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A folliculometri and Laparoscopic Oocyte Pick-Up in a patient with congenitally malpositioned Ovaries. (Archieve of Assist. Prof. Gazi YILDIRIM)

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Contents

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Editorial

Dear Colleagues,

The IX. Turkish - German Gynecology Congress which has been held biennially for more than 15 years was conducted in Antalya this year, with a high scientific quality and great number of national and international participants from all over the world. The great interest in our congress from the gynecology and obstetrics society has shown itself with 1450 participants from 20 different countries. The feedbacks of almost each participant were very positive about the organization, social activities, hotel selection and the high level of scientific program. These feedbacks and positive comments are encouraging and stimulating us to make even better and better activities and meetings in the near future.

The congress started with six pre-congress courses and hands-on training sessions in different interests. The courses on Colposcopy, Ovulation Induction, Perinatology, Robotic Surgery, Urogynecology, and ISUOG have taken great interest from the participants, followed by the impressive opening conference, presented by the honorary president of our ninth congress, Prof. Camran Nezhat about "When will operative laparoscopy replace almost all laparotomies? Or will it?". The scientific program of our congress was quite satisfactory for all participants in all topics of interest in the field of obstetrics & gynecology, and related disciplines. The program was presented in 41 sessions in 4 parallel halls by 150 Turkish and 45 international faculty members. The experts have shared their knowledge and experiences widely with the participants from the basic terms to the latest innovations in the topics of obstetrics & gynecology, perinatology, infertility, reproductive medicine, oncology, gynecologic surgery, laporoscopy, embryology and urogynecology. Special sessions cooperated with some national and international establishments such as NOGGO, Turkish Society of Menopause & Osteoporosis and Bourn Hall Clinic also took place in our congress. Three satellite symposiums and a meet the expert meeting were also organized at our congress and the participation was very satisfactory for these meetings. Many prestigious pharmaceutical, instrumental and appliances firms found a great opportunity to introduce their current and brand new products and services to the participants in our congress. On the other hand, the participants had a chance to be informed of the latest innovations and technology in scientific medicine and devices industry at the exhibition area, even making a test drive with the robotic surgery system during four days.

A total of 370 abstracts have been collected by the online abstract submission system for poster, oral and video presentations from many different countries. From these submitted abstracts; 325 poster, 28 oral, and 5 video abstracts have been accepted and presented at the congress. Best three abstracts, evaluated and selected by the scientific secretariat of the congress, were financially awarded with a total of 6.000 TL.

The closing ceremony and the gala dinner of the congress with the great performance of Behzat Gerçeker and Enbe Orchestrate was a great relaxation and a cream of the crop for the participants before going back to their home after four days of scientific program.

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

Best regards,

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

Effect of polymyxin B on gram-negative bacterial infection during pregnancy

Gebe fare modelinde gram-negatif bakteriyel enfeksiyona polimiksin B'nin etkisi

Mukesh Kumar Jaiswal, Varkha Agrawal, Yogesh Kumar Jaiswal

Molecular Biology and Reproductive Immunology Laboratory, School of Studies in Biochemistry, Jiwaji University, Gwalior, India

Abstract

Objective: Polymyxin B (PB) is a naturally occurring cationic cyclic decapeptide which is highly bactericidal to Gram-negative bacteria. The objective of this study was to investigate the effect of PB on the viability of developing embryos during pregnancy and to validate its protective effect on the embryotoxic effect of Gram-negative bacterial lipopolysaccharide (LPS).

Material and Methods: Animals were injected intraperitoneally *(i.p.)* with PB (5-100 μg/animal), (Minimum effective dose) MD of LPS and MD of LPS+PB (5-100 μg/animal) on day 0.5 of pregnancy. The percentage of normal gestational sacs and histopathologic analysis were assessed.

Results: PB treatment of pregnant females disturbs the pregnancy in a dose dependent manner and increases the substantial risk of congenital abnormalities in the growing fetuses of the mother. However, PB does not show any adverse effect on implantation of embryos. The embryotoxic effect of LPS can be prevented completely by 25 μg PB/animal; however other lower and higher doses of PB were not able to protect against the effect of LPS on pregnancy.

Conclusions: Our results demonstrate that PB has the ability to protect the LPS-induced pregnancy loss but may not be recommended as a safe drug for the treatment of a mother suffering from Gram-negative bacterial infection during pregnancy.

(J Turkish-German Gynecol Assoc 2011; 12: 64-70)

Key words: Gram-negative bacterial infection, lipopolysaccharide, polymyxin B, pregnancy loss, implantation failure

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Özet

Amaç: Polimiksin B (PB) Gram-negatif bakterilere karşı son derece bakterisidal olan, doğada bulunan bir katyonik siklik dekapeptiddir. Bu çalışmanın amacı PB'nin gebelik süresince gelişmekte olan embriyonun yaşayabilirliği üzerine etkisini araştırmak ve Gram-negatif bakteriyel lipopolisakkaridin (LPS) embriyotoksik etkisi üzerine koruyucu etkisini doğrulamaktı.

Gereç ve Yöntemler: Hayvanlara gebeliğin 0.5'inci gününde intraperitoneal olarak (i.p.) PB (5-100 μg/hayvan), minimum etkili doz (MD) LPS ve MD LPS+PB (5-100 μg/hayvan) enjekte edildi. Normal gebelik keselerinin yüzdesi ve histopatolojik analiz değerlendirildi.

Bulgular: Gebe dişilerin PB tedavisi doz bağımlı bir şekilde gebeliği kötü yönde etkiler ve annelerin büyümekte olan fetüslerinde konjenital anormalliklerin riskini belirgin olarak artırır. Bununla beraber, embriyoların implantasyonu üzerine PB olumsuz bir etki göstermez. LPS'nin embriyotoksik etkisi 25 μg PB/hayvan ile tamamen önlenebilir; bununla beraber daha düşük ve daha yüksek diğer PB dozları gebelik üzerine LPS'nin etkisine karşı koruyucu olamadı.

Sonuçlar: Sonuçlarımız PB'nin LPS'nin tetiklediği gebelik kayıplarını önleyebilme becerisine sahip olduğunu fakat gebelik süresince Gram-negatif bakteriyel enfeksiyon geçiren bir annenin tedavisi için güvenilir bir ilaç olarak önerilemeyeceğini göstermektedir.

(J Turkish-German Gynecol Assoc 2011; 12: 64-70)

Anahtar kelimeler: Gram-negatif bakteriyel enfeksiyon, lipopolisakkarit, polimiksin B, gebelik kaybı, implantasyon başarısızlığı

Geliş Tarihi: 18 Şubat 2011 **Kabul Tarihi:** 15 Mart 2011

Introduction

Preterm labor and delivery continues to be the most important unsolved problem in obstetrics. 10-30% of women with preterm labor have clinically evident and subclinical Gram-negative bacterial infection (1-3). The bacterial endotoxin, lipopolysaccharide (LPS), is the major antigen of the outer membrane of Gram-negative bacteria that possess a toxic effect. Gram-negative bacteria colonize in the genitourinary tract of women and contribute to creating a distinct microbial environment (4). The endotoxins, continually released into the genitourinary tract of the infected pregnant females, may be associated with preterm labor and birth and other perinatal complications.

Several different model systems for inducing preterm labor in mice have been developed over recent years. These involve local (intrauterine or intracervical), extrauterine (e.g., renal), and systemic administration of a variety of substances, such as bacteria, bacterial products from Gram-positive and Gram-negative organisms, inflammatory cytokines, prostaglandins, and others (5). The use of killed bacteria, components of the cell wall (LPS or Lipoteichoic acids) or proinflammatory cytokines (interleukin (IL)-1) create an inflammatory state in the absence of an overt infection. Both of these methods can promote preterm delivery in rodents and non-human primates (6). We developed a Gram-negative bacterial infection model in mouse by the systemic administration of 'minimum dose' (i.e., 250 μg/kg body weight, i.p. on day 0.5 of pregnancy) of

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LPS which is sufficient to cause embryonic cell death (7) and leads to implantation failure on day 5.5 of pregnancy (8). LPS exhibits a variety of toxic and proinflammatory activities that are related to the pathogenesis of Gram-negative bacterial infection (9-11). LPS inhibits the blastocyst implantation in mouse by modulating the level and pattern of expression of different cytokines such as tumor necrosis factor (TNF)-α, (12) IL-1 (13) and growth factors such as colony stimulating factor (CSF)-1 (7). LPS-induced DNA damage in preimplantation stage embryos and uterine cells leads to poor pregnancy outcome (14).

Polymyxin B (PB) is a naturally occurring cationic cyclic peptide isolated from *Bacillus polymyxa* (15, 16). PB is highly bactericidal to Gram-negative bacteria and is considered to be one of the most efficient cell-permeabilizing compounds (17). This capacity is due to its high-affinity binding to the lipid A moiety of LPS in Gram-negative bacteria. PB forms a heptapeptide ring by an amide bond between the C-amino group of diaminobutyric acid (DAB) at position 4 and the carboxyl group of the C-terminal, and a tripeptide tail which is attached to a small fatty acyl chain via a peptide bond. PB is an amphiphilic compound due to the presence of both a polycationic heptapeptide ring containing five positively charged DAB residues and a hydrophobic acyl chain. These positively charged DAB residues of PB interacts with the Lipid A moiety of LPS with an ensuing loss of many of the biological properties of LPS by forming a LPS-PB complex (18). This property of PB may be used in developing it as a novel anti-Gram-negative bacterial drug. However, the therapeutic applications of PB are very limited because of its relatively high toxicity (19, 20).

