre-embryo, role of the family and genetic link problem, rights of sperm/gamete donors and surrogate mothers and gender selection (5).

Right to have a child and rights of the child

The desire to have a child is a complicated need concerning the self-identity of an individual, nature of parenthood and his/her idea of family structure. The right to benefit from scientific developments in order to have a child due to this need is considered as a responsibility of governments and has been discussed in recent years not only in terms of reproductive rights, but also the assurance and subsidization of reproduction by medical science and the state. However, reproduction also has social and symbolic functions that imply familial and ethical references. No cultures can degrade bloodline to birth, motherhood to pregnancy or rights of the child to the desires of parents (4).

Although there are more problematic fields for donor artificial insemination, in which a third party is included in the process, operations between married couples, which gained general acceptance in many countries, are not free of problems. As methods of embryo cryopreservation develop, discussions have been initiated concerning the rights of the embryo.

While these developments were emerging, ethical disagreements were inevitable; the problem was not only the simplification of reproduction, it was about turning reproduction into a matter of choice; life could be deposited in the bank like an investment. It was also possible to make choices about the number of transplantations; and second twins were born in Melbourne 16 months after the birth of the first twins. The family tree of these children revealed results which were startling to some people; some dramatic results caused new polemics concerning multiple pregnancies and their family lives. Everything seemed possible; embryos could be manipulated and unused embryos could be used for in vitro fertilization experiments. The argument passed to the problem of the moment when an individual was created as a human being. In other words, what was the status of the embryo? (3)

There is no clear statement regarding the legal status of an unborn human being in the European Convention of Human Rights. Similarly, the Turkish Constitution (1982) does not cover a clear provision concerning this matter (6).

Legal situation in Turkey

Remarkable information regarding the approach to the fertilized human egg in the Turkish law is found in the Civil Code, Population Planning Law, its relevant legal arrangements and the Penal Code. Such arrangements do not accept an unborn human being as an individual. However, again in the framework of this legal arrangement, an unborn human being is provided with a limited protection. For instance, intentional abortion after the 10^{th} week of pregnancy, an abortion induced by some other person, although the mother's consent is given, has been defined as a crime in the Penal Code. The Civil Code also includes a provision that states that a fetus holds rights during the period when it is present in the mother's uterus until its live birth. When the legal qualification of the fertilized human egg after it leaves the uterus is evaluated, provisions which imply the non-acceptance of a person's existence are worthy of attention in the legal arrangements.

Concerning the embryos outside of uterus, provisions are made in the Regulation of Assisted-Reproduction Treatment Centers. This regulation indicates that embryos which are not used/cannot be used/are not agreed to be used by the applicants they are taken from need to be disposed of according to a specific procedure. It is clear here that the embryo is not protected by a right to live. It is clearly stated in the Regulation of Assisted-Reproduction Treatment Centers that unwanted embryos cannot be used for any other purposes except for assisted-reproduction treatment method (6).

Although "Protection of Human Rights and Human Dignity in Terms of Biology and Medical Practices, Human Rights and Biomedicine Agreement" (Biomedicine Agreement) does not

prohibit research to be conducted on an embryo in a tube, it specifies that, in the event that it is allowed by the laws, adequate protection needs to be provided for the embryo. However, the Agreement also indicates that researches need to be conducted within the framework of the laws. A legal arrangement regarding this matter does not exist in Turkey. Our country is in need of a legal arrangement in this respect.

Creation of an embryo for the purpose of research has been prohibited in the Biomedicine Agreement. Accordingly, creation of an embryo with the sole purpose of research is not possible in Turkey. Contrary acts cause contradiction with the Biomedicine Agreement (6).

The Biomedicine Agreement signed by Turkey on April 4th, 1997, has been approved by the Turkish Grand National Assembly on 3.12.2003 and the relevant law has entered into force on the same date with the name, "Protection of Human Rights and Human Dignity Agreement in Terms of Biology and Medical Practices: Law Concerning Approval of Human Rights and Biomedicine Agreement" after having been published on the Official Gazette numbered 25311 on December 9th, 2003 with the Law number 5013 (6).

In terms of the Turkish judicial system, the place of international agreements within municipal law is determined by the 90th article of the Constitution. According to this article; "Approval of agreements to be made with foreign states and international organizations on behalf of the Republic of Turkey is subject to the confirmation of the Turkish Grand National Assembly through a law". In the continuation of this article, it is specified that international agreements put into force in due form are statutory and lawsuits cannot be brought against them with a plea of unconstitutionality. On the other hand, with an addition made to the 90th article of the Constitution on 7.5.2004 through law no. 5170, it is stated that, in the case of a contradiction arising due to different provisions taking place in international agreements and laws concerning fundamental rights and freedoms, provisions of international agreements shall be the basis. "Human Rights and Biomedicine Agreement", which is a part of Turkish municipal law, sets an example for such international documents (6).

Preimplantation genetic diagnosis according to medical law

In this context, the most controversial subjects regarding the usage of assisted reproductive techniques can be specified as: who can be sperm donors, if the set criteria are reliable in terms of genetics and biology, if the criteria are reliable in terms of health or aesthetic and social terms, whose sperm shall be used to fertilize, whose consent shall be obtained, if the sperm donor holds a right of fatherhood, if this right can be taken from this person and if so, who shall remove this right, how the selling of sperm and therefore commercialization shall be prevented, if the surrogate mother or the biological mother holds rights on the child; when embryos are created with more than one gamete for in vitro fertilization practices and one or more embryo(s) is/are placed in the mother's uterus, what shall happen to the other embryos, if they shall be disposed of or why, how and for what purpose they shall be kept. These problems form the most significant and current central points of legal arguments in this field, as shall be discussed here (7).

First of all, assisted-reproduction tools and methods must be arranged in accordance with the laws in our country. Legal loopholes create serious problems and misappropriation not only in penal law but also in private law. In fact, we cannot take action according to an assumption/postulate that anything we are able to do as human beings is allowed (7).

Apart from a Law Concerning Protection of Embryo, a Law Concerning Stem Cell Studies, a Law Concerning Protection of Personal Data, a Data Bank Law generally known as the conception of DNA Bank in our country which has got a limited meaning and a Bio-bank Law need to be legislated.

Lack of legal arrangements and punishment norms, especially in terms of serious violations, will also open the doors for other negative results: Just as individuals can perform the acts that are prohibited by Turkish regulations in North Cyprus, Greece or Belgium and avoid their responsibilities in Turkey, Turkey may become a paradise of experimental acts on embryos in the event that a legal arrangement is not made; Turkey may be deemed to have helped since it shall not inflict punishments on such acts, may be deemed not to have done her part in terms of international law and not to have performed the liabilities imposed by the international agreements. Persons visiting Turkey may conduct acts on embryos such as experiments, heterologous insemination, surrogate motherhood, fertilizing human eggs or sperms with the dead or animals or other acts that we examine within this study (6).

Since the decision made by the High Council of Health in 1987 regarding the approval of in vitro fertilization practices, the allowed acts in Turkey are; the limited permission of such acts and allowing them in the institutions and organizations approved by the Ministry of Health under control/supervision, in a very limited area.

Laws have not yet been legislated and this subject is managed through legislations, memorandums and regulations. Considering positive texts, "*In Vitro Fertilization and Embryo Transfer Centers Regulation*" has been published by the Ministry of Public Health and Welfare for the first time in 1987 and first practices started after this date. This regulation has been changed many times and it has been renamed "*Assisted-Reproduction Treatment Centers Regulation*" as it is presently called. This lack of law causes many misappropriations and legal loopholes, prevents the punishment of offenders and leads to the limitation of the rights and freedoms of individuals arbitrarily through legislations or memorandums/regulations; which contradict the 13th article of the Constitution that requires limitations not to affect the rights and freedoms and to be in accordance with the relevant laws (implicitly, also with the international laws when the 90th article of the Constitution is taken into consideration) (6).

According to the practices conducted since 1987 under the control of the Ministry of Health; in case the mother is infertile, fertilizing the mother's egg with the father's sperm in a tube and placing it in the mother's uterus is allowed, only for married couples and with the couple's eggs and sperms. Although lacking legal grounds, deontologists who mistake the law for customs and traditions or; morals for law, describe the artifi-

cial insemination made with eggs or sperms taken from other people as 'illegal' faultily, without any exceptions and any legal grounds. In addition to such justifications, there are concerns regarding the possibilities such as, the mother or father may claim rights to the child afterwards, the child may have negative feelings towards his/her mother and/or father, possible marriages between brothers and sisters or between the children and the parents. However, even those who object to such opinions acknowledge that these problems can be solved in a modern society in legal and ethical terms, cannot bring forward their arguments about contradiction with ethics and law; and close the doors of opposition to such methods (6).

According to the Regulation *(Assisted-Reproduction Treatment Centers Regulation)*, assisted-reproduction centers require the couples to be married, to use only their own germ cells and cannot have children through the current treatment methods stipulated in the Regulation for married couples. There are no positive texts arranging the legal status for people except for the aforementioned couples (6).

As we know, preimplantation genetic diagnosis allows the genetic characteristics of embryos to be studied before being transferred to the uterus to prevent the birth of a child with genetic defects in couples with high genetic risk. This technique is useful in couples who have a high risk of passing on certain genetic diseases or chromosome mutations to their children. It is also indicated in some couples coming from an IVF programme.

Monogenic diseases are those caused by a specific gene mutation (cystic fibrosis, thalassemias, fragile X syndrome, etc.). To prevent the transmission of this disease by use of PGD, it is essential to know the mutation causing the disease.

If a family has a disease associated with X chromosome but the specific gene alteration is not known, PGD can be conducted by means of gender selection. Gender selection for social reasons is prohibited.

The presence of a chromosome reorganisation (Robertsonian translocations, reciprocal translocations and inversions) in one member of the couple may lead to difficulties in conceiving, miscarriages or congenital malformations. The use of PGD in these couples is extremely useful (8).

It is also indicated in cases of numerical chromosome abnormalities, pure or mosaic.

Both in the case of monogenic diseases and those associated with chromosomal reorganisation, it is necessary to conduct a genetic informativity study before the PGD cycle to confirm that the diagnosis is reliable and to adjust the technique to each individual case.

PGD permits the screening for chromosomes most commonly involved in prenatal abnormalities and miscarriages during the first trimester. The aim is to improve rates of live home births by means of increased implantation rates and a reduction in the number of miscarriages and conceptions with chromosomal diseases.This may be indicated in various situations such as; advanced maternal age (>37 years), altered male meiosis, couples with repeated miscarriage and couples with repeated implantation failures (8).

It is known that mutations of some genes predispose individuals to certain diseases that may appear at different life stages, such as neurofibromatosis, familial adenomatous polyposis or genetic breast cancer (BRCA1, BRCA2), etc.

When a hereditary disease component is confirmed, the possibility of PGD would allow the possible appearance of this disease in the next generation to be avoided.

In order to apply the *preimplantation* genetic diagnosis (PGD), it is necessary to obtain embryos from the couple using IVF techniques, even when there are no infertility issues. Embryo biopsy is conducted three days after insemination, when the embryo has about 6-10 cells. It consists of extracting one or two cells from the embryo, but without affecting its normal development. Using a laser fitted to a microscope, the outer layer of the embryo (zona pellucida) is dissected and the biopsy is then performed. Once the biopsy has been performed, the embryo is maintained in culture until the time of transfer (day +5) (9).

The biopsy obtained is then processed for analysis by means of Fluorescent In Situ Hybridization (FISH) or Polymerase Chain Reaction (PCR), depending on the disease being analysed. Cytogenetic analysis of interphase nuclei allows us to detect both numeric and structural chromosome mutations. The FISH technique consists of applying DNA probes which are specific for the chromosomes being analysed and this enables the chromosomes analysed to be counted, detecting possible aneuploidy (missing or extra chromosomes).