In the field of reproductive medicine, PB has been used as a chemotherapeutic drug in some gynecological pathologies, for treating pregnant females suffering from severe pyelonephritis, endometriosis, tubular obstruction in fallopian tubes and genital tract infections caused by Gram-negative bacteria (21). It has been reported that exposure of mothers to PB in the first trimester of pregnancy does not lead to any congenital abnormalitie (21). However, the use PB during pregnancy is debatable as to whether use of PB as an antagonist of LPS should be promoted or not. Here we investigate the effects of PB on developing embryos and pregnancy outcomes in a Gram-negative bacterial infection mouse model.

Materials and Methods

Animals

Park strain mice (6-7 weeks, ~ 20-21 g) used in the study was maintained in our animal care facility at 25±2°C with 12:12 hr light: dark period. They were regularly fed with pelleted diet (Amrut Laboratory Animal Feed, Pranav Agro Industries, Sangli, MH, India) and drinking water *ad libitum*. Normal mature adult females were selected for the present study. This study was conducted in accordance with the institutional ethics committee guidelines for the care and use of animals in research.

Design of experiment

The reproductive cycle was checked and females in proestrus were caged individually overnight with proven fertile male for mating. Vaginal plug was checked next day morning at 9:00 A.M. and the vaginal plug positive females were considered as being on day 0.5 of pregnancy.

Determination of the effect of PB on implantation

The effect of PB on the implantation of blastocysts was evaluated. Females were divided into two groups of five animals each. The animals of group I received 100 μl of sterile normal saline as a control and group II received 100 μg PB/animal (InvivoGen, California, USA) in a 100 μl volume through i.p. route on day 0.5 of pregnancy. Pontamine Blue dye test was performed on day 5.5 of pregnancy to observe the effect of this dose on implantation.

Determination of the effect of PB on post Implantation period of pregnancy

Pregnant females were divided into five groups of six animals each. Different doses of PB (5, 25, 50 and 100 μg, i.p.) were given to individual groups on day 0.5 of pregnancy. Control animals received 100 μl of normal saline in a similar manner. The effect of PB on the status of pregnancy was assessed on days 9.5 and 14.5 of pregnancy by examining individual uterine horns and gestational sacs for live (pink, round, uniform) and dead (abnormally shaped, hemorrhagic sacs) pups and for resorption (very small, pale and gray sacs with no discernible fetus). Five pregnant females of control and 100 μg PB-treated groups were maintained up to the day of parturition to monitor the effect of PB on development of the implants during the post implantation period of pregnancy.

Determination of PB dose effective in preventing the embryotoxic effect of LPS

Pregnant females were divided into six groups of four animals each. The animals of group I received sterile normal saline, group II received LPS (5 μg/animal), group III received LPS and PB (5 μg/ animal), group IV received LPS and PB (25 μg/ animal), group V received LPS and PB (50 μg/ animal) and group VI received LPS and PB (100 μg/ animal) i.p. in 100 μl volume on day 0.5 of pregnancy. The dose of LPS was the same i.e., 5 μg/ animal in each LPS and PB treated group. Status of pregnancy was assessed on day 14.5 of pregnancy. Five pregnant females of group I, II and IV were maintained up to the day of parturition to check that normal gestational sacs observed on day 14.5 of pregnancy develop into normal pups or not.

Histopathologic analysis of uterus on day 14.5 of pregnancy in animals treated with LPS and different doses of PB

Histopathologic analysis of uterine horns was carried out according to a standard procedure (22). Uterus of selected groups was collected on day 14.5 of pregnancy and fixed in Bouin's fixative for 22-24 hours. The fixed tissues were processed in a tissue processor (Leica Tissue Processor 1020, Leica Microsystems, Wetzlar, Germany) and embedded in the paraffin wax at 60°C with the use of tissue embedder (Leica Tissue Embedder, Wetzlar, Germany). Tissue blocks were sectioned at 4.5 μm using a microtome (Leica EG 1106 Microtome, Semi automated, Microm, Wetzlar, Germany). Tissue ribbons were processed in an autostainer (Leica Autostainer XL,

Wetzlar, Germany) and stained with Hematoxylin and Eosin. Slides were mounted with DPX and observed under a light microscope (Leica DM IL, Wetzlar, Germany) at X5 magnification and photographed.

Statistical analyses

The results of all the experiment were analyzed by using one way analysis of variance (ANOVA) with Duncan's multiple range test for comparison of the significance level (P) among control and treated values. $p<0.05$ was considered to be a significant difference among the values compared.

Results

Effect of PB on implantation

The effect of PB on implantation was assessed by the presence of positive Pontamine Blue sites in uterine horns on day 5.5 of pregnancy. Uterine horns were recovered from control (Figure 1A) and 100 μg PB-treated (Figure 1B) animals on day 5.5 of gestation. No significant difference was observed in the number of implantation sites between control (9.45±0.48) and PB-treated (9.33±0.88) animals on day 5.5 of pregnancy (Table 1). However, overcrowded conceptus with abnormal spacing was observed on day 5.5 on pregnancy as compared to controls.

Effect of PB on post-implantation period of pregnancy

The effect of PB (100 μg/animal) on the viability of embryos was assessed by examining the gestational sacs for live and dead pups and resorption during the post-implantation period of pregnancy. The uterine horns were recovered from control (Figure 1C) and 100 μg PB-treated animals (Figure 1D) on day 9.5 of gestation. No significant difference was observed between the number of gestational sacs present in control (9.41±0.41) and 100 μg PB-treated (8.50±0.50) animals on day 9.5 of gestation (Table 1).

The effect of PB was checked on the viability of embryos on day 14.5 of gestation. Uterine horns were recovered from control (Figure 2A) and 100 μg PB-treated animals (Figure 2F) on day 14.5 of gestation. A significantly lower number of normal gestational sacs were observed in 100 μg PB-treated animals (2.50±1.07) compared to the controls (8.58±0.23) (Table 1). Developmentally normal gestational sacs with no sign of dead pups and resorptions were recovered from the control animals (Figure 2K), whereas only 29.14±12.46% of gestational sacs were normal in 100 μg PB-treated animals (Figure 2K) on day 14.5 of pregnancy.

The animals treated with normal saline and 100 μg PB was kept up to the day of parturition to check the delivery outcome. No pups were recovered from 100 μg PB-treated animals, whereas normal pups were observed in control animals (8.5±0.19) (Table 1). This observation clearly suggests that the gestational sacs, which were normal on day 14.5 of pregnancy in 100 μg PB-treated animals, had undergone resorption during the later stages of development.

The effect of lower doses of PB (i.e., 5, 25 and 50 μg PB/animal) on pregnancy was evaluated by visual examination of uterine horns and individual gestational sacs on day 14.5 of pregnancy. We found that percentages of normal gestational sacs were only 66.04±21.63% in 5 μg PB-treated animals (Figure 2C, 2K), 40.79±13.87% in 25 μg PB-treated animals (Figure 2D, 2K) and 43.03±14.36% in 50 μg PB-treated animal (Fig. 2E, 2K).

Present observations show that PB treatment in pregnant females disturbs the normal pregnancy in a dose dependent manner and increased the substantial risk of congenital abnor-

Table 1. Effect of PB (100 μg/animal) on embryonic loss during different stages of pregnancy

Treatment	Number of animals used	No. of implantation sites /animal on day 5.5 of pregnancy*	No. of gestational sacs/animal on day 9.5 of pregnancy*	No. of gestational sacs/animal on day 14.5*	Number of pups born/animal *
Control	5	9.45±0.48[a]	9.41±0.41[a]	8.58±0.23[a]	8.5±0.19[a]
100 μg PB treated animals	5	9.33±0.88[a]	8.50±0.50[a]	2.50±1.07[b]	0.00±0.00[c]

Data are expressed as mean ± 1SEM
[a-c]Values bearing non-similar superscripted alphabets differ from each other at $p \leq 0.05$ (based on Duncan's multiple-range test)

Table 2. The effect of protective dose of PB (25 μg/animal) in LPS-induced pregnancy loss

Treatment	Number of animals used	No. of implantation sites on day 5.5 of pregnancy*	No. of gestational sacs on day 14.5 of pregnancy*	Number of pups born*
Control animals	5	8.77±0.33[a]	8.58±0.23[a]	8.5±0.19[a]
LPS-treated animals	5	0.32±0.09[b]	0.25±0.13[b]	00±0.00[c]
25 μg Polymyxin B+ LPS-treated animals	5	8.46±0.13[a]	8.00±0.00[a]	8.33±0.33[a]

*Data are expressed as mean±1SEM
[a-c]Values bearing non-similar superscripted alphabets differ from each other at $p \leq 0.05$ (based on Duncan's multiple-range test)

malities in the developing fetus. However, none of the tested doses of PB showed any adverse effect on the implantation of blastocyst in mouse.

Effect of PB on LPS

The effect of PB in LPS-treated females was analyzed on day 14.5 of pregnancy by visual examination of individual gestational sacs recovered from the animals treated with normal saline, LPS and LPS with different doses of PB (i.e., 5, 25, 50 and 100 μg PB/animal).