In the case of chromosome reorganisation, the chromosomes analysed are only those involved in the reorganisation. For aneuploidy screening in IVF patients, the 13, 15, 16, 18, 21, 22, X and Y chromosomes are studied, which permits a large number of abnormalities to be ruled out. The PCR technique is used for the diagnosis of monogenic diseases. With this process, the presence of the altered gene is detected using specific DNA sequence amplification. The diagnostic efficiency with the PGD technique is approximately 95% (9).

On the other hand, according to the latest contemporary experiments, Array-CGH will detect approximately 50% more abnormalities than 12 probe FISH and 20% more abnormal embryos (abnormalities tend to concentrate on the same embryos). Being quantitative, Array-CGH can detect all aneuploidies. It should be noted that Array-CGH cannot detect polyploidy, but this would result in only 0.2% missed abnormalities. Array CGH can detect deletions and duplications of small pieces of DNA. Presently, we are using Array-CGH not only to detect whole chromosome numerical abnormalities (aneuploidy) but also for translocations, inversions and other chromosomal abnormalities (10).

The choice of which embryos to transfer is based on the genetic test results and the embryonic viability characteristics. If there are extra viable pre-embryos which are not transferred in this cycle these are cryopreserved for subsequent cycles. After a PGD cycle, Prenatal Diagnostic testing is advisable during the first weeks of pregnancy (9).

Although *preimplantation* genetic diagnosis (PGD), makes a great contribution to the decrease of genetic disease risks, disposal of such embryos spark ethical and legal debates. In our country, a human being cannot be mentioned as long as a

human-specific distinctive such as implantation and primitive band on the uterus wall becomes evident and diagnosed by scientists, and the 28th article of Turkish Civil Code states that a human being cannot be mentioned as long as the fetus is born live from the uterus, penal law protection norms peculiar to human life cannot function here; this embryo is considered as a part of the human body and as a tissue within this period, it is only protected within the scope of the norms that protect human organs and tissues, in other words, the special law against trading of organs and tissues; and the articles 91-93 of Turkish Civil Code. However another opinion claims that human life starts with insemination; and when this opinion is accepted, existence of a human being shall be accepted immediately after the insemination process is completed and relevant norms shall be applied as an act has been conducted against an individual (11).

According to the Swiss Civil Code (art. 31), when a person is born live, legal protections regarding his/her life start at that moment. Although rights are accorded to the embryo, which holds a life capability, to be used after it is born, it is indirectly protected through penal law protection, regulations concerning crime types of abortion or miscarriage; and norms protecting the life and physical integrity of the mother. The actual direct protection starts when it is an individual, after its birth. Interventions on the physical integrity of the embryo are not considered as physical violence or abortion acts; there are no penal laws concerning such crimes. We see the same lack of protection in genetic manipulations conducted on the embryo. The embryo can even be taken from the mother's uterus with her consent until a certain period of time and the law does not impose any responsibility judgments on this act. Sex determination on the fetus is also an unpunished illegal act; and limitless permitted artificial insemination among unmarried couples will cause bloodline problems and ethical and legal problems such as parent-child or brother-sister marriages in the future. This subject needs to be juridically arranged (8).

Despite the prohibitory provisions of the Biomedicine Agreement (art. 11 vd, art. 13 and art. 15 vd) and without any technical differentiations, some writers argue that such researches should be permitted in our country on the condition that they shall not exceed 25 days after insemination. It must be noted that the 18th article of the European Council Biomedicine Agreement is related especially to in vitro fertilization and according to the text within this article, in cases where the law allows researches to be conducted on the embryo, appropriate protection shall be provided for the embryo (paragraph: 1) and creation of human embryos only for research purposes is forbidden (paragraph: 2) (6).

Ethical Conflicts

In the case of preimplantation genetic diagnosis, healthy infants are chosen and this intervention is considered as ethical behavior and conducted by doctors in Turkey in order to raise healthy generations. Although it is stated with general expressions that '*if transmittance of the disease cannot be prevented even though all necessary precautions are taken, the operation must not be conducted*', such diseases need to be interpreted as serious diseases that can leave the child disabled, irremediable diseases or genetically hereditary serious diseases (7).

However, due to the fact that these operations are not inspected as needed, such interventions are conducted with payment in order to fulfill the desire of the families that want to have male children, on women of advanced ages on whom assisted reproductive techniques have been conducted previously, on couples who are known to carry genetic diseases or who have children with genetic diseases, or for sex determination purposes (generally for a high payment).

Such interventions clearly contradict the 14th article of the European Council Biomedicine Agreement (6).

During such operations, it is possible that embryos with unwanted genders are generally disposed of or used for illicit trade and sold to unmarried or other married couples (third persons) for artificial insemination. Such acts contradict the European Council Biomedicine Agreement, which was also signed and approved by Turkey. The Agreement allows such operations only in case of venereal diseases and prohibits them in any other cases. Since Turkey did not legislate a law as a sub-norm after signing the agreement, such interventions are not punished by penal law and are outside the enforcement-scope of protection (7).

Moreover, the opposite of such an intervention is also possible. For instance, it is possible for a handicapped - dwarf couple to use the method of embryo scanning and look for the embryo that will be a physically disabled child like them. In this example, we face a "handicapped baby order" instead of "perfect baby order". The desire to have a child like them, to lead an easier life with the baby and look after the baby effortlessly can make couples go to any extremes. This method, which has been developed for fighting against genetic diseases, intervened chromosomes due to the egoistic, unethical and illegal desires of parents and an 'order' has been given for a child that will spend his/her whole life as a disabled person. This incident occurred in Chicago in 2006, Cara and Gibson Relnolds from New Jersey, ordered a dwarf baby in a clinic through this embryo scanning method (Pre-implantation Genetic Diagnosis=PGD), which is normally used for diagnosing serious genetic diseases before the embryo is placed in the mother's uterus (12).

Some claim that such demands are numerous and they may be fulfilled in some clinics. Fundamentally, although these types of interventions are not for research purposes and have the characteristics of a known method or tool, such practices contradict 13th article of European Council Biomedicine Agreement, which prohibits researches intended to change the genetic structure of human breeds, the 11th article which prohibits discrimination and the 12th article which specifies that genetic tests only need to be conducted for human health and treatment purposes (6).

The 18th article of the Biomedicine Agreement is related to researches to be conducted on embryos in tubes and states that provisions of law may allow researches to be conducted on embryos in tubes. In such cases, the embryo needs to be provided with the appropriate protection, but creation of embryos only for research purposes is prohibited. A similar regulation also takes place in the UNESCO Declaration and EU Charter of Fundamental Rights (7).

Medical interventions for sex determination are prohibited in Germany and are considered as a violation of human dignity in doctrine.

Sex determination interventions on embryos are only allowed for gender-related genetic diseases; and even this provision, which is actually compatible with the European Council Biomedicine Agreement, has been criticized in the German doctrine due to its potential For misappropriation.
Medical interventions for sex determination are also prohibited in Britain, Spain and many other European countries (6).

Conclusion

Reproductive health is one of the most important elements of general health. Its effects can be seen and followed through generations in not only productive periods of life, but also in all stages of life, from newborn infancy to teenage,and from teenage to old-age.
Being able to control their own reproduction process as men and women, planning their fertility without risking their lives with effective, reliable, affordable and acceptable contraceptive methods, having safe pregnancies and giving safe births for women, having a live infant and providing the infant with the best possible health services for a healthy growth are the main purposes of health services.
Yet in the research conducted on embryos, it is not the embryo itself but others (its parents) who need to give consent, and the aforementioned research processes mean the end of the embryo's 'life'. This situation increases the importance of researcher responsibilities; if scientists give objective and fair information to adults who shall decide whether embryos will be volunteers/experimental subjects or embryos, they will increase the confidence placed in results and the support to be given in the public conscience.
For the present, it does not seem easy to reach a compromise which will not prevent the benefits of science to humanity, yet will enable individuals to make their own choices according to their beliefs and value judgement.

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

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Natural Orifice Surgery (NOS)-the next step in the evolution of minimally invasive surgery

Doğal Açıklık Cerrahisi (DAC)-minimal invaziv cerrahinin evriminde bir sonraki adım

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Abstract

Endoscopy, which was introduced in the 20th century, changed the outcome of surgery by reducing the need for analgesia and shortening hospital stay. Any new surgical method should improve safety and outcome. At the beginning of the 21st century, the use of natural orifice surgery is a promising progress. The transgastric and transdouglas approaches are currently being investigated and evaluated. The transgastric approach still has a long way to go due to objective problems such as infections, stomach acidity, and the optimal way to ensure the safe closure of gastrotomy. The transdouglas approach, however, is already starting to establish itself and it seems that with the construction of designated instruments it will prevail in the 21st century.

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Key words: Endoscopy, natural orifice surgery

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Özet

Yirminci yüzyılda kullanıma sunulan endoskopi, analjezi ihtiyacını azaltarak ve hastanede yatış süresini kısaltarak cerrahinin akibetini değiştirdi. Yeni herhangi bir cerrahi metodun güvenliliği ve akibeti iyileştirmesi gerekir. Yirmi birinci yüzyılın başında, doğal açıklık cerrahisinin kullanımı umut verici bir ilerlemedir. Transgastrik ve transdouglas yaklaşımlar halen araştırılmakta ve değerlendirilmektedir. Enfeksiyonlar, mide asiditesi ve gastrostominin güvenli kapatılmasının optimal yolu gibi objektif problemlerden dolayı transgastrik yaklaşımın önünde hala uzun bir yol vardır. Bununla birlikte, transdouglas yaklaşım halihazırda kendini kabul ettirmeye başlamıştır ve tasarlanmış enstrümanların yapımıyla birlikte 21. yüzyılda yaygınlaşacak gibi görünmektedir. (J Turkish-German Gynecol Assoc 2012; 13: 56-60)

Anahtar kelimeler: Endoskopi, doğal açıklık cerrahisi

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Introduction

Endoscopic surgery achieved high standards during the 20th century as proved by reducing morbidity, improving recovery and shortening hospital stay. Although endoscopic procedures are less invasive than open surgery, they still require several incisions for port placements and incision enlargement for specimen retrieval (1). Most of the discomfort and complications associated with open and endoscopic surgery are caused by the abdominal incisions: The longer they are, the stronger the pain intensity and the higher the risk for wound infection and herniation (2). Despite the high standards of endoscopy already achieved, it seems that surgery can be made safe and efficient by using various natural orifices such as the mouth or the vagina as an access to the abdominal cavity. This natural orifice surgery (NOS) concept seems to be the next step in the evolution of minimally invasive surgery and may further reduce the invasiveness of surgical procedures by eliminating abdominal wall incisions and their implications, such as the afore mentioned postoperative abdominal pain, wound infection, and herniation (3, 4). This and other potential benefits of this approach have been proposed and debated, and are the driving force for extensive research in this emerging discipline.

Several areas were recognized as being potential barriers to the further development of the NOS concept. These include the creation of safe access and closure of the incision of the abdominal cavity, and the development of devices to facilitate these interventions (5). The transvaginal and transgastric approaches were the common routes used for the first NOS applications in human.

The access to the abdomen

While the American NOSCAR (Natural Orifice Surgery Consortium for Assessment and Research) working group decided to concentrate on the transgastric approach (6), many researchers focused on the use of the transvaginal route in women because the pouch of Douglas offers an easy and safe access into the peritoneal cavity (7). This was also the preferred route for some innovative surgeons to perform cholecystectomies or appendectomies using hybrid techniques (8-11). The NOSCAR group as well as other authors promoted the transgastric access, which turned out to be a technical challenge, because current flexible endoscopes

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and instruments are quite restricted in design and too unstable when introduced into the peritoneal cavity (12).

Transgastric approach

The transgastric access remains an appealing approach because it is more universally available than the transvaginal one and may be also more appealing to patients. This approach is feasible but associated with several problems: stomach acidity, problems which might arise from iatrogenic penetration of the stomach, the bacteriological contamination of the instruments introduced through the mouth and oesophagus, and the limitations involved with the limited diameter of the instruments in use due to the oesophageal diameter. Furthermore, several technical challenges inherent to the transgastric approach exist, including the creation of a gastrotomy, maintaining the necessary pneumoperitoneum, manipulating abdominal organs, retrieving specimens, and safe closure of the wall of the stomach.

Transgastric access into the peritoneal cavity

Several transgastric access procedures within the stomach have been described using the needle-knife method, sphincterotome and balloon dilatation. The procedure itself begins with the use of a standard single-channel endoscope for gastroscopy and placement of an overtube. The stomach is then disinfected, although the exact clinical benefit of thus reducing the bacterial load has not been studied or quantified; however, this step seems logical to maintain sterility in the abdominal cavity (13).

Various techniques for gastrotomy have been reported, with the most common location for the incision being the anterior gastric wall. Wagh et al. initially used endoscopic ultrasound (EUS) to mark the location of the gastrotomy, but this technique was abandoned after initial experiments found it not particularly useful. A percutaneous endoscopic gastrostomy (PEG) technique has also been described to help prevent damage to adjacent structures (13).

In their original report, Kalloo et al. described the following gastrotomy technique (14): A forward-viewing endoscope (GIF-160; Olympus America Corp., Melville, N.Y.) is inserted into the stomach. Access to the peritoneal cavity is made by using a needle-knife (KD-10Q-1.A; Olympus) to create an initial 2-mm incision in the anterior wall of the stomach. A flexible-tip guidewire (Jagwire 5658; Microvasive Endoscopy, Boston Scientific Corp., Natick, Mass.) is then advanced through the incision into the peritoneal cavity under fluoroscopic guidance. The incision is enlarged, either by extending it with a pull-type sphincterotome (210Q-0720; Olympus) to 20 mm or by dilation with an 8-mm dilation balloon (CRE esophageal balloon 5838; Microvasive) which is inserted over the guidewire. The endoscope is then advanced into the abdominal cavity, which is insufflated to lift the anterior abdominal wall and to expose the abdominal viscera (14).

Pneumoperitoneum

The transgastric insufflation is even more complex, not just because of the lack of the hand guided feedback but also due to the different anatomical considerations. When the abdominal wall is insufflated during laparoscopy, it is elevated, but when an incision is made transgastrically there is no direct way to make sure that there is a safe space beyond and there is no way to control the presence of intestinal loops just behind the incision. Hybrid manoeuvres are of course possible. A Verres needle can be introduced prior to stomach penetration, but without an optical device inserted into the peritoneal cavity prior to the penetration, safety cannot be guaranteed.

The pneumoperitoneum is currently maintained out of convenience with simple insufflation via the endoscope, because there is no readily available pressure regulated insufflator for NOS adapted to the flexible endoscopic system.

Closure of the gastric incision

Ensuring adequate closure of the gastric incision seems to be the most crucial part of transgastric surgery and is regarded as the biggest challenge in the passage from preclinical studies to human application. A leak from the stomach could lead to significant complications; hence, a reliable closure with minimal risk of leak must be achieved. At present there is no evaluated way of providing the optimal closure of the stomach that is needed for an endoscopic transluminal approach. Contemporary closure techniques described include endoscopic suturing, tissue opposition and clipping, and PEG tube closure (5).

Endoclips are the accessory most commonly used for gastric closure; however, endoclips are primarily designed for haemostasis and not for approximating edges of incisions (13).

Various sophisticated devices are being developed to ensure closure. Examples include prototype devices, e.g. the Stringer Device (LSI Solutions, Victor, NY) (15), the Eagle Claw (Olympus America, Inc., Center Valley, PA) (16, 17), the NDO Plicator (NDO Surgical, Mansfield, MA, USA) (18), and the three-channel device based on ShapeLock technology (USGI Medical, Inc., San Clemente, CA) (12, 19).

The Stringer Device is a prototype incision and closure device that was used by Fong et al. to assess the transcolonic approach as a means of accessing and systematically exploring the abdominal cavity in a pig survival study design (15). After advancing the hand-activated device under visualization to the desired incision site, a purse string suture is deployed around the planned incision site using an integrated dual metal ring mechanism. This is followed by the creation of a 20 mm incision with a blade mechanism at the tip of the device. For closure, a suction mechanism brings the tissue into a chamber at the tip of the device. Two needles (single arrows) pass through the tissue to engage a single-stranded suture with metal rings in the distal tip to create a purse string (2-0 polypropylene).

The Eagle Claw was developed by Olympus Medical Systems in collaboration with the Apollo Group (16, 17). It was originally described for endoscopic control of major arterial bleeding. A major problem with endoscopic suturing devices has been that the placed sutures were often too superficial to allow good approximation and permanent healing. This was due to the superficial bites that suction capsules could achieve and also because suturing could not be performed under direct vision.

The Eagle Claw that can be mounted alongside a standard endoscope uses large curved needles and allows suturing under direct vision. The introduction of an opposable jaw allows the new suturing device to grasp the tissue sufficiently r to achieve full-thickness sutures. The grasping forceps function also allows placing of sutures more precisely (16, 17).

The NDO Plicator is a reusable endoscopic tissue-plicating device designed to handle gastroesophageal reflux disease (GERD) by reducing the inner diameter of the gastroesophageal junction with sutured, full-thickness, tissue plications. The device consists of two articulating jaws and a retractable tissue grasper that accommodates a thin endoscope which is passed through a channel in the device. Single-use suture implants are preloaded on the jaws of the device prior to wire-guided access into the stomach. Once the stomach is intubated by the Plicator, the access wire is exchanged for a thin endoscope, which provides visualization. For treatment of GERD, the NDO device is then retroflexed to 180° for grasping, opposing, and plicating tissue with an implant. The implant consists of two expanded polytetrafluoroethylene (ePTFE) pledgets bound to form a U-stitch with pretied 2-0 polypropylene sutures and two titanium retention bridges. Clinical studies have demonstrated improvement in GERD symptoms for patients undergoing NDO plication at the gastroesophageal junction (18).

The Transport platform scope (Transport, USGI Medical, San Capistrano, CA, USA) has 7-, 6-, and two 4 mm working channels, which has allowed the creation of 5-mm graspers with 2.5 cm jaws similar to those of laparoscopic tools. Such graspers enable retraction of organs and large ''bites'' of tissue to allow approximation and closure (12, 19).

Altogether, the transgastric access seems to be complicated for the surgeon and risky for the patient. The most significant concern associated with an endoscopic transluminal approach is secure closure of the wall of the organ that is traversed in order to gain access to the abdominal cavity. Although some preclinical studies have addressed the efficacy of gastrotomy closure, the relatively low number of experimental subjects leaves these studies inadequately empowered to derive meaningful comparisons between closure techniques. A study comparing the best viscerotomy closure practice between multiple endoscopic clips and a proprietary device would require hundreds of operations to show minor differences between them. Moreover, the reports of pigs surviving NOTES without any viscerotomy closure raise the question as to whether the pig is an optimal model to study closure techniques (20). An endoluminal method of determining closure security at the end of a transgastric procedure remains an unresolved issue. Nonetheless, all above mentioned methods should make access and closure via the stomach or colon nearly as safe as the transdouglas route. Should these challenges be solved, training programmes will have to be developed, preferably using designed simulators before- and if - it becomes mainstream therapy.

Transdouglas approach

The transdouglas access has been used for more than 100 years by gynaecologists for diagnostic and therapeutic purposes, thus being well established and accepted. Opening and closure of the vaginal wall is safe and is done from the outside under vision by using standard surgical techniques. In every vaginal hysterectomy, with or without prolapse, the opening of the pouch of Douglas is carried out easily by cutting the vaginal wall transversally about 1-2 cm above the external os and then lifting the posterior aspect of the cervix with a tooth tennaculum, identifying the pelvic peritoneum between the sacro-uterine ligament, pulling it with surgical forceps, cutting it with round scissors, inserting the scissors into the peritoneal cavity, and pulling the widely opened scissors out using both hands (21). This method has been proved to be safe, does not require insufflation prior to the manoeuvre, and can be done under epidural and/or spinal anaesthesia.

It is well-known that the vaginal wall repairs itself without leaving any visible scars and without causing long-term dysfunction. Even if closure of the access site at the apex of the vagina were to fail, there would be little if any clinical significance. The extremely low risk of hernia is evidenced by the fact that many gynaecologists do not routinely suture the posterior colpotomy when performed during pelvic operations. Furthermore, experience of gynaecologists performing transvaginal hysterectomy has demonstrated safety in regards to rarity of pelvic infection (22).

The advantages of the transvaginal approach are as follows:

1) The easy and relatively non-traumatic entry into the abdominal cavity;
2) The possible wide diameter of the inserted instruments;
3) When performing vaginal hysterectomy the pouch of Douglas can be opened under vision, and the traditional 15 mmHg pressure is not needed. For some procedures much lower intra-abdominal pressure is needed, therefore these procedures can be performed with epidural anaesthesia;
4) The vaginal wall lining repairs without leaving scars and without any long-term discomfort or dysfunction;
5) Large specimens can be retrieved.
6) Optimal ergonomics: the transdouglas approach can be performed while the surgeon is seated comfortably.

For all these reasons the transdouglas approach seems actually to be the preferred route for NOS procedures by many authors, since it does not necessitate any sophisticated devices for opening and closure of the posterior colpotomy. It is easy for the surgeon and safe for the patient.

Clinical application of NOS

We believe that due to the relatively uncomplicated entry into the abdomen and its safety, the use of the pouch of Douglas will become more prevalent during the 21st century when adapted instruments have been introduced. Various abdominal operations have already been done using the transdouglas route.

In recent years, the pouch of Douglas has also been used as an entry for infertility evaluation and treatment using the so-called fertiloscope (23).

I In 2001, a preliminary report on culdo-laparoscopy was published (24), and in 2003 a procedure of a combined transvaginal hysterectomy and hybrid cholecystectomy in an 81-year-old woman was reported (25). Later, other hybrid transvaginal cholecystectomies were reported in Brazil (9), the United States (10), and France (11).

The transdouglas approach for urological, gynaecological and surgical indications is establishing itself gradually, not just because of the relatively uncomplicated access but also due to the relatively wide diameter of the entry, which enables the usage of wide instruments and easy retrieval of specimens. In our own study, which was conducted in order to estimate the potential usage of the pouch of Douglas, the mean diameter was measured to be 2.6 cm with a range of 2.0-3.4 cm (26). This was an anatomical study, but it seems that in living patients the elasticity of the pouch of Douglas is even higher. These results are important when instruments are being designed which could be used without causing damage to the pelvic floor due to over-stretching.

The feasibility of hybrid transdouglas nephrectomy combined with mini-laparotomy has already been evaluated in five patients (27). Although the average operation time was long (120 minutes), the blood loss was minimal and all the reported operations performed were uneventful.

Recently, with accumulated experience, more sophisticated transvaginal procedures are being done, like hybrid hemicolectomy (28) or nephrectomy (29, 27), and even a combined abdominal and transvaginal sleeve gastrectomy in morbidly obese women has been reported, although in six of them a conversion (to laparoscopy) became necessary (30). Different gynaecological procedures such as the removal of uterine fibroids are routinely done transvaginally (31, 32). Apart from all these reported studies, cholecystectomy nowadays is the most widely performed NOS procedure, mostly due to its easiness, its history as an initial target for minimally invasive surgery, and the fact that laparoscopy is a ready fallback (33). However, it is widely felt that removing the gallbladder is not the ideal target for widespread NOS adoption. This is due to the already minimally invasive nature of the laparoscopic gold standard. There is consensus, however, that cholecystectomy is a worthwhile model for the initial exploration of the potential of NOS.

The largest experience with NOS procedures has been in Germany, where well over 1500 transvaginal cholecystectomies have been performed. A report on 551 cases of natural orifice transluminal endoscopic surgery from the German NOTES registry was recently published (34). From South America, reports are emerging concerning a large and diverse experience with transvaginal cholecystectomy, in particular, approaching a clinical norm in several centers (33).

Transvaginal hybrid NOS is a safe method with a low complication rate, even in old or obese patients. It can be performed with rigid endoscopes and conventional trocars, which seem to be favorable for surgeons, as both instruments are common in surgical practice (34).