Gestational sacs were developmentally normal with no sign of dead pups and/or resorption in the control animals on day 14.5 of pregnancy (Figure 2A, 2K). In LPS-treated animals, only 2.19±1.52% gestational sacs were normal (Figure 2B, 2K). Percentages of normal gestational sacs were 46.62±24.26% in LPS+5 μg PB-treated animals (Fig. 2G, 2K) and 94.87±3.1% in LPS+25 μg PB-treated animals (Figure 2H, 2K). The numbers of gestational sacs were the same in uterine horns recovered from LPS+25 μg PB treated (8.00±0.00) and normal saline (8.58±0.23) treated animals (Table 2). Percentages of normal gestational sacs were 25.00±12.21% in LPS+50 μg PB/animal (Figure 2I, 2K) and 32.23.00±12.32% in LPS+100 μg PB/animal (Figure 2J, 2K).

The present investigation showed that the embryotoxic effect of LPS can be significantly prevented with the dose of 25 μg PB/animal ($p<0.05$). The pregnancy outcome from the normal gestational sacs present on day 14.5 of pregnancy in control and LPS+25 μg PB-treated animals was analyzed. No significant difference was observed in the number of pups recovered from both groups (Table 2). These observations show that the treatment of animals with 25 μg PB can protect the embryotoxic effect of LPS (Figure 2K). However, the other tested lower and higher doses of PB with LPS failed to prevent the high percentages of fetal loss. It may also be due to the embryotoxic effect of either LPS and PB or both.

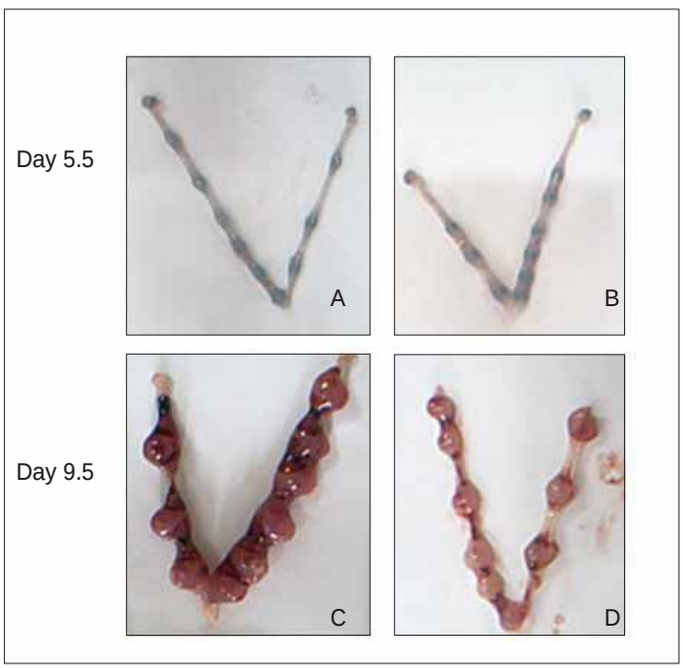

Figure 1. Photographs of the uterine horns showing gestational sacs on day 5.5 and 9.5. Pontamine Blue dye test showing implantation sites in the uterine horns on day 5.5 of pregnancy in (A) control and (B) 100 μg PB- treated animals. Uterine horns recovered on day 9.5 of pregnancy in females treated with (C) normal saline and (D) 100μg PB- treated animals

Histopathologic analysis of uterus on day 14.5 of pregnancy in LPS and PB-treated animals

The histopathologic analysis of the uterus recovered from LPS + PB treated animals was carried out on day 14.5 of pregnancy to evaluate the state of development of fetus and its interaction with uterine epithelium. The cross sections of uterine horns recovered from animals treated with normal saline, LPS, 25 μg and 100 μg PB, LPS+25 μg PB and LPS+100 μg PB/animal were examined. The normal developing fetus was observed in the cross sections of uterine horns recovered from the control animals on day 14.5 of pregnancy (Figure 3A). The cross-sections of the uterine horns recovered from animals treated with LPS showed uterine lumen closure, hyperplasia of the luminal epithelium, few glands in stromal region, and with no ectoplacental cones (Figure 3B). However, the cross sections of uterine horns recovered from animals treated with 25 μg (Figure 3C) and 100 μg (Figure 3E) PB showed no fetus, reduced deciduas with none to few degenerated glands in deciduas. Moreover, the deciduas with reduced frond like villous outgrowth were observed in the cross sections of uterine horns recovered from the animals treated with 100 μg PB as compared to 25 μg PB-treated animals.

The cross sections of the uterine horns recovered from LPS+25 μg PB-treated animals showed normal fetal membrane and developing fetus (Figure 3D) as observed in control pregnancy. However, the cross sections of uterine horns recovered from LPS+100 μg PB-treated animals displayed no fetus and deciduas as compared to control (Figure 3F).

Discussion

PB has been used widely for treating certain bacterial infections such as the meningial infections caused by *Haemophilus influenzae*, urinary tract infections of *E. coli* and bacteremia caused by *Enterobacter aerogenes* etc. and more so for treating the endotoxic or septic shock which is caused by endotoxin (18). Endotoxin is an overwhelmingly powerful poison, the actions of which target virtually every cell-type in the susceptible animal, and in this way endotoxin evokes a multitude of biological responses. Gram-negative bacterial endotoxin can induce both local and systemic activation of immune response, and in extreme cases, this leads to septic shock. Gram-negative bacterial infections of the genito-urinary tract of pregnant women are known to cause fetal abortions or pregnancy loss (23, 24).

Some of the obstetricians recommended PB treatment in clinics for severe infection caused by Gram-negative bacteria. (21). However, in the present study, it has been observed that PB shows embryotoxic effects during the post-implantation period of normal pregnancy and increases the substantial risk of congenital abnormalities in the developing fetus in a dose

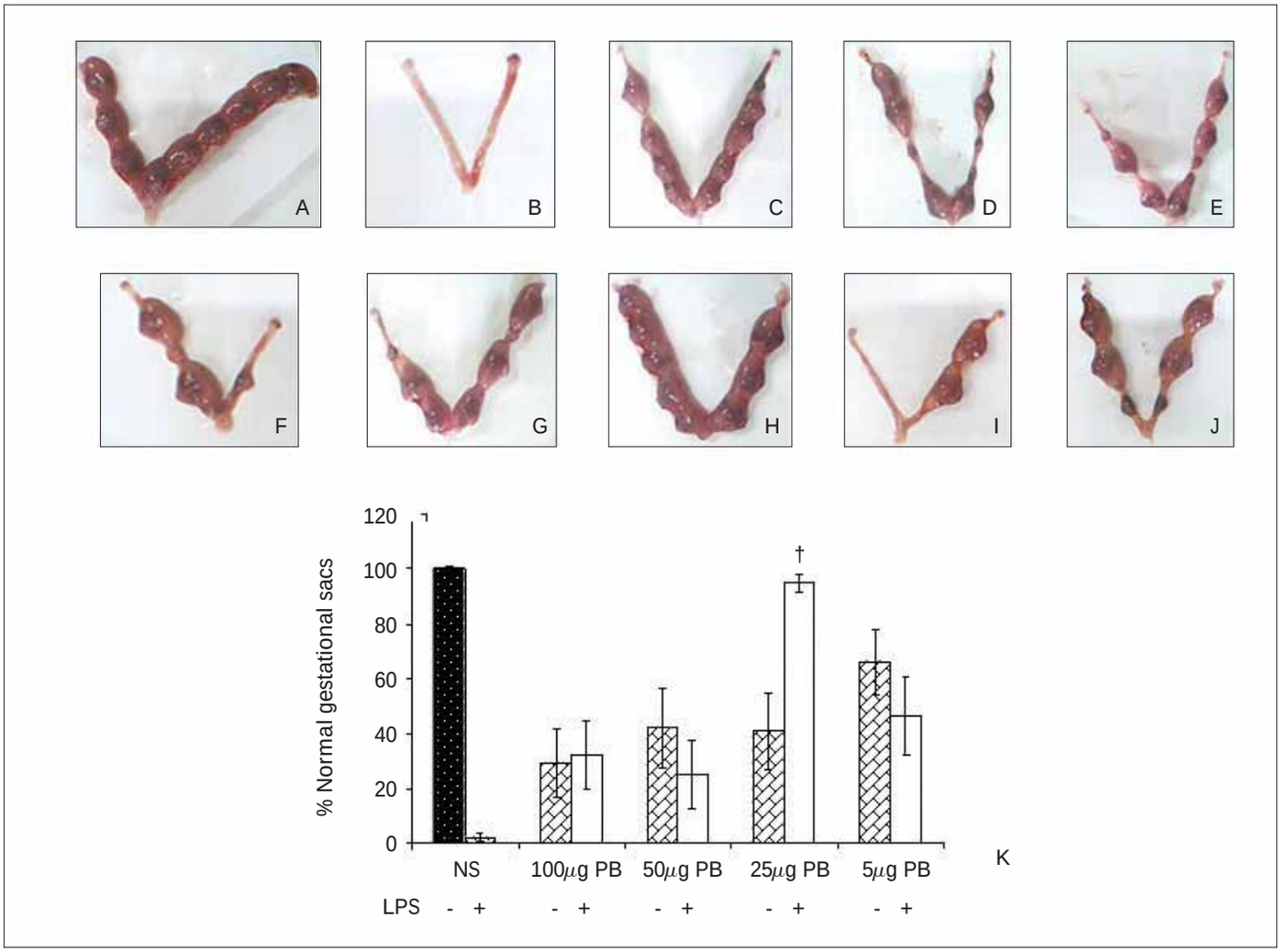

Figure 2. **Photographs of the uterine horns showing gestational sacs on day 14.5 of pregnancy in females treated with (A) normal saline, (B) LPS, (C) 5 μg PB, (D) 25 μg PB (E) 50 μg PB, (F) 100 μg PB, (G) LPS+5 μg PB, (H) LPS+25 μg PB, (I) LPS+50 μg PB, (J) LPS+100 μg PB; (K) Percentage of normal gestational sacs on day 14.5 of pregnancy in PolymyxinB and LPS-treated animals**