The future of NOS

At the time when reports concerning surgical procedures are emerging from all over the world, it becomes clear that, in order to avoid hybrid operations, the introduction of designed instruments is necessary, which will provide the safety and perfection of the surgery. These instruments should contain optics, irrigation and suction, coagulation, triangulation, and stability.

Special instruments which provide stability and enable suturing or coagulation must be designed in order to perform single port transdouglas procedures with safety.

Today's challenge is to secure optimal vision, stability and accuracy, which is difficult and needs a high degree specialization.

When performing operations according to evidence-based methods with the same steps and sequence, the matched outcome variability should not deviate significantly. However, when operating in a location that is far away from the entry point without optimized instruments, different individual skills might lead to variation concerning the operative and postoperative outcome (bleeding, damage to neighbouring organs, febrile morbidity, and hospital stay).

The conditio-sine-qua-non of secure standardized performance of single port or hybrid procedures is the adapted optimized tools.

Any new surgical method should provide benefits when compared to the previously existing ones. The benefits of the transgastric approach still have to be evaluated. However, the transdouglas approach is a very promising one which, even with the existing instrumentations, has already proved its benefits. When designed instruments are introduced (7), it seems that the transdouglas approach will become the state of the art for abdominal operations in women in the 21st century.

Conflict of interest

No conflict of interest was declared by the authors.

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Extramedullary hematopoiesis in leiomyoma uteri

Myoma uteri içerisinde ekstramedullar hematopoiezis

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Abstract

Extramedullary hematopoiesis (EMH) that often occurs as a compensatory reaction to an underlying hematologic abnormality is a non-neoplastic proliferation of hematopoietic tissue outside the bone marrow and peripheral blood. Rarely, EMH may be seen in hematologically normal individuals. EMH is most commonly (95%) seen in reticuloendothelial organs such as the spleen, liver, and lymph nodes but has rarely been reported in other locations. EMH is extremely rare in the uterus. In this case report, we present EMH in leiomyoma uteri in patients without any underlying hematologic abnormalities. Very rare clinical conditions like EMH can be observed in cases of myoma uteri and therefore should be kept in mind. There is currently no consensus regarding the pathogenesis and clinical management of this uncommon pathology and further reports on this topic are needed.

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Key words: Extramedullary hematopoiesis, leiomyoma uteri

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Özet

Kemik iliği ve periferik kan dışında hematopoietik dokuların neoplastik olmayan proliferasyonu olarak tanımlanan ekstramedullar hematopoiezis (EMH) nadir olarak sağlıklı kişlerde görülür. Yine EMH çok nadir olarak uterusta görülebilir. Bu sunumda hematolojik olarak normal izlenen bir olguda myoma uteri içerisinde saptanan EMH durumu tartışılmıştır. Dejenere 8x10 cm büyüklüğünde intramural-subseröz myoma uteri saptanan 43 yaşındaki hastaya histerektomi operasyonu uygulanmıştır. Patoloji örneğinin histolojik incelemesi sonucunda mitotik aktif leiomyoma uteri ile birlikte EMH saptanmıştır. Takiben yapılan sistemik araştırmada, periferik yayma ve kemik iliği biopsisini de içeren detaylı laboratuvar bulguları normal olarak izlenmiştir. EMH gibi nadir klinik durumlar myoma uteri içerisinde izlenebilir. Günümüzde bu nadir durumun patogenezi ve klinik yaklaşımı konusunda fikir birliği yoktur. Bu konuda yeni yayınlara ihtiyaç vardır.

(J Turkish-German Gynecol Assoc 2012; 13: 61-3)

Anahtar kelimeler: Ekstramedullar hematopoiezis, myoma uteri

Geliş Tarihi: 28 Ağustos 2011 **Kabul Tarihi:** 19 Eylül 2011

Introduction

Extramedullary hematopoiesis (EMH) is a non-neoplastic proliferation of hematopoietic tissue outside the bone marrow and peripheral blood (1). EMH often occurs as a compensatory reaction to an underlying hematologic abnormality (2). Rarely, EMH may be seen in hematologically normal individuals. EMH is most commonly (95%) seen in reticuloendothelial organs such as the spleen, liver, and lymph nodes but, rarely, has been reported in other locations, such as serous membranes and the uterus (3-7). In this case report, we present EMH in leiomyoma uteri in patients without any underlying hematologic abnormalities.

Case Report

A 43-year-old woman had undergone hysterectomy because of a degenerated intramural-subserosal uterine leiomyoma about 8X10 cm in size. Histological examination of the specimen revealed a mitotically active cellular leiomyoma with EMH (Figure 1, 2). Erythroid precursors were stained for glycophorin (Figure 3). There was no evidence of any hematological disease. The laboratory findings of the patient are reported in Table 1. An extensive hematologic and systemic evaluation was performed after the pathology report of EMH in myoma uteri. Bone marrow biopsy was performed and was evaluated as normal (Figure 4). Cellularity was observed as 70%, including three series of haematopoietic cells in bone marrow.

Cranial, neck, thoracic, upper and lover abdominal computed tomography scans showed no obvious pathology. Despite high levels of rheumatoid factor, rheumatological and physical examination revealed normal findings. Although the patient has an increased platelet count of lower than 450 x 10^3/ml, clinical management for thrombocytosis was not considered, because other hematological evaluations of the patient, including bone marrow biopsy and peripheral blood smear, were all normal.

Discussion

EMH is extremely rare in the uterus. In the English literature, Creagh et al. reported four cases of EMH in the endometrium

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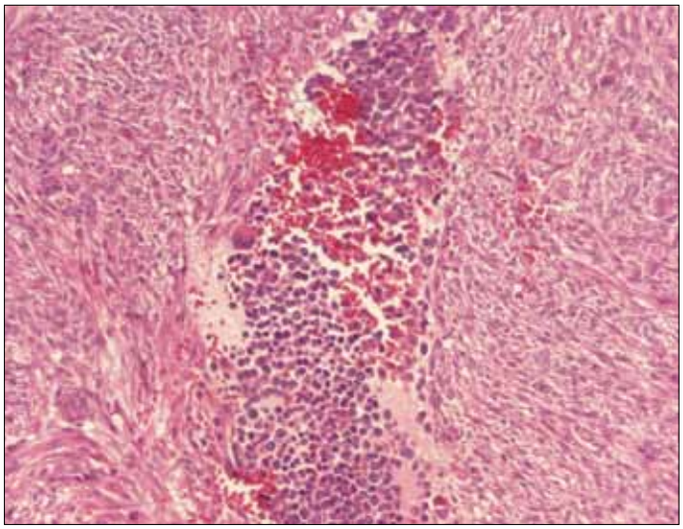

Figure 1. Extramedullary hematopoesis in leiomyoma. Hematopoietic cell groups are seen among spindle cells of leiomyoma (H.E. x 100)

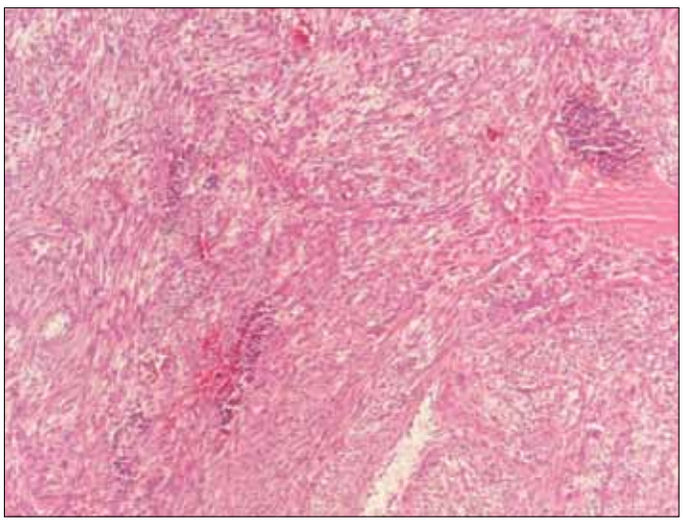

Figure 2. An extramedullary hematopoetic focus in cellular leiomyoma. This micrograph shows a megakaryocyte and the other hematopoietic cells among spindle mesenchymal cells (HE x 200)

associated with hematological disease, including myeloproliferative disorder, thalassaemia trait, chronic myeloid leukaemia and multiple myeloma (8), and other authors reported EMH in the endometrium or cervix with no underlying haematological abnormality (4-6).

Schmid et al. described EMH in leiomyoma of the uterus in patients with no hematological disorder (7). We observed EMH in leiomyoma in a hematologically normal individual, similar to Schmid et al.

Theories accounting for the occurrence of haemopoietic foci in extramedullary locations consider two mechanisms. One is the presence of a precursor uncommitted mesenchymal cell and the other is seeding of distant sites by circulating haemopoietic cells (8, 9). Supporting the former mechanism in leiomyoma uteri, Sun et al showed that blast colony-forming cells exhibiting bilineage (hematopoietic and vascular) potential and

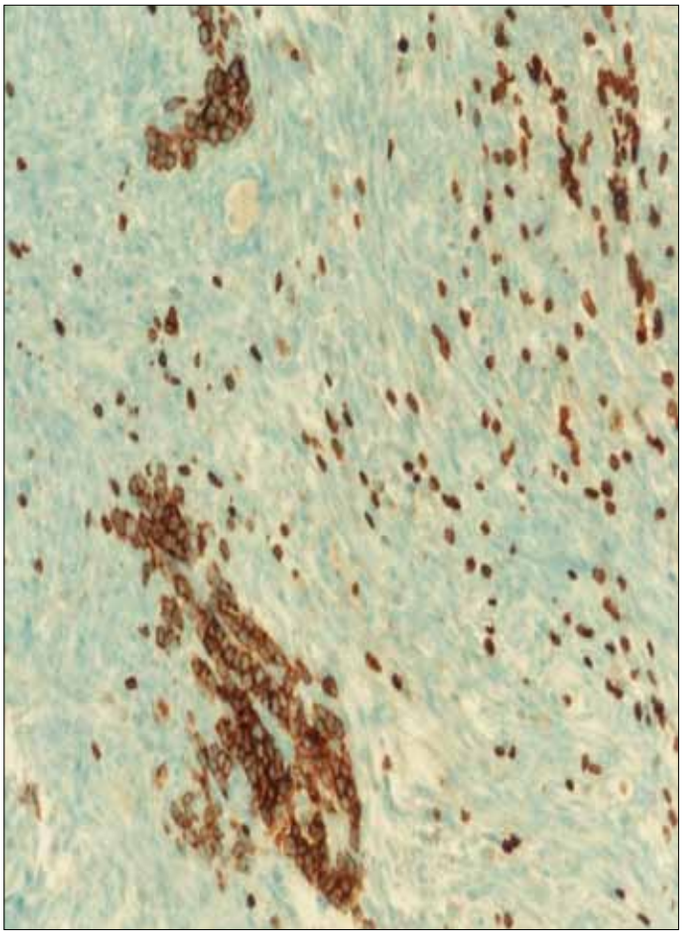

Figure 3. Extramedullary hematopoesis in leiomyoma. Erythroid precursors are stained for glycophorin (Glycophorin x 200)

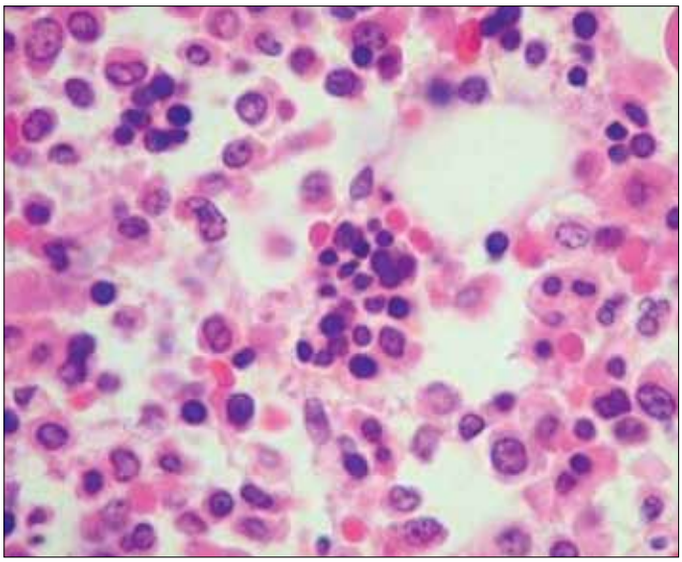

Figure 4. Normal bone marrow tissue

long-term self-renewal originate from the uterus in the mouse (10). Currently, Zhou et al. hypothesised that hypoxia might be a novel driving force for leiomyoma as an indirect inducer of differentiation of myometrial stem cells into leiomyoma cells,