Note: Data is expressed as mean±1SEM

†Non-significant to the PBS group (p=0.57), based on Duncan's multiple-range test

NS=Normal saline, PB=Polymyxin B

dependent manner. We have also shown that PB treatment may protect pregnancy in LPS-treated animals. The molecular mechanism involved in this process is not known. It is not even well established how PB disturbs the pregnancy, because it does not possess any specific and selective toxicity. Our observation of the Pontamine blue test on day 5.5 of pregnancy suggests that PB (100 μg) does not affect the blastocyst implantation. However; this dose leads to abortion of the developing fetus in later stages of gestation. Furthermore, treatment of other PB doses shows a very high percentage of fetal rejection in a dose dependent manner.

It has been reported that PB induces hypothermia by modulating the thermoregulatory mechanism in rodents. In addition, it has been demonstrated that an i.v. injection of PB decreases the metabolic rate and heat loss response in rodents, which may be responsible for PB induced hypothermia that may lead to hemorrhagic condition in rodents (25). It has also been reported that PB induces neuromuscular blockade and its action might be responsible for the decrease in metabolic rate, especially in skeletal muscles. The PB induced myoneural effect is due to its pre-synaptic action that inhibits the release of acetylcholine, which suggests the presence of its receptor at the pre-synaptic sites (25). These observations suggest that PB induced pregnancy loss might be due to a reduction of metabolic rate, heat loss and neuromuscular blockade which may reduce the growth and development of embryos and uterus during pregnancy. We observed that the uterus recovered after PB treatment was abnormal, with loss of flexibility and hemorrhagic gestational sacs as compared to the normal uterus. Our histopathologic observations of uterus on 14.5 day of gestation also support that PB possesses an abortifacient property in a dose dependent manner.

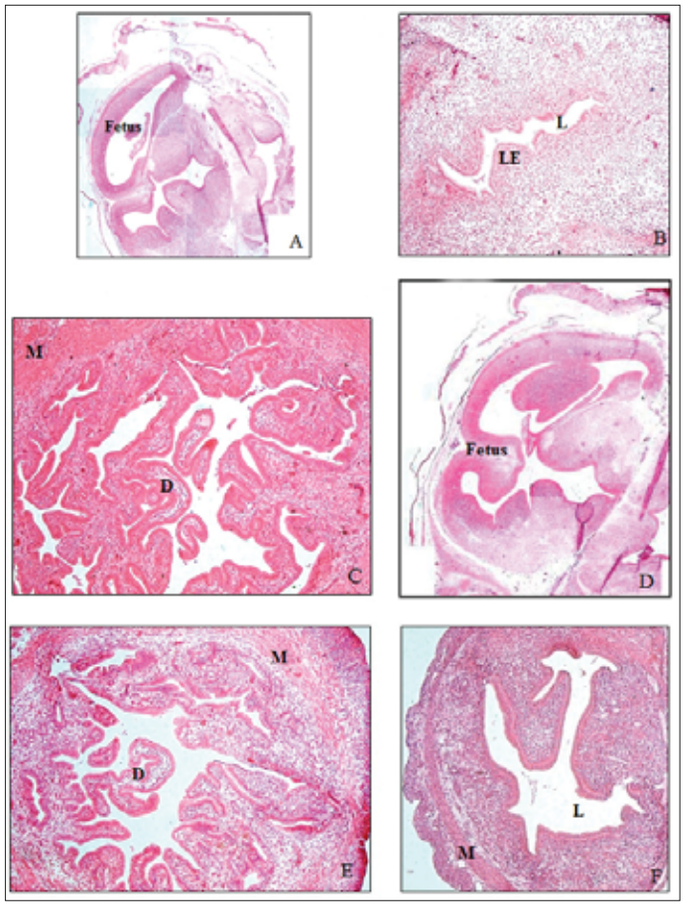

Figure 3. **Cross-sections of uterine horn through developing fetus on day 14.5 of pregnancy. Uterine horns recovered from animals treated with (A) normal saline, (B) LPS, (C) 25 μg PB, (D) LPS + 25 μg PB (E) 100 μg PB, (F) LPS + 100 μg PB. X50 magnification; M = Myometrium; L = Lumen; LE = Luminal epithelium; D = Decidua (Panel A and D has been edited with the help of Adobe Photoshop software)**

In the present study, we observed that 25 μg PB is efficient in protecting the effect of LPS-induced early pregnancy loss, whereas its other tested lower and higher doses failed to do so. Our studies show that the embryotoxic effect of LPS can be completely prevented by 25 μg PB/animal. It has been found that 94.87 ± 3.1% developmentally normal gestational sacs present in the animals pre-exposed with LPS and treated with 25 μg PB/animal on day 14.5 of gestation, were able to develop into normal and healthy pups, whereas this was not observed with other lower and higher doses of PB. It has been suggested that PB binds to LPS at multiple places and neutralizes its effect. PB is a natural cationic cyclic peptide antibiotic containing a lipophilic and hydrophilic groupment (lipophobic) that binds to the lipid A region of LPS (18). PB binds with high affinity to the lipid A portion and alters the three-dimensional conformation of the LPS molecule. The alteration in the conformation of LPS may possibly inhibit the binding of complex endotoxin-PB to CD14 receptor on monocytes and abrogate the liberation of inflammatory mediators such as the TNF-α (26). Due to this property; PB is used to prevent septic shock (27, 28) and it has been used to neutralize LPS-induced TNF-α production (26).

In addition, PB down regulates the expression of various cytokines such as TNF-α, IL-1, IL-10 (29). It has been shown that PB prevents the lethal effect of endotoxin in chick embryos, mice, dogs, goats, foals and horses (30). PB was shown to inhibit LPS-induced intramuscular coagulation, macrophage production of interferon-γ, TNF-α, IL-1, the generalized Shwartzman reaction and LPS-mediated shock (27). These evidences suggest that the protective property of PB against the LPS-induced embryopathy might be due to its ability to inhibit the production of LPS-induced cytokines during the preimplantation period of pregnancy and thus may prevent LPS-induced early pregnancy loss in mouse.

Our present observation suggests that 25 μg PB may be an efficient dose for the treatment of Gram-negative bacterial infection without any adverse affect on ongoing pregnancy. However, other doses of PB, with or without LPS, induce a high percentage of fetal resorption. Therefore, PB may not be recommended as a safe drug for the treatment of Gram-negative bacterial infection during pregnancy, because other studied doses are not effective and are even associated with fetal resorption. However, more work is being carried out in our laboratory to determine the mechanism of action of PB during pregnancy. The outcome of the investigation may provide a deeper insight to our current understanding about the roles of the antibiotic as a chemotherapeutic drug for the treatment of women suffering from vaginitis and other urinary tract infections during pregnancy.

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Conflict of interest

No conflict of interest was declared by the authors.

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The positivity of *Helicobacter pylori* Stool Antigen in patients with Hyperemesis gravidarum

Hiperemezis gravidarumlu hastalarda gaitada Helicobacter pylori antijeni saptanması

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Abstract

Objective: We aimed to investigate the possible association between *Helicobacter pylori* infection and Hyperemesis gravidarum.

Material and Methods: Thirty-six pregnant women with Hyperemesis gravidarum with severe vomiting (more than 4 times a day), weight loss (≥3 kg), ketonuria and 36 pregnant women gestational age-matched, without nausea and vomiting attending our outpatient clinic for antenatal care were enrolled the study. Demographic data of the patients were registered. Blood samples for hemogram, serum electrolytes (sodium, potassium, chloride, and calcium), alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), creatine, thyroid stimulating hormone (TSH), free T3-T4, total T3-T4, and urine samples for ketonuria, stool samples for HpSA were studied. The data of both groups were compared.

Results: Eight Hyperemesis gravidarum patients (22.2%) and 1 control patient (2.8%) were established HpSA positive and it was statistically significant (p:0.037). There was no significant difference between Hyperemesis gravidarum and control subjects in terms of age, gestational week, parity, educational level, socioeconomic status and smoking. There was anemia in 5 Hyperemesis gravidarum patients, 4 of them were HpSA positive. HpSA positivity was more prevalent in Hyperemesis gravidarum patients with anemia (p=0.003). Severe vomiting (more than 4 times a day), heartburn, epigastric pain, duration of hospitalization (more than 4 days) and weight loss (≥5 kg) were not correlated to HpSA positivity.

Conclusion: The pregnant women with Hyperemesis gravidarum have a significantly higher prevalence of *Helicobacter pylori* compared with control subjects.

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Key words: Hyperemesis gravidarum, *Helicobacter pylori*, *Helicobacter pylori* stool Antigen

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Özet

Amaç: Hiperemezis gravidarumu ile *H. pylori* enfeksiyonu arasındaki ilişkinin araştırılması amaçlandı.

Gereç ve Yöntemler: Şiddetli kusma (≥4/gün), kilo kaybı (≥3kg) ve ketonürisi olan Hiperemezis gravidarum tanılı 36 gebe kadın ile bulantı ve kusması olmayan antenatal bakım için polikliniğe başvuran gebelik haftasına göre eşleştirilmiş 36 gebe kadın çalışmaya alındı. Hastaların demografik verileri kaydedildi. Hemogram, serum elektrolitleri, ALT, AST, BUN, kreatin, TSH, fT3, fT4, total T3, total T4, için kan örneği, ketonüri için idrar örneği, *H. pylori* antijeni (HPSA) için gaita örnekleri çalışıldı. Her iki grubun verileri karşılaştırıldı.

Bulgular: Hiperemezis gravidarum grubunda 8 hastada (%22.2), kontrol grubunda 1 hastada (%2.8) HPSA pozitif saptandı. Bu istatistiksel olarak anlamlı idi (p:0.037). Hiperemezis gravidarum ve kontrol grubu arasında yaş, gebelik haftası, parite, eğitim düzeyi ve sosyoekonomik düzey ve sigara kullanımı açısından istatistiksel olarak anlamlı bir fark bulunmadı. Çalışma grubunda anemisi olan (Hg<11g/dL) beş hasta vardı ve bu hastaların 4'ünde (%80) HPSA pozitif idi. Hiperemezis gravidarumda anemisi olan hastalarda daha yüksek oranda HPSA pozitifliği saptandı (p=0.003). Şiddetli kusma (>4/gün), mide yanması, epigastrik ağrı, hastanede kalma süresi (≥4gün), kilo kaybı ile HPSA pozitifliği arasında korelasyon saptanamadı.

Sonuç: Hiperemezis gravidarumu olan gebe kadınların önemli bir oranında *Helicobacter pylori* enfeksiyonunun ile birlikte olduğu görüldü.

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Anahtar kelimeler: Hiperemezis gravidarum, *Helicobacter pylori*, gaitada *Helicobacter pylori* antijeni.

Geliş Tarihi: 02 Şubat 2011 **Kabul Tarihi:** 02 Mart 2011

Introduction

Nausea and vomiting are the most common gastrointestinal diseases affecting women during pregnancy. It is encountered in approximately 80% of all pregnancies. Hyperemesis gravidarum is a clinical entity including nausea, vomiting which causes loss of weight, dehydratation, ketosis, electrolyte and acid-base imbalance and occasionally renal or hepatic failure. Hyperemesis gravidarum with the incidence of 0.3-1.5% of all live births is the most common indication of hospitalization during the first trimester (1).

Little is known about the etiology of nausea and vomiting in pregnancy. Recently, several studies performed in different populations revealed a significantly high prevalence of *Helicobacter pylori* among pregnant women with Hyperemesis gravidarum. The purpose of the study is to investigate the possible association between *Helicobacter pylori* infection and Hyperemesis gravidarum.

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Material and Methods

The present study was conducted prospectively between September 2009 and February 2010. The pregnant women with Hyperemesis gravidarum between 10 and 14 weeks of pregnancy were recruited in the study group. The inclusion criteria for the study group consisted of severe vomiting (more than 4 times a day), weight loss (≥3 kg) and ketonuria. Control subjects were selected randomly among the pregnant women without nausea and vomiting of similar gestational age, attending our outpatient clinic for antenatal care during the same period of time.

The study was approved by the medical ethical committee and informed consent was obtained from all cases. Demographic data of both groups were recorded. Patients who have thyroid disease, multiple pregnancy, infection, psychological and gastrointestinal disease and patients on anti-acid or antibiotic treatment were excluded. Gestational age was determined using the first date of the last menstrual period and confirmed by ultrasonography. Blood samples were obtained for hemogram, serum electrolytes (sodium, potassium, chloride, and calcium), alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), creatine, thyroid stimulating hormone (TSH), free T3-T4, total T3-T4. Urine samples for ketonuria and stool samples for HpSA were collected. Stool samples were tested for *Helicobacter pylori* stool antigen (HpSA) using HpSA enzyme-linked immunosorbent assay (*Helicobacter Antigen Quick Castte, GENERIC ASSAYS GmbH*, *Germany*) according to the manufacturer's instructions.

Statistical analysis was carried out using SPSS 16.0 for Windows (SPSS Inc, Chicago, III, USA) statistical software. Categorical variables were compared by Chi-square and Fisher's exact test. For continuous variables, descriptive statistics were calculated and reported as mean±standard deviation. Categorical variables were described using frequency distribution. Student-*t* and Mann-Whitney-U tests were used to compare mean scores of continued variables between two groups. P value of less than 0.05 was considered as statistically significant.

Results

Thirty-six pregnant women with Hyperemesis gravidarum and gestational age-matched 36 control subject were enrolled the study. The demographic data of the study and control groups were summarized in Table 1. There were no statistically significant differences between the study groups with Hyperemesis gravidarum and control groups in terms of age, gestational week, parity, body mass index, educational level and socio-economic state, and smoking. Hyperemesis gravidarum patients were found to have a significantly higher HpSA prevalence compared to control subjects (22.2% versus 2.8%; p=0.037). Mean duration of hospitalization in the Hyperemesis gravidarum group was 2.7±1.8 days. Their mean weight loss was 3.5±1.7 kg. The gastrointestinal symptoms, ketonuria, hematological, and biochemical values of 36 patients with Hyperemesis gravidarum is presented in Table 2. In Hyperemesis gravidarum patients, anemia (Hgb<11g/dL) was encountered in 5 patients, 4 of them were HpSA positive. The Hyperemesis gravidarum patients with anemia were found to have a significantly higher HpSA positivity compared to patients without anemia (p=0.003).

Heartburn, epigastric pain, duration of hospitalization (more than 4 days) and weight loss (≥5 kg) were not correlated to HpSA positivity (Table 3). There was no statistically significant relation between HpSA positivity and hyponatremia, hypokalemia, elevated alanine/ aspartate aminotransferase.

Table 1. The comparison of demographic data and the result of HpSA test between the study group pregnancies with Hyperemesis gravidarum and the control group pregnancies

Characteristics	Study groups with HG[a] (n=36)	Control groups (n=36)	p value
	mean± standard deviation	mean± standard deviation	
Age (year)	25.1±3.8	26.7±5.5	NS[b]
Gestational week	9.3 ±2.9	9.6±2.5	NS
Body mass index (kg/m^2)	22.9±4.7	24.1±3.6	NS
	Number (Percent)	Number (Percent)	
Nulliparity	22 (62.8%)	17 (47.2%)	NS
Primiparity	10 (27.7%)	16 (44.4%)	NS
Multiparity	3 (8.3%)	3 (8.3%)	NS
Education <8 year	11 (30.5%)	20 (55.6%)	NS
Low socio-economic level	4 (11.1%)	9 (25.0%)	NS
Smoking	0	4 (11.1%)	NS
HpSA[c]	8 (22.2%)	1 (2.8%)	0.037

[a]HG: Hyperemesis gravidarum
[b]NS: Non-significant, p>0.05
[c]HpSA: *Helicobacter pylori* Stool Antigen

Table 2. The gastrointestinal symptoms, ketonuria, hematological, and biochemical values of 36 patients with Hyperemesis gravidarum

Clinical and laboratory characteristics	Number	Percent
Epigastric pain	11	30.5
Heartburn	18	50
Serious ketonuria (3+, 4+)	10	27.7
Anemia (Hg<11 g/dl)	5	13.8
Hyponatremia(<136 mmol/l)	4	11.1
Hypokalemia (<3.6 mmol/l)	2	5.5
Elevated level of AST (>65 U/L)	3	8.3
Elevated level of AST (>37 U/L)	3	8.3

Table 3. The relations between HpSA positivity and serious vomiting, epigastric pain, heartburn, serious ketonuria anemia, hospital stay and weight loss

Characteristics	HpSA[a] (+) n:8		HpSA(-) n:28		p value
	number	percent	number	percent	
Epigastric pain	3	37.5	9	32.1	NS
Heartburn	4	50.0	15	53.6	NS
Severe ketonuria (3+, 4+)	3	37.5	8	28.6	NS
Anemia (Hg<11 g/dl)	4	50.0	1	3.6	0.003
≥4 days hospitalization	3	37.5	4	14.3	NS
≥5 kg weight loss	1	12.5	5	17.8	NS

[a]HpSA: *Helicobacter pylori* Stool Antigen
NS: Non-significant, p>0.05

Discussion

This study was planned to investigate the association between *Helicobacter pylori* infection and Hyperemesis gravidarum in our hospital based population.

During pregnancy, the increased level of steroid hormones and human chorionic gonadotrophin change the pH and motility of gastrointestinal tract. These changes favor activation of *Helicobacter pylori* infection. In addition, the altered humoral and cell-mediated immunity also contribute to the manifestation of a latent *Helicobacter pylori* infection (1). Observational studies indicated the possibility of an increased susceptibility to *H. pylori* infection in pregnancy (1, 2).