Table 1. Clinical characteristics of the patient with extramedullary haematopoiesis in leiomyoma

	Results	Reference Values
Hemoglobin (g/dl)	13	12.3-15.4
Leukocytes ($10^3/\mu l$)	8.8	4.1-10.3
Thrombocytes ($10^3/\mu l$)	437	158.7-387.7
Glucose (mg/dl)	86	70-109
Creatinine (mg/dl)	0.067	0.57-1.11
Albumin (g/dl)	4.42	3.5-5.0
ALT (U/l)	23	3-55
LDH (U/l)	211	125-243
Total bilirubin (mg/dl)	0.57	0.2-1.2
Direct bilirubin (mg/dl)	0.25	0.0-0.5
Anti HCV	Negative	Negative
Anti HIV	Negative	Negative
Hbs Ag	Negative	Negative
Direct Coombs Anti Ig G	Negative	Negative
Anti C3d	Negative	Negative
CCP (Units/ml)	4.24	0-15
CRP (mg/l)	7.63	0-5
RF (IU/ml)	65	0-15
CMV PCR-2 (copy/ml)	<235	<235
Ig A (g/l)	2.25	0.7-4
Ig M (g/l)	2.64	0.4-2.3
Ig E (IU/ml)	59	0-100

ALT: alanine amino transferase, LDH: lactate dehydrogenase, HCV; Hepatitis C virus, HIV: Human immunodeficiency virus, HBsAg: hepatitis B surface antigen, CCP: cyclic citrullinated peptide, CRP: C-reactive protein, RF: rheumatoid factor, CMV: cytomegalovirus, PCR: polymerase chain reaction, Ig: immunoglobulin

which could be activated by aberrant activation of estrogen signaling pathways (11). In this case, hypoxia could be an insult stimulating differentiation of stem cells, which exist in the leiomyoma tissue, into hemapoietic cell.

In conclusion, very rare clinical conditions like EMH can be observed in cases of myoma uteri and therefore should be kept in mind. There is currently no consensus regarding the pathogenesis and clinical management of this uncommon pathology and further reports on this topic are needed.

Conflict of interest

No conflict of interest was declared by the authors.

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Dichorionic twin pregnancy discordant for fetal anencephaly: a case report

Fetal anensefali ile komplike dikoryonik ikiz gebelik: Olgu sunumu

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Abstract

Dichorionic twin pregnancy discordant for fetal anencephaly is a serious condition that threatens the normal co-twin's life by causing polyhydramniosis, preterm labor and sudden death of one or both of the fetuses. We report a case of dichorionic twin pregnancy discordant for fetal anencephaly delivered at the 32nd week of gestation because of preterm labor and nonreassuring fetal monitoring. The aim of this case report is to summarize management options in this situation.

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Key words: Anencephaly, twin pregnancy, discordant fetal growth

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Özet

Fetal anensefali ile komplike dikoryonik ikiz gebelik polihidramnioz, preterm eylem ve bir ya da iki fetusun birden ani ölümü şeklinde sonuçlara yol açabilen potansiyel tehlikeli bir durumdur. Bu çalışmada 32. gebelik haftasında preterm eylem ve güven vermeyen fetal kalp hızı patterni nedeni ile doğum yapan fetuslardan birinin anensefal olduğu dikoryonik ikiz gebelik olgusu sunulmuş ve tedavi seçenekleri tartışılmıştır. (J Turkish-German Gynecol Assoc 2012; 13: 64-6)

Anahtar kelimeler: Anensefali, ikiz gebelik, uyumsuz fetal büyüme

Geliş Tarihi: 05 Eylül 2010 **Kabul Tarihi:** 15 Ekim 2010

Introduction

In twin pregnancies discordant for anencephaly, there are risks of development of polyhydramnios, severe preterm delivery and death of the anencephalic fetus. In dichorionic twins discordant for anencephaly, there are three management options: selective fetocide, serial ultrasound examination for polyhydramnios or expectant management (1). In this paper, we present our experience with a case of twin pregnancy discordant for anencephaly which was managed conservatively.

Case Report

A-27-year old woman was admitted to our emergency unit with preterm contractions at 32nd week of gestation. She was hospitalized with the diagnosis of twin pregnancy and preterm labor. She was primary infertile and conceived with gonadotropine treatment and intrauterine insemination after 7 years of infertility. She was observed in another center with the diagnosis of preterm labor and was treated with tocolytic drugs and corticosteroids for fetal lung development. An ultrasound scan showed a dichorionic diamniotic twin pregnancy. Twin A's development was concordant to 30 weeks and it was structurally normal but twin B was anencephalic and concordant to 29 weeks. This situation was noticed at the14th weeks of gestation, the couple was informed and the expectant management option was chosen. The amniotic fluid index of the second fetus was increased (300 mm). Fetal heart rates of both babies were bradycardic (60-80/min), thus the patient was delivered by emergency cesarean section. The anencephalic female infant died soon after birth (birth weight 920 gr). The surviving male infant weighed 1520 gr and had an Apgar scores of 6 and 7 at 1 and 5 mins, respectively (Figure 1). The surviving infant was admitted to the neonatal intensive care unit (NICU) and given nasal continuous positive airway pressure for two days and supplemental oxygen for further day. During the NICU stay, the developing bronchopulmonary dysplasia was treated with synthetic surfactant therapy. At the 2nd day in NICU, icterus appeared and was treated with phototherapy. The infant was discharged after 26 days weighing 2010 gr.

Discussion

Anencephaly, together with spina bifida, is the most common and multifactorial neural tube defect, occurring in about 1 in 1000 births (2). In a twin pregnancy when one of the fetuses is anencephalic, this situation multiplies the risks and complications twin pregnancies already have.

Prenatal detection of anencephaly by ultrasound is possible in almost 100% of cases (3, 4). In singleton pregnancies, almost all of these conceptions are terminated since anencephalic infants have no chance to survive. However, in the case of multiple gestations the management is not that clear. In twin pregnancies complicated with one anencephalic fetus, there is

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Figure 1. Postnatal image of discordant anencephaly in dichorionic twins: Anencephalic and normal twins

an increased risk of either neonatal death due to severe preterm delivery secondary to development of polyhydramnios or intrauterine death. Another major risk is development of discordant fetal growth. The prevalence of discordance for anencephaly is higher in monochorionic than in dichorionic twins (5, 6).

In their retrospective study, Ben Ami et al. asked the question "Is there an increased rate of anencephaly in twins?". In this study a higher rate of anencephaly was currently found among IVF-ICSI pregnancies compared to spontaneous pregnancies. This is attributed neither to assisted conception technique nor to recent folic acid supplementation, but rather to the twinning itself (7). In twin pregnancies the prevalence of anencephaly is higher than in singletons (5, 6).

After diagnosis of an anomaly in one fetus with a normal co-twin, the general management options are the following: abortion of both fetuses, continuation of pregnancy without intervention or selective fetocide of the abnormal twin. In the presented case, the family did not approve of the selective fetocide treatment. A multicenter study revealed that selective fetocide after 16 weeks' gestation is related with a higher risk of miscarriage (5%-14%), but it reduces the risk of severe preterm delivery in the remaining fetus before 16 weeks (8). In dichorionic twins, Vandecruys et al. recommend serial ultrasound examinations for early diagnosis of polyhydramnios, which can then be treated either by amniodrainage or selective fetocide (9). In the presented case, polyhydramnios was detected on admission and an emergency cesarean section due to the fetal bradycardia was performed at 32 weeks of gestation; and therefore amniodrainage was not performed. Leeker et al. emphasised that selective fetocide of the anencephalic fetus before 15 weeks of gestation prevents the development of polyhydramnios and might reduce the risk of prematurity and increase the chance for survival for the healthy fetus (1). In addition, there are a few studies carried out on expectantly managed dichorionic twins with one anencephalic fetus where the rates of pregnancy complications are similar to those reported in normal twin gestations (10, 11).

In monochorionic twin pregnancies, conventional selective fetocide is not possible because of the high risk of subsequent death of the normal co-twin, and treatment options are expectant management and selective fetocide by cord occlusion (1).

There are a few studies describing outcomes in twin pregnancies discordant for anencephaly. The largest ones are rewieved by Vandecruys et al. (63 cases) and Leeker et al. (43 cases) (1, 9). Neither group used selective fetocide for monochorionic twins and expectant management was the common choice. Survival rates of the normal co-twin in these studies were 90% and 88%, respectively. Lust et al. (11) introduced treatment options in 86 cases of monochorionic and dichorionic twin pregnancies complicated with anencephaly. The treatment options reported were selective fetocide or expectant management in all cases. In dichorionic twins, selective fetocide was performed by intracardiac instillation of potassium chloride, whereas in monochorionic twins, it was carried out by bipolar coagulation of the umbilical cord. No significant differences were found in survival of nonaffected twin between the management options in monochorionic and dichorionic twins although a statistically significant difference was found between the two groups in mean gestational age and birth weight at delivery supporting the selective fetocide. Survival rates in the dichorionic group were found as 94.1% and 95.1% for the fetocide and expectant management groups, respectively. In this study, the authors concluded that "While selective fetocide does not reduce perinatal mortality, it does result in significantly longer gestations and higher birth weight, and appears to be a choice in dichorionic twins discordant for anencephaly. In monochorionic twins, selective fetocide also increases birth weight, but in view of the complexity of this group, no clear recommendations can be made" (11).

As a result, dichorionic twin pregnancy discordant for fetal anencephaly is a rare case but may have serious consequences. Although both expectant management and selective fetocide have good outcomes, close follow-up of these patients is very important in management.

Conflict of interest

No conflict of interest was declared by the authors.

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Isolated fetal liver calcifications

İzole fetal karaciğer kalsifikasyonları

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Abstract

Hepatic calcification in the fetus is considered an uncommon occurrence and the clinical significance is not fully known. We describe five cases with isolated hepatic calcification. The causes and postnatal outcome of the fetal liver calcifications detected by ultrasound imaging are discussed. Isolated fetal liver calcifications with no aneuploidy and infection have a good prognosis.

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Key words: Isolated fetal liver calcifications, fetal ultrasound, prognosis

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Özet

Fetusta, hepatik kalsifikasyonlar nadir gözlenirler ve klinik önemleri ise tam olarak bilinmemektedir. Biz, beş olguda izole olarak hepatik kalsifikasyon saptadık. Bu olguları tanımlarken ultrasonografi ile saptanan fetal karaciğer kalsifikasyonlarının nedenleri ve postnatal durumları tartışıldı. Kromozomal anomali ve enfeksiyon saptanmayan izole fetal karaciğer kalsifikasyonlarının prognozları oldukça iyidir.

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Anahtar kelimeler: Fetal ultrasonografi, hepatik kalsifikasyon, prognoz

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Introduction

Hepatic calcifications in the fetus are hyperechogenic areas that are detected by ultrasonography imaging known as fetal liver calcifications (FLCs). Recent advances in fetal ultrasonographies have allowed these lesions to be diagnosed prenatally. These lesions have been previously described as isolated findings or in association with other abnormalities. Although isolated liver calcifications are relatively common, clinical significance and management are not known exactly (1, 2). We report here, a series of five cases with isolated fetal liver calcification and discuss the clinical significance.