Most of the studies were case-control designs testing the hypothesis. The majority of them used *Helicobacter pylori* immunoglobulin G (IgG) antibody specific serologic tests to identify exposure to Hyperemesis gravidarum. The overall prevalence of seropositivity was between 65.0-91.5% in pregnant women with Hyperemesis gravidarum (3-8). Although some studies suggested a positive association between Hyperemesis gravidarum and *Helicobacter pylori* seropositivity (5-8), others could not find any association (3, 4). Serologic tests cannot differentiate acute and chronic infections of *Helicobacter pylori*, because seroconversion lasts up to months or years after recovery of the disease (9).

The HpSA test, which is an enzymatic immunoassay, detects bacterial antigens of an actual ongoing infection in the stool (10). There were a few studies which used HpSA tests to identify exposure to Hyperemesis gravidarum. The overall prevalence of HpSA was between 22.6-52.53 % in pregnant women with Hyperemesis gravidarum (11-15). The prevalence was found lower by HpSA tests than serologic tests. We determined that the pregnant women with Hyperemesis gravidarum have a significantly higher prevalence of *Helicobacter pylori* compared with control subjects (22.2%). The prevalence in Karadeniz et al's. study (22.6%) was nearest to the rate of our study (11). However, the difference in prevalence between study and control groups did not reach significant level due to the small number of subjects.

Some of the patients who had *Helicobacter pylori* seropositivity were asymptomatic. Asymptomatic patients were thought to have mild infection or ongoing seroconversion. The studies did not demonstrate any significant association between *Helicobacter pylori* seropositivity and gastrointestinal symptoms such as heartburn, epigastric pain or the duration and severity of symptoms and objective data which demonstrate severity of the disease (14, 16). HpSA tests are qualitative tests and show the presence of antigen. Our data also did not demonstrate any significant association between HpSA positivity and objective data which show severity of the disease such as weight loss, ketonuria and duration of hospitalization in pregnant women with Hyperemesis gravidarum. However, the study in which *Helicobacter pylori* infection was diagnosed by endoscopic evaluation and biopsy also revealed higher prevalence of *Helicobacter pylori* infection in pregnant women with severe Hyperemesis gravidarum than control subjects (17). Furthermore, the severity of *Helicobacter pylori* infection associated with the degree of gastric complaints and the symptoms of Hyperemesis gravidarum were suggested (17).

Various prevalence rates for *Helicobacter pylori* infection were reported depending on differences in the patient population studied. Although lower socio economic status was stated to be an important risk factor for *Helicobacter pylori* infection in pregnant women with Hyperemesis gravidarum (11), we did not find any association between low socioeconomic state and *Helicobacter pylori* infection. We also did not find a statistically significant relation between socio-economic status and Hyperemesis gravidarum. This may due to the low number of cases in both research and control groups. So, there is a need to carry out research with higher a number of patients.

Helicobacter pylori infection leads to iron deficiency anemia by decreasing iron absorption, bleeding of gastritis or to capture and use of iron by *Helicobacter pylori* (18). The positive relationship between iron deficiency anemia and *Helicobacter pylori* infection was also demonstrated in pregnant patients (19, 20). In this study, it was found that the pregnant women with Hyperemesis gravidarum and positive HpSA have higher prevalence of iron deficiency anemia.

Although the studies on this topic were considerably heterogenic, a recent review of the studies indicates that exposure to *Helicobacter pylori* was associated with a 3.32 times increased risk of Hyperemesis gravidarum (95% CI:2.25-4.90) (16). Our data also demonstrated that the prevalence of *Helicobacter pylori* was significantly higher in pregnant women with Hyperemesis gravidarum compared with control subjects. The limitation of the study was the small number of cases. Understanding the role of *Helicobacter pylori* infection in the pathogenesis of Hyperemesis gravidarum and why some patients were asymptomatic need more endoscopic studies.

Conflict of interest

No conflict of interest was declared by the authors.

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Histopathological analysis of the placental lesions in pregnancies complicated with IUGR and stillbirths in comparison with noncomplicated pregnancies

İUGR ve ölü doğumlarla komplike olmuş gebeliklerde plasental lezyonların histopatolojik analizi ve nonkomplike gebeliklerle karşılaştırılması

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Abstract

Objective: Placental factors and hypoxemia are the keys to intrauterine growth restriction (IUGR) and stillbirth. The aim of the study is to analyze histological changes in placentas of IUGR fetuses in pregnancies with no apparent etiologic factor and unexplained intrauterine fetal deaths.

Material and Methods: A total of 110 placentas were collected; 26 placentas of IUGR fetuses with no apparent cause, 58 placentas from unexplained intrauterine deaths over 20 weeks of gestation, and 26 placentas from uncomplicated pregnancies who delivered a healthy live baby. Microscopic examinations of placentas were performed for histopathological analyzes.

Results: Gestational age at delivery was 33.67 ± 4.37 weeks, 29.15 ± 8.36 weeks, and 39.0 ± 1.52 weeks in women in group I, group II and group III, respectively ($p < 0.01$). Infarction and intervillous thrombosis are significantly more frequent in placentas of Group I and group II. Chronic villitis occurred in 69%, 63% and 30% of group I, group II, and group III, respectively. Placental intravascular thrombi (Group I, 31% and group II, 26%), perivillous fibrin deposition and fibrinoid necrosis (65% in Group I and 53% in group II), infarction, intervillous thrombosis, chronic villitis, hemorrhagic endovasculitis, placental intravascular thrombi, perivillous fibrin deposition, fibrinoid necrosis, erythroblastosis and villous edema were found in the study group.

Conclusion: The results reported here indicate that a relationship exists between morphological changes in the placentas of IUGR and intrauterine fetal deaths (J Turkish-German Gynecol Assoc 2011; 12: 75-9)

Key words: Stillbirth, intrauterine growth restriction, histopathology, placenta, light microscopy

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Özet

Amaç: Plasental faktörler ve hipoksemi, intrauterine gelişme geriliği (İUGR) ve intrauterine eksituslar da ana nedenlerdir. Çalışmanın amacı etyolojik nedeni tespit edilemeyen IUGR'li fetüslerin ve açıklanamayan intrauterine ölümlerin plasentalarındaki histolojik değişikliklerin analizini yapmaktır.

Gereç ve Yöntemler: Bilinen bir sebebi olmayan 26 İUGR'lı fetüsün plasentası, 20 haftanın üzerinde 58 açıklanamayan intrauterine eksituslu fetüsün plasentası ve 26 sağlıklı nonkomp-like canlı yenidoğanın plasentası olmak üzere toplam 110 plasenta toplandı. Histopatolojik analiz için plasentaların mikroskopik incelemesi yapıldı.

Bulgular: Grup I, grup II ve grup III'ün doğum anındaki gestasyonel haftaları, sırasıyla, 33.67 ± 4.37 hafta, 29.15 ± 8.36 hafta ve 39.0 ± 1.52 hafta idi. İUGR'li fetüsler ve ölü bebek-lerin plasentalarında enfarktüs ve intervillöz trombozis anlamlı olarak daha fazlaydı. Kronik villitis, grup I, Grup II ve grup III'de sırasıyla %69, %63 and %30 oranında idi. Çalışma grubunda plasental intravasküler trombüs (Grup I'de %31, grup II'de %26), perivillöz fibrin depozitleri ve fibrinoid nekroz (Grup I'de %65 ve grup II'de %53) ve ayrıca enfarktüs, in-tervillöz tromboz, kronik villitis, hemorajik endovaskülitis, plasental intravasküler thrombus, perivillöz fibrin depozitleri, fibrinoid nekrozis, eritroblastozis ve villöz ödem varlığı tespit edildi.

Sonuç: Çalışmamızın sonuçları, intrauterin gelişme gerilikli fetüsler ile intrauterine ölüm olan fetüslerin plasentalarındaki morfolojik değişiklikler arasında ilişki olduğunu göstermektedir.

(J Turkish-German Gynecol Assoc 2011; 12: 75-9)

Anahtar kelimeler: Ölü doğum, intra uterin gelişme geriliği, histopatoloji, plasenta, ışık mikroskopi

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Introduction

Fetal growth and viability depends on the maternal supply of nutrients and oxygen through the placenta into the umbilical circulation. Placental factors and hypoxemia are keys to intrauterine growth restriction (IUGR) and fetal death. IUGR is a condition associated with placental insufficiency (1). Adaptive changes in IUGR may fail at some point, leading to fetal death. Conditions resulting in placental dysfunction can be recurrent. The placental complications may manifest in different ways in different pregnancies; IUGR in one pregnancy and fetal death in another pregnancy (2, 3). Faulty placentation has been linked to the pathogenesis of preeclampsia, preterm births, abortions, IUGR and intrauterine death (4, 5). IUGR may be caused by fetal, placental, or maternal factors. These factors are usually multiple and overlapping.

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The most common causes of stillbirth between 24 and 27 weeks of gestation are infection, abruption and fetal anomalies. Unexplained stillbirth is a fetal death that cannot be attributed to an identifiable fetal, placental, maternal, or obstetrical etiology, and accounts for 60% of fetal deaths (6, 7). The most frequent cause of stillbirth after 28 gestational weeks is unexplained fetal loss. Unexplained fetal loss includes stillbirths associated with growth restriction and placental abruption (6). Information on placental abnormalities may reveal the presence of chronic fetal insults and allow their differentiation from acute (peripartum) stresses (8). The aim of the present study is to analyze histological changes in placentas of IUGR fetuses, in placentas of unexplained intrauterine death fetuses and in uncomplicated pregnancies.

Material and Methods

Patients

A total of 110 placentas were collected; 26 placentas of IUGR fetuses (Group I) with no apparent cause, 58 placentas from unexplained intrauterine deaths over 20 weeks of gestation (Group II) and 26 placentas from uncomplicated term pregnancies in whom a healthy live baby was delivered (Group III). Gestational ages were 33.67±4.37 weeks, 29.15±8.36 weeks, and 39.0±1.52 weeks in women with group I, group II and group III, respectively ($p<0.01$). (Table 1). The study was approved by the Institutional Review Board and informed consent has been obtained from all the patients.

Multiple pregnancies, fetuses with a chromosomal anomaly or congenital anomaly, and fetuses with hydrops fetalis were excluded from this study. Maternal exclusion criteria were preeclampsia, diabetes mellitus, infection, placental tumors and trauma. In addition, women with antenatal hemorrhage (placental abruption, vasa previa and placenta previa), and women diagnosed with any systemic disorder were excluded from the study. Fetal death was defined as a lack of fetal heart activity diagnosed by ultrasound examination. IUGR was defined on the basis of an estimated fetal weight of less than the third percentile for gestational age (9), reduced amniotic fluid volume or Doppler ultrasound of the umbilical artery demonstrating absent end diastolic flow velocity. The diagnosis of IUGR was established by serial ultrasonographic examination of fetal biometric measurements (weight, biparietal diameter, head circumference, femur length, and abdominal circumference).

Sample collection and histological analyses

Immediately after delivery, placentas were fixed with 10% formalin for 24 hours and processed for routine paraffin embeding. Multiple random samples were taken from each placenta from a macroscopically normal central portion of the placenta, including two samples of umbilical cord (one close to the distal end and one from within 10 cm of the insertion on the chorionic plate), two samples of the extra placental membranes (one from the edge of the site of rupture when identifiable, one from a membrane roll extending from the site of rupture to the placental margin), and at least one sample of chorionic plate consisting of chorionic vessels. For all cases, 4 sections of 4 μm thickness were cut on a rotary microtome from the middle of each specimen, and were mounted on clean gelatinized slides, and stained with H & E. Sections were analyzed by light microscopy and in each of the placental slides, the 10 smallest terminal villi (each less than 80 μm in diameter) in 10 different fields were examined (magnification ×400). The observations were recorded by digital camera (Olympus® DP70, Japan). Microscopic evaluation of placentas included non-inflammatory changes of amnion, acute inflammatory changes, infarction, intervillous thrombosis, chorionic villitis, hemorrhagic endovasculitis, placental intravascular thrombi, trophoblast degenerative knots, perivillous fibrin deposition and fibrinoid necrosis, erythroblastosis and villous edema (Table 2) (8). (Fig. 1, 2). The researchers examining the tissue sections were blinded to the clinical details of the cases.

Table 1. Demographic and obstetric characteristics of patients

	Group 1	Group 2	Group 3	p
Mean of age	26.5±6.05	26.77±6.08	25.8±4.94	p>0.05
Gravida	2.27±1.64	2.60±1.90	2.58±1.38	
Parity	0.81±1.2	1.1±1.35	1.66±1.08	
Gestational age at delivery	33.67±4.37	29.15±8.36	39.0±1.52	p<0.01

Statistical analysis

Statistical analyses were performed using the chi-square test for categorical variables. Continuous variables were compared by the Student's t-test. $p<0.05$ was considered significant. All computations were carried out with SPSS software 13.0 (SPSS inc. Chicago, Illinois, USA).

Results

Means of age, gravida and parity of patients were similar in group I, group II and group III. Gestational age at delivery was 33.67±4.37 weeks, 29.15±8.36 weeks, and 39.0±1.52 weeks in women in group I, group II and group III, respectively ($p<0.01$). (Table 1).

Microscopic examination revealed no significant difference between the three groups in respect to non-inflammatory changes of amnion, acute inflammatory changes and trophoblastic degenerative knots ($p>0.05$). Statistically significant light microscopy findings are shown in Table 3. Infarction and intervillous thrombosis are significantly more frequent in placental cotyledons of group I and group II fetuses. The most common associated pathologic condition with infarction and thrombosis was chronic villitis in both groups (Fig. 2).

Chorionic villitis occurred in 69%, 63% and 30% of group I, group II, and group III, respectively. There was one case (3.8%) of hemorrhagic endovasculitis in the control group ($p<0.05$). Thirty one percent of placentas in group I and 28% of placentas in group II had hemorrhagic endovasculitis. The volume of affected tissue was similar in group I and group II.

There was no case of intravascular thrombi in the control group. However, 31% and 26% of placentas of group I and group II had placental intravascular thrombi ($p<0.01$). Thrombi were only detected in chorionic vessels (Fig. 2). The number of affected vessels is shown in Table 3. Perivillous fibrin deposition and fibrinoid necrosis was more common in group I (65%) and group II (53%) compared to group III (11%, $p<0.01$). (Fig.1). The incidence of erythroblastosis and villous edema were significantly higher in the group I and group II than the control one ($p=0.01$).

Table 2. Parameters for microscopic evaluation of placenta (8) (Figure 1 and 2)

Non-inflammatory changes of amnion
amnion nodosum
meconium histiocytosis
Acute inflammatory changes
pattern of spread of organism extra amniotic or intraamniotic
maternal vs. fetal inflammatory response
discordance btw. maternal-fetal inflammatory responses
Infarction, intervillous thrombosis
age
associated pathologic condition [chorionic villitis, decidual thrombosis]
Chorionic villitis
type of cellular infiltrate
presence of intervillositis
volume of affected tissue [grade]
associated features [vasculitis, intravascular thrombi, hemorrhagic endovasculitis]
Hemorrhagic endovasculitis
type of vessel[s] involved [chorionic, major stem]
volume of affected tissue [grade]
associated changes[chronic villitis, placental intravascular thrombi]
Placental intravascular thrombi
type of vessel[s] involved [chorionic, major stem]
number of involved vessels
associated features [vasculitis, hemorrhagic endovasculitis]
Trophoblast degenerative knots
proportion normal, increased or decreased relative to gestational age
diffuse or focal
associated changes [abnormal villous fibrosis or vascularity, decidual vasculopathy]
Perivillous fibrin deposition and fibrinoid necrosis
proportion normal, increased or decreased relative to gestational age
diffuse or focal
associated changes [X-cell proliferation, acute inflammation, villous and decidual pathologic conditions]
Erythroblastosis and villous edema
proportion of nucleated-anucleate erythrocytes relative to gestational age
proportion of affected villi [edema]
severity of edema [diameter of affected relative to gestational age]
associated changes [acute or chronic inflammation, decidual vasculopathy, abruptio placenta]

Discussion

Placental pathology in intrauterine growth restriction and fetal demise after 20 weeks of gestation are investigated in this study. In this analysis, we intentionally did not include any women with a particular clinical risk factor (such as preeclampsia, or gestational diabetes) along with intrauterine death or intrauterine growth restriction. Although gestational ages of the groups were different, this study provides important results in placentas of IUGR fetuses and intrauterine death fetuses. Amnion nodosum is commonly regarded as a placental hallmark of severe and prolonged oligohydramniosis (10). Meconium histiocytosis reflects the duration of exposure to meconium before delivery. Acute placental inflammation is usually related to clinical situations such as premature rupture of the membranes and preterm delivery. Although these lesions are associated with chronic uterine vascular insufficiency, neither of them was found to be significantly different among the groups.

Placental infarction can be observed in many normal pregnancies. It is usually of no significance unless it affects more than 10-20% of the placental volume (11). The existence of a relation between fetal hypoxia and placental infarction has been shown (12). In the present study, placental infarction was detected in 58% and 62%, 4% of group I, group II and group III, respectively. Intervillous thrombosis was only observed in placentas of intrauterine death fetuses and may be feature of intrauterine death (Fig. 2).

It is reported that fetal thrombotic vasculopathy and fetal stem vessel thrombosis are common findings in women with adverse pregnancy outcomes (13). In the present study, no major stem occlusion was found in the placentas of group I or group II fetuses. Thirty-one percent of group I fetuses and 26% of group II fetuses had chorionic vessel occlusion. However, none of the placentas from healthy babies had placental intravascular thrombi ($p<0.05$). In contrast, occlusion of <50% of the lumen are not uncommon at term and this is reported to be a preparation for parturition (8).