Case Report

During 2006-2009, 1800 detailed second trimester ultrasonographies between the 18th-23rd weeks were performed in our clinic. Seven cases of FLCs were detected incidentally. Complete anomaly surveys of other organs of each case were conducted on the fetus to detect the presence of calcifications and to detect whether the calcifications were located in the liver parenchyma or liver surface. The findings were recorded. Parental and fetal cystic fibrosis (CF), mutation analysis and maternal STORCH (syphilis, cytomegalovirus, herpes virus 1 and 2, rubella, and Toxoplasma) were performed. The fetal karyotype was demonstrated by amniocentesis. The findings and results were explained and discussed with each patient. During the pregnancies, calcification size and growth of the fetus was followed and reported. After the birth, neonatal controls were carried out.

In two of the seven cases, FLCs were found to be associated with other abnormalities on ultrasonographic examination, such as ventriculomegaly, ventricular septal defect, ascites, echogenic gut. One of the two cases was reported as Trisomy 13 the other Trisomy 21. Therapeutic abortion was thus performed by consent of the parents. This study therefore focused on the prognoses of the five remaining cases. Each of the five cases was found to have isolated FLCs. In three of the five cases; the fetal calcifications were in the parenchyma (Figure 1), the others on the surface (Figure 2). In all five cases, there were no additional ultrasonographic abnormalities, no abnormal karyotypes and no genetic abnormalities of cystic fibrosis. The maternal STORCH screening was found negative for each case. The calcification size remained unchanged during the pregnancy of each case. All newborns were delivered spontaneously at term (38-41 weeks gestation) and all of the neonates were healthy. The growth development of each newborn was followed after birth for at least 6 months by pediatricians, and no developmental problems were recorded.

Discussion

The incidence of fetal hepatic calcifications is undetermined (1, 3). Most available information has been derived from

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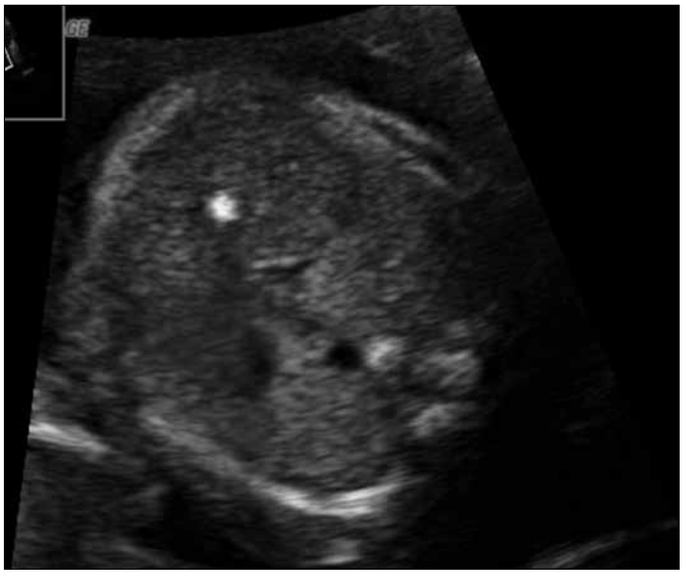

Figure 1. Parenchymal fetal liver calcification

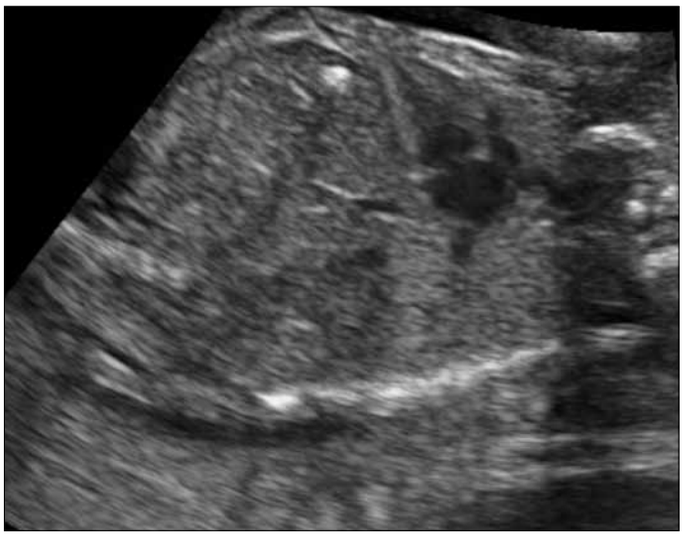

Figure 2. Surface fetal liver calcification

spontaneously aborted fetuses (4). Recent advances in ultrasonography resolution and sonographic monitoring of intrauterine pregnancies has resulted in an increased prenatal detection of these lesions. In the literature, Bronstein et al reported an incidence of 1 in 1750 at the gestational ages of 15-26 weeks (3). Similar results were found by Achiron et al. 1 in 2000, however, they emphasized that their result was biased as they included only live fetuses (2). In the current study, incidences found were higher than other publications. This may be due to the fact that our clinic is a referral center and many patients were referred with suspected fetal abnormalities.

Prenatal diagnosis of FLCs requires careful ultrasonographic, genetic and microbiological evaluation. According to the literature, possible causes for these lesions have been associated with infection, ischemic insults, portal and hepatic vein thromboemboli, tumors, chromosomal abnormalities, CF, and sludge or lithiasis in the gallbladder (1- 3). Severe malformations were reported in 21-85% of these cases (1-3). Simchen et al showed that 11/61 patients had abnormal karyotypes, the most common being Trisomy 13, and 10 of 11 patients with abnormal karyotypes had other abnormalities (1). Intrauterine fetal infections, especially cytomegalovirus, were associated with FLCs (3). A few previous reports concerning CF as a cause for intraabdominal fetal calcifications have been published. Thus we evaluated CF in our patients. Some authors suggested that there are no reports that link CF to prenatally detected isolated liver calcifications. Therefore, this test should be provided mainly to populations with high carrier rates of CF (1).

Fetal ultrasound hyperechogenities in the area of the liver can be categorized according to their location as peritoneal, parenchymal and vascular. It was emphasized that the location of calcification may be affected by the causes (3). Peritoneal hepatic calcifications as calcified masses on the liver surface were reported as a feature of meconium peritonitis and some of these cases were complicated by fetal CF. Parencymal hepatic calcifications have been previously associated with intrauterine infection and primary or metastatic tumors. Vascular causes have been related to the result of thrombosis or ischemia, which has also been associated with parenchymal hepatic calcification (1, 2). However, Simchen et al. showed that there were no differences between parenchymal and surface liver calcifications regarding the cause and outcome. They therefore divided groups with FLCs into isolated (when no other abnormalities were detected) and non-isolated (in which additional abnormalities were present) cases (1). Furthermore, FLCs may be single or multiple. In the literature, cases with multiple FLCs were associated with other abnormalities and with poor prognosis (1).

According to the literature, there are several studies that reported the outcome of fetuses with FLCs. The clinical implications from the literature review are that hepatic calcifications may represent various fetal conditions and the outcome depends on the causes and additional major abnormalities. Physicians should therefore give serious attention to this spectrum. In the current study, we present five cases of isolated fetal liver calcification with no other abnormalities; two of them were on the surface, the others in the parenchyma. The common result of all studies, including the current study, was that cases with isolated FLCs had good outcomes (1-3, 5).

In conclusion, the clinical implication from our cases and the literature suggest that isolated FLCs with no associated morphological abnormalities, abnormal karyotypes or intrauterine infection may be favorable.

Conflict of interest

No conflict of interest was declared by the authors.

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Approach to concomitant rectal and uterine prolapse: case report

Uterin prolapsusa eşlik eden rektal prolapsusa yaklaşım: Olgu sunumu

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Abstract

The classic description of rectal prolapse is a protrusion of the rectum beyond the anus. Peaks of occurrences are noted in the fourth and seventh decades of life, and most patients (80-90%) are women. The condition is often concurrent with pelvic floor descent and prolapse of other pelvic floor organs, such as the uterus or the bladder. In this study, two cases having contraindication to general anesthesia with rectal and uterine prolapse are presented. These cases were operated on under local anesthesia with support of sedation by Leforte and Delorme's operation at the same time. In conclusion; pelvic floor disorders should be considered as a whole, and surgical correction of rectal prolapse and uterine prolapse may be done at the same time under local anesthesia with the support of sedation. Performance of these operations by experienced and trained pelvic reconstructive surgeons may be advocated.

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Key words: Rectal prolapse, Delorme's operation, local anesthesia
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Özet

Rektal prolapsusun klasik tanımı rektumun anüsün dışına sarkmasıdır. Yaşamın dördüncü ve yedinci dekatında pik yaptığı gösterilmiştir ve hastaların %80-90'ı kadındır. Bu durum genellikle pelvik tabanda iniş ve diğer pelvik taban organlarının prolapsusu (mesane ve uterus gibi) ile birliktedir. Bu çalışmada genel anestezi için kontrendikasyonu olan ve rektal ve uterin prolapsuslu iki olgu sunulmuştur. Bu olgular lokal anestezi ve sedasyon altında, aynı seansta, Delorme ve Lefort operasyonlarıyla opere edildi. Sonuç olarak; pelvik taban hastalıkları bir bütün olarak düşünülmelidir ve rektal ve uterin prolapsusun cerrahi düzeltilmesi lokal anestezi altında aynı seansta yapılabilir. Bu operasyonları deneyimli ve eğitimli pelvik rekonstrüktif cerrahların yapması daha doğru olabilir. (J Turkish-German Gynecol Assoc 2012; 13: 70-3)

Anahtar kelimeler: Rektal prolapsus, Delorme operasyonu, lokal anestezi
Geliş Tarihi: 11 Ağustos 2010 **Kabul Tarihi:** 24 Eylül 2010

Introduction

The rectal prolapse or procidentia is defined as a protrusion of a part or all of the rectum beyond the anus (1). The rectal prolapse is associated with pelvic floor disorders in approximately 18-27%, and floor disorders are also associated in 36-80% with stress urinary incontinence, 36% anorectal disease, 19% fecal incontinence and constipation (2-6). Therefore, genital organ prolapse and rectal prolapse may coexist, and each of them is a part of pelvic floor disorders (4, 5). The patients with pelvic organ prolapse apply to a gynecologist more frequently. Under the circumstances, not only genital organ prolapse but also all of the pelvic floor should be examined and concomitant rectal prolapse and/or fecal incontinence should not be overlooked.

In the present study, the surgical approach applied to two cases with concomitant rectal prolapse and pelvic organ prolapse operated on in our clinic is discussed.

Case Reports

Case 1

A 77-year-old woman patient was admitted to our department with complaints of anal distension and a mass prolapsed from the vagina. The patient had had these complaints for three years but her complaints had increased recently. She had had five babies delivered vaginally and forceps was only used in the second birth in her obstetric history. Stage IIIa prolapse according to POP-Q (pelvic organ prolapse quantitative) and stage I rectal-mucosal prolapse according to Altemeier's staging were determined in her pelvic examination. In addition, she had had hypertension for twenty years and chronic obstructive lung disease for fifteen years.

Case 2

A 83-year-old woman patient was admitted to our department with complaints of anal distension, with a mass prolapsed

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from the vagina. The patient stated that she had had these complaints for five years and recently she was having trouble and stress while doing housework. In her obstetric history, largest one was 4100 gr, she had had six vaginal deliveries at home performed by a midwife. In her pelvic examination, stage IIIb prolapse according to POP-Q, stage I rectal-mucosal prolapse according to Altemeier's staging and loss of anal circular muscle tone were determined. In addition, she had had a history of heart failure for five years and was receiving medical treatment because of heart failure.

Operative technique

Preoperatively, local estrogen preparations were given twice a day for ten days. Mechanical bowel preparation was started 48 h before surgery and ceftriaxone 1 g and metronidazole 500 mg were administered intravenously 30 min before surgery.

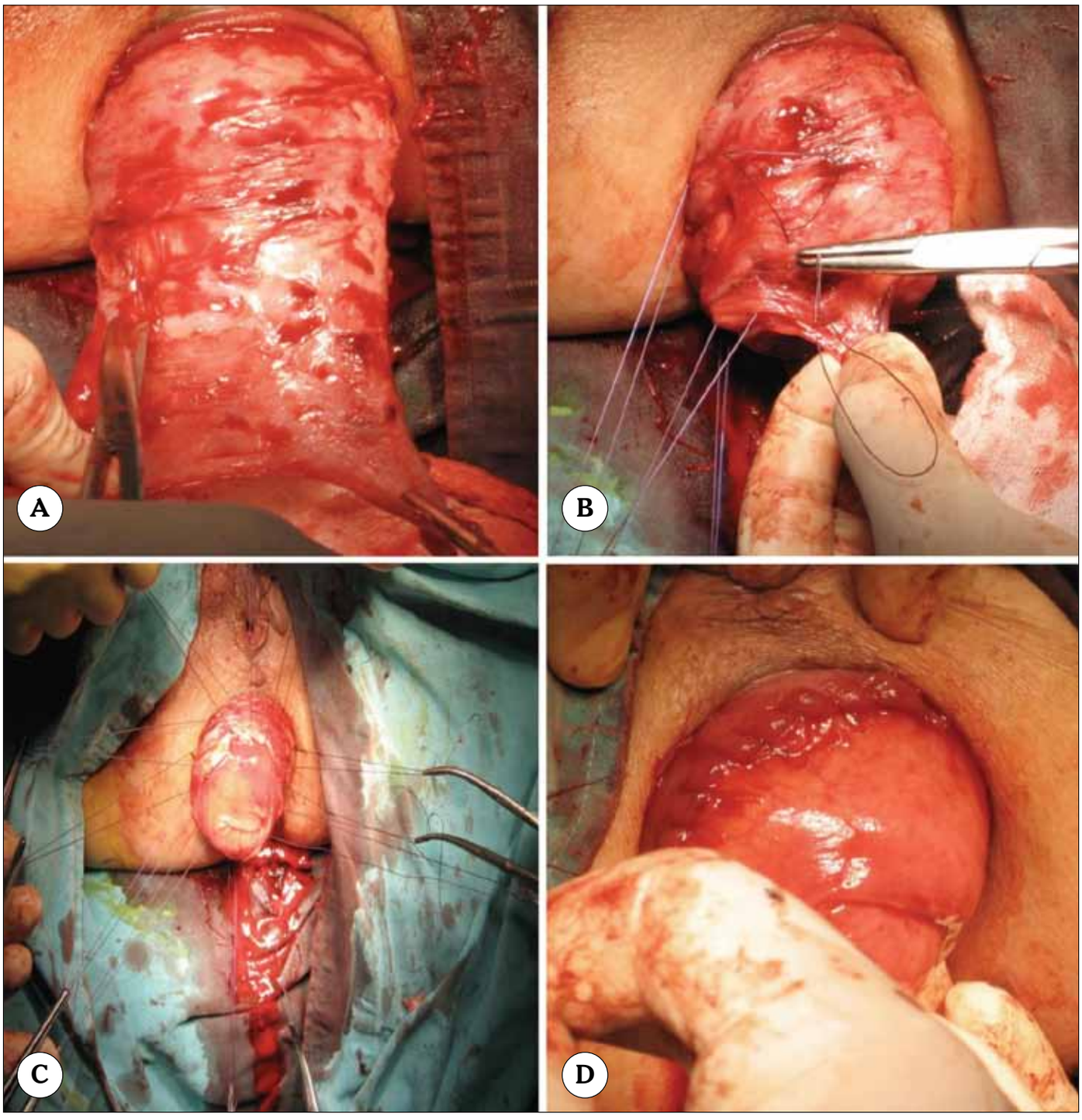

Figure 1. (Delorme's operation). Rectal mucosa was stripped by sharp dissection (A), vertical plications were made to rectal mucosa (B-C) and rectal mucosa was sutured, end to end (D)

The operation was carried out under local anesthesia with the support of sedation due to systemic decompensation. Lefort's operation (colpocleisis) for uterine prolapse and Delorme's operation for rectal prolapse were carried out at the same time. For rectal prolapse, the prolapse was fully extended using Babcock forceps and sufficient 1:400 000 adrenaline solution was injected submucosally to elevate all the exposed mucosa. This facilitated dissection in that plane and greatly reduced bleeding. Later, a circumferential mucosal incision was made 10-15 mm from the mucocutaneous junction and the mucosa was dissected proximally, the mucosa was stripped from the rectum to the apex of the prolapse and excised (Figure 1A).

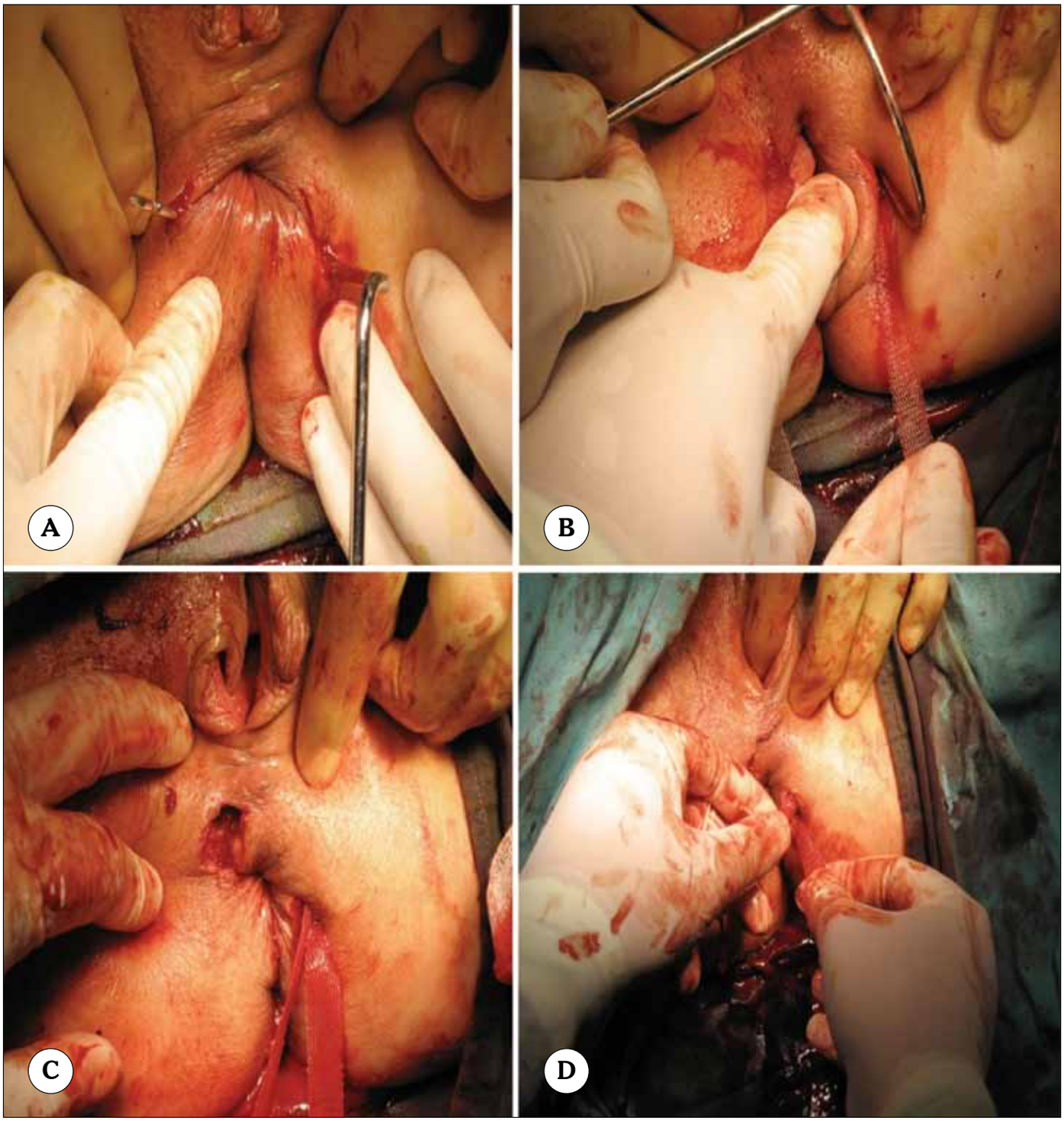

Figure 2. (Thiersh's operation) The curved needle was entered at 3 o'clock point by moving forward behind the sphincter and removed at 9 o'clock point (A), and was withdrawn by placing mesh at the apex (B), this process was repeated by passing in front of the sphincter and the mesh was placed around the anal sphincter circularly (C). Finally, the apexes of the mesh were connected to each other (D)

Between 8 and 12 plicating sutures of an absorbable material were inserted in the muscular wall of the rectum between the mucosal cut edges at the mucocutaneous junction and the apex (Figure 1B, 1C). Then, the denuded prolapsed muscle was pleated with a suture and was reefed up like an accordion (Figure 1B, 1C). The transected edges of the mucosa were then sutured together (Figure 1D). Later, Thiersh's procedure was used and the purpose of this procedure was to keep the rectum from prolapsing by restricting the size of the anal lumen. A polypropylene mesh was placed for anal canal encirclement. A curved needle was inserted at the 3 o'clock position around the anal canal, progressed subcutaneously, removed at 9 o'clock (Figure 2A) the and curved needle was withdrawn by placing mesh at the apex (Figure 2B). This process was repeated in the front and back of the anal canal, and as a result, mesh material was positioned to surround the external anal sphincter (Figure 2C). Finally, the polypropylene mesh was connected end to end (Figure 2D) and operation was completed. Postoperatively, antibiotic therapy was continued for 7 days, low molecular weight heparin was started in the twelveth hour. Oral feeding was stopped for the first two days. After this period, liquid nourishment was started and rapidly advanced to a regular diet. Difenoxylate + atropine sulfate was implemented four times a day to prevent rectal contamination.

Discussion

Rectal prolapse is an anatomical disease that is a component of pelvic floor dysfunction and it affects the quality of life adversely. This disease is seen in the elderly population more frequently or in patients who have a contraindication to general anesthetia. Our cases were at high risk for operations under general anesthesia, Lefort and Delorme operations were carried out at the same time under local anesthesia with the support of sedation. In this manner, possible mortality and morbidity due to general anesthesia were minimized and rectal prolapse was repaired at the same time. In the surgical treatment of rectal prolapse, abdominal procedures and perineal procedures can be preferred. The Delorme operation can be performed in elderly candidates or in patients for whom general anesthesia would constitute a high risk because of cardiac or pulmonary co-morbidities (8, 9). The Delorme procedure is expected to be sufficient for correction of anatomic structure, if the degree of rectal prolapse is stage I according to Altemeier's staging (10). Thiersh's procedure can be made around the anal sphincter for the treatment or prophylaxis of anal incontinence (9).

In conclusion, in elderly patients who have additional medical conditions and have concomitant rectal and uterine prolapse, the Delorme and Leforte operations may be applied under local anesthesia with the support of sedation. In addition, subcutaneous placement of mesh around the external anal sphincter may contribute to anal continence. The pelvic floor disorders should be considered as a whole, and evaluation of the pelvic floor should be made by urogynecologists who are experienced and trained.

Conflict of interest

No conflict of interest was declared by the authors.

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The future of telesurgery: a universal system with haptic sensation

Uzaktan cerrahinin geleceği: Dokunma duygusu hissi veren evrensel bir sistem

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Dear editor;

The 19th century will be remembered as the era of abdominal surgery, and the 20th as that of endoscopy. The 21st century has a potential to become the era of telesurgery, should the technical developments bring added value to the existing surgical methods. The optimal telesurgical system should be suitable to any kind of surgical procedure and provide tactile sensing, 3D vision as well as cost-effectiveness.

The Joint Research Centre (JRC) of the European Commission, in collaboration with SOFAR S.p.A. in Milan, Italy, initiated a project to meet these demands, the Telelap Alf-x. The New European Surgical Academy is providing the academic background to such a demanding project. This system enables universal telesurgical procedures with optimal ergonomy and haptic sensation. The preclinical studies have proven an optimal outcome and it seems that the system will replace several endoscopic procedures in the 21st century.

During the 19th century, along with the development of general anaesthesia (1), surgical procedures became routine, and certain novel operative methods were developed, some of which are in use even today, such as the Billroth or the Wertheim operations (2, 3).

Georg Kelling, a German surgeon, was the first to perform an experimental laparoscopy (4). Throughout the 20th century, the introduction of endotracheal intubation (5), the insufflator (6), light sources (6) and other designed instruments enabled the development of many endoscopic procedures.

Today, most operations can be performed endoscopically (7), especially the gynaecological ones, namely, the laparoscopically assisted vaginal hysterectomy (8) and the total abdominal hysterectomy (9). The patients undergoing endoscopic procedures need less postoperative analgesics and present decreased morbidity with shorter hospital stay (10).

At the end of the 20th century, it seemed that surgery had reached its peak.

The potential and vision of future remote operations in space and on other planets led to the development of telesurgical devices (11). Although we are still on Mother Earth, this idea stimulated the development of various systems. The era of telesurgery started in 1988 when the PUMA telesurgical system was used for a controlled neurosurgical biopsy (12). Other systems in use at present or in the past are the Da Vinci (13), Probot (14), Robodoc (15), and Zeus (16).

The term "robotic" prevails in the literature. However, it is misleading since none of the existing telesurgical systems is equipped with artificial intelligence. The term "telesurgery" should be preferred.

The accumulated advantages of the existing telesurgical systems are improved dexterity and accuracy, 3D stereo-vision, lack of tremor, and the potential of telementoring and operating from remote cities and countries. The most important disadvantage in all existing systems is the lack of haptic feedback.

Exactly like musicians who use their fingers for producing the desired sound and, in case of string instruments, feel the vibrations of the strings, it is of utmost importance for a surgeon to be able to feel the consistency and anatomical structures and evaluate the tensility of the suture during knot-tying.

Haptic sensation during surgery should be part of any telesurgical system, even if its relevance in telesurgical procedures is controversial, and it has been claimed that the results of visual force feedback and haptic feedback are comparable (17). In a recent study, differences between strand-to-strand knots and loop-to-strand knots were detected when telesurgical and manual knot-tying were compared (18).

In the past, surgeons used their fingertips to hold and manipulate instruments. In endoscopy, the trocar as well as other instruments are manipulated with the fists or the proximal parts of the fingers.

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Telesurgical systems should provide safety, accuracy, optimal short and long time outcomes and optimal ergonomy. Cost-effectiveness should always be considered.

Any surgical development should only be applied if it provides added value to the existing systems.

To meet these demands, the EU commission, in collaboration with SOFAR S.p.A. in Milan, Italy, has initiated a different telesurgical system, the Telelap Alf-x, which has been designed in order to meet the needs of patients and surgeons with the aim to provide added value to existing procedures (Figure 1).

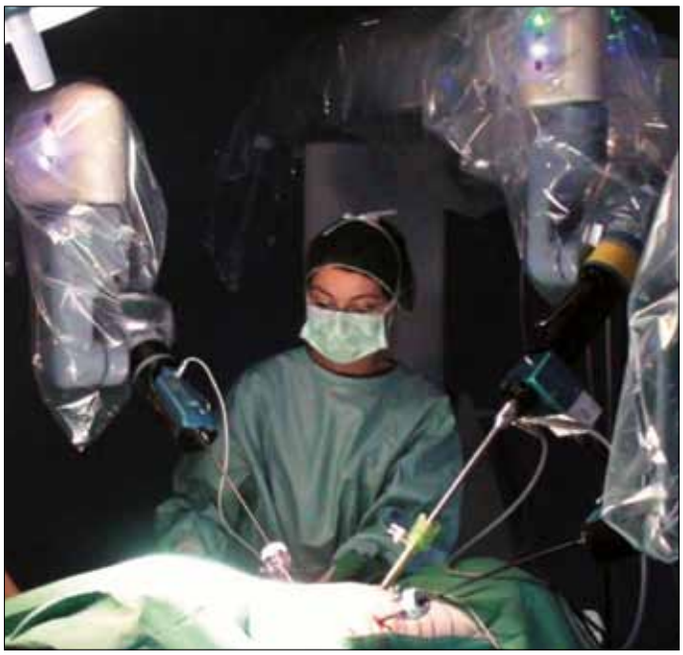

Figure 1. The Telelap-Alf-x system in work

Figure 2. Eye-tracking system

The features of the system are as follows:

1. 3 or 4 arms combined with 1 or 2 consoles, according to the needs. As the arms are separately moveable, immediate access to the patient is possible in case of an emergency;
2. Fast docking: all instruments are connected to the arms with magnets - immediate exchange of needed instruments;
3. The system detects within seconds the optimal pivot point of each inserted instrument. This point becomes the axis of the arm movement, preventing extension of the entry point;
4. Avoidance of tremor, advanced control and limitation of applied forces;
5. Haptic sensation and newly designed handles enabling manipulation of the instruments with the fingers;
6. Placing the instruments at any given angle needed. The system can access the abdominal cavity from the abdomen and, in women, through the pouch of Douglas, therefore transdouglas surgery is possible with this system;
7. A console with unobstructed view onto the screen with 3D vision and an ergonomic seat enabling a comfortable position during long operations;
8. Cost-effectiveness: the surgeon can use low-cost disposable instruments, however, the system provides reusable instruments;
9. Universality: any existing endoscopic instrument (articulated tip, monopolar, bipolar, laser etc. instruments) can be adapted. Therefore, surgeons do not have to change their operative habits and can even use the system for training;
10. An unique eye-tracking system. Next to the 3D vision, the surgeon controls the insertion of instruments by looking at the corresponding icon on the screen, the picture is magnified when his/her head approaches the screen, and any point looked at moves to the centre of the screen (Figure 2).

In the first experimental operations performed using the Telelap Alf-x, the average time for cholecystectomy was 31.75 min as compared to 91 min using a conventional telesurgical system (1). We strongly believe that haptic sensation provided more confidence to the surgeon, which explains the shorter operation time.

No surgical system can provide an optimal outcome when the surgical steps are not taken according to an evidence-based programme (20). At the same time, only standardized and optimized surgical methods will allow valuable meta-analysis and enable a comparison of surgical outcome in different institutions and by different surgeons (21). Therefore, a group of internationally renowned opinion leaders was assigned to design evidence-based surgical procedures in various disciplines.

The Telelap-Alf-x provides a combination of unobstructed 3D vision, haptic feedback and universality which offers all the advantages of laparotomy along with those of endoscopy. Therefore, this system will be the basis of novel surgical developments during the 21st century.

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What is your diagnosis?

A cesarean scar ectopic pregnancy (CSEP) is a pregnancy embedded in the myometrium of a previous cesarean scar, outside the uterine cavity. Its incidence is reported to be as high as from 1 in 1800 to 1 in 2200 (1, 2). A delay in diagnosis and/or treatment can lead to uterine rupture, major hemorrhage secondary to placenta accreta or percreta, a need for hysterectomy and serious maternal morbidity (3-5).Therefore, the main objectives in the management of a cesarean scar ectopic pregnancy should be early and accurate diagnosis and prevention of severe blood loss while preserving fertility.

The diagnosis can be made initially by ultrasonography. However, as presented in this quiz, MRT can be also very useful (Figure 1, 2). Differential diagnosis has to be made with ectopic intramural pregnancy.

There is no consensus about the method of choice for managing CSEP. The use of blind or ultrasound-guided surgical evacuation, medical management with methotrexate (MTX), administered either systemically or locally, and expectant management have all been reported in the literature, as have combined treatments (1-7).

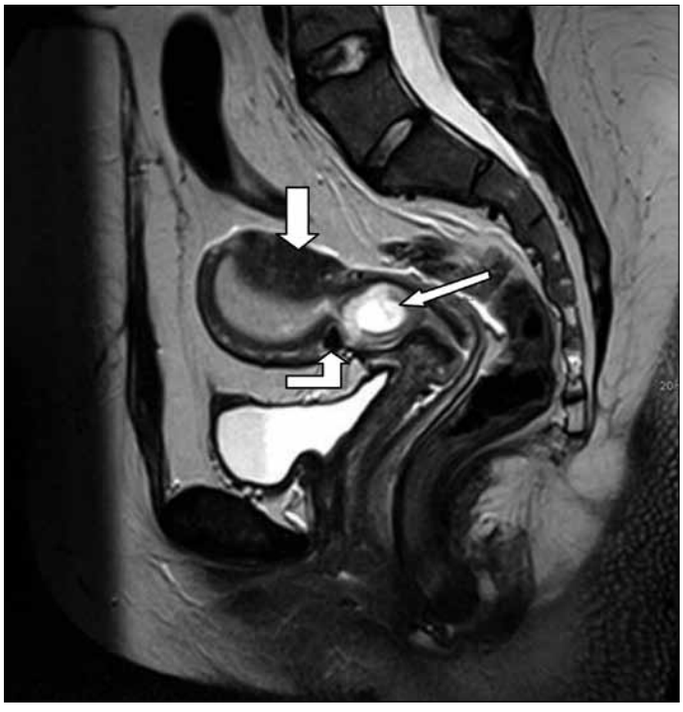

Figure 1. Please name the structures pointed by the arrows on MRI of the pelvis

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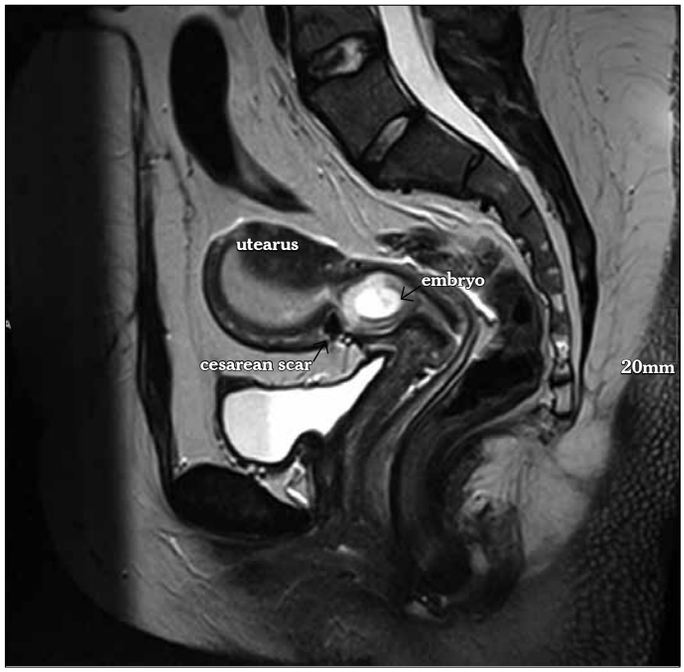

Figure 2. MRI imaging of cesarean scar pregnancy

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CONGRESS CALENDAR

28-31 March 2012	**9th European Congress on Menopause and Andropause (EMAS)** Athen, Greece http://www2.kenes.com/emas
19-21 April 2012	**5. Ege Jinekolojik Endoskopi Sempozyumu Crowne Plaza** İzmir, Turkey http://www.ege2012.org
26-29 April 2012	**Comprehensive Colposcopy (American Society for Colposcopy and Cervical Pathology) Rhode** Island, USA http://www.asccp.org
9-12 May 2012	**ACOG 60th Annual Clinical Meeting (ACOG)** San Diego, CA USA http://www.acog.org/acm
13-15 September 2012	**NESA Palmaplanas Innovation Days 2012** Palma de Mallorca, Spain www.uspnesadays.com
21-23 September 2012	**SGI Summit Turkey 2012: Innovations in Obstetrics and Gynecology** Istanbul, Turkey www.sgiturkey2012.org
11-14 October 2012	**The 8th National Congress of Turkey Maternal Fetal Medicine and Perinatology Association** Istanbul, Turkey www.tmftpkongre2012.org
9-11 November 2012	**2nd Asian Conference on Endometriosis** Istanbul, Turkey www.ace-2012.org