Table 3. Statistically significant light microscopy findings in placentas of fetuses with intrauterine growth restriction (Group I) and placentas of stillbirths (Group II), and healthy live babies (Group III) (Student's t-test)

Comparision of Histopathological Analyses	Group I n [%]	Group II n [%]	Group III n [%]	P value
Infarction, intervillous thrombosis	15 [58]	39 [67]	1 [4]	<0.01
Infarction	15 [57.7]	36 [62]	1 [4]	
intervillous thrombosis	- -	2 [3]	-	
Infarction and intervillous thrombosis	- -	1 [2]	-	
associated pathologic condition	14 [54]	34 [59]	1 [4]	
chorionic villitis	10 [39]	21 [36]	1 [4]	
decidual thrombosis	4 [15]	9 [16]	- -	
chorionic villitis and decidual thrombosis	- -	4 [7]	- -	
Chorionic villitis	18 [69]	37 [64]	8 [31]	<0.5
Hemorrhagic endovasculitis	8 [31]	16 [28]	1 [4]	<0.5
type of vessel[s] involved				
chorionic	8 [31]	15 [26]	1 [4]	
major stem	- -	1 [2]	- -	
volume of affected tissue[grade]				
grade 1	1 [4]	4 [7]	1 [4]	
grade 2	6 [23]	10 [17]	-	
grade 3	1 [4]	2 [3]	-	
Placental intravascular thrombi	8 [31]	15 [26]	- -	<0.5
type of vessel[s] involved				
chorionic	8 [31]	15 [26]		
major stem	- -	- -		
number of involved vessels				
1	2 [8]	2 [3]		
2	5 [19]	12 [21]		
more than 2	1 [4]	1 [2]		
Perivillous fibrin deposition / fibrinoid necrosis	17 [65]	31 [53]	3 [12]	<0.01
Perivillous fibrin deposition	16 [62]	29 [50]	- -	
fibrinoid necrosis	1 [4]	2 [3]	3 [12]	
Perivillous fibrin deposition with fibrinoid necrosis	- -	- -	- -	
proportion relative to gestational age				
normal	8 [31]	16 [28]	2 [8]	
increased	8 [31]	12 [21]	1 [4]	
decreased	1 [4]	3 [5]	- -	
diffuse or focal				
diffuse	12 [46]	18 [31]	2 [8]	
focal	5 [19]	13 [22]	1 [4]	
Erythroblastosis / villous edema	12 [46]	28 [48]	7 [27]	<0.01
Erythroblastosis	5 [19]	5 [9]	- -	
villous edema	3 [12]	7 [12]	6 [23]	
Erythroblastosis and villous edema	4 [16]	16 [28]	1 [4]	

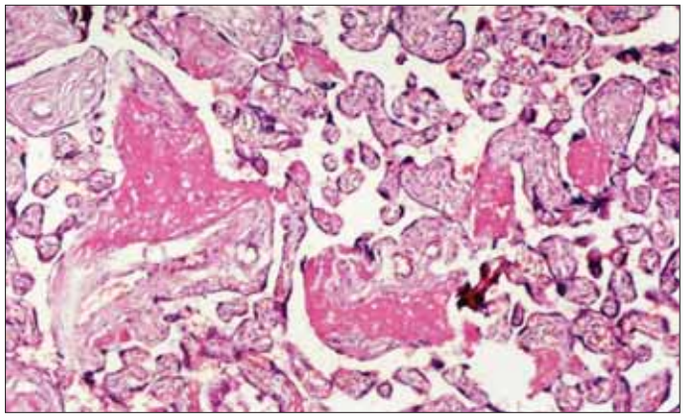

Figure 1. Histopathological section of perivillous fibrin deposits in placenta of IUGR fetus. H&E, x400

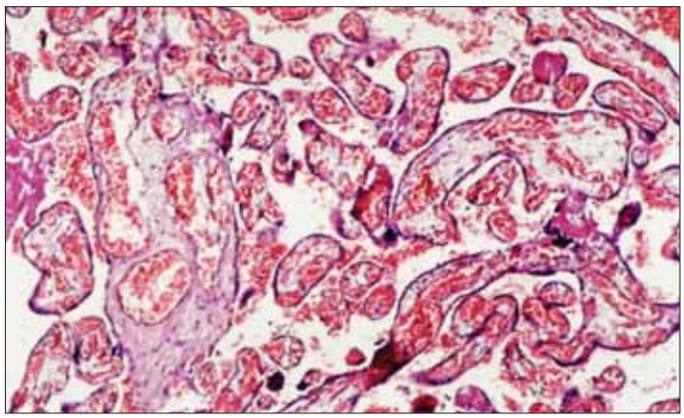

Figure 2. Histopathological section of villous thrombosis in intrauterine death fetus pla-centas. H&E, x400

Large areas of the placenta may be affected by different lesions without any obvious danger to the fetus. It is evident that the number of different types of lesions that are seen is far more strongly associated with fetal growth restriction or intrauterine death than the presence or severity of any one lesion (14). It is more likely that accumulation of placental injury for a sufficient duration leads to IUGR and fetal death (15).

Fibrinoid necrosis and perivillous fibrin deposition are associated with IUGR, autoimmune processes, infection, toxic insult, a known abnormal host-placenta interaction, genetic disorders and confined placental mosaicism (16-19). Perivillous fibrin deposition and fibrinoid necrosis were significantly more frequent in group I and group II compared to group III (Fig. 1). The presence of excess erythroblasts in the placental circulation suggests a response to hematopoietic stress (14). Villous edema may be observed in several situations like hydrops fetalis and acute intraamniotic infection (20). In this study erythroblastosis together with villous edema were found to be significantly more common in complicated pregnancies.

Infarction, intervillous thrombosis, chorionic villitis, hemorrhagic endovasculitis, placental intravascular thrombi, perivillous fibrin deposition, fibrinoid necrosis, erythroblastosis and villous edema were found to be the types of lesions that cause a normal fetus to become growth restricted or die (Fig. 1, 2). However, the extent of these lesions and clinical outcomes could not be clearly defined. The results reported here indicate that a relationship exists between morphological changes in the placentas of IUGR fetuses and intrauterine death fetuses.

Conflict of interest

No conflict of interest was declared by the authors.

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Shortening the induction delivery interval with prostaglandins: a randomized controlled trial of solo or in combination

Prostaglandinler ile indüksiyon-doğum aralığının kısaltılması: tek başına veya kombine olguların randomize kontrollü karşılaştırılması

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Abstract

Objective: To compare the efficacy and safety of misoprostol alone with dinoprostone followed by misoprostol, all inserted intravaginally in induction of labor at term and the obstetrical outcome.

Material and Methods: A pilot study comprising 111 primigravidae, >37 gestational weeks with singleton pregnancy in cephalic presentation having an unfavorable Bishop score admitted for labor induction, were considered and randomly allocated into two groups. In group I (n=55) with intravaginal 25mcg misoprostol 4 hourly (six doses at the most) and and group II (n=56), with dinoprostone 0.5mg followed eight hours later by 25mcg misoprostol induction to vaginal delivery time was found to be significantly different, being 14.8 h in group- I and shorter in group-II with a mean of 11.6 h. Vaginal delivery rates within 12 h (groups-I and –II: 47.2%, as compared to 60.7%, respectively) were found to be higher with dinoprostone-misoprostol induction, as well as vaginal delivery rates in 24 h, 80.0% and 91.1%. The need for oxytocin augmentation was more frequent in the misoprostol than in the dinoprostone-misoprostol group, (61.8%, and 39.3%), and all these observations were statistically significant. Abnormal foetal heart rate pattern occurred more frequently (18.2%) in group-I in contrast to 5.3% in group-II, as was the incidence rate of (18.2%) who had passage of meconium in group-I, this rate being significantly different from group-II having meconium passage in 3 cases, a rate of 5.3%.

Conclusion: Using dinoprostone followed by vaginal misoprostol is safe and effective for induction of labor with less need for oxytocin augmentation and shorter induction delivery interval.

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Key words: Labor induction, prostaglandin, intravaginal

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Özet

Amaç: Termde doğumun indüklenmesinde intravajinal olarak kullanılan tek başına misoprostol ile misoprostolün izlediği dinoprostonun etkililik ve güvenliliklerini ve obstetrik sonucu kıyaslamak.

Gereç ve Yöntemler: >37 gebelik haftasında, sefalik prezentasyonda tekli gebeliği olan ve uygun olmayan Bishop skoru olup doğum indüksiyonu için başvuran 111 ilk gebeliği içeren bir pilot çalışma düşünüldü ve randomize olarak iki gruba ayrıldı. İntravajinal 4 saatte bir (en fazla altı doz) 25 mcg misoprostol alan grup I (n=55) ve 0.5 mg dinoproston ve takiben sekiz saat sonra 25 mcg mizoprostol alan grup II (n=56)'de vajinal doğum indüksiyon süresi anlamlı olarak farklı bulundu; grup I'de 14.8 saat, grup II'de ortalama 11.6 saat ile daha kısa olarak. 12 saat içinde vajinal doğum oranları (grup I ve II'de sırasıyla %47.2'ye kıyasla %60.7) dinoproston-misoprostol indüksiyonu ile daha yüksek bulundu; 24 saatteki vajinal doğum oranları da sırasıyla %80.0 ve %91.1 idi. Oksitosin ilavesi gereksinimi misoprostolde dinoproston-misoprostol grubundan daha sıktı (%61.8 ve %39.3) ve bütün bu gözlemler istatistiksel olarak anlamlıydı. Anormal fötal kalp hızı paterni grup-I'de grup II'ye kıyasla daha sıklıkla görüldü (sırasıyla %18.2 ve %5.3); benzer şekilde grup I'de mekonyum pasajı olanların insindans hızı (%18.2), 3 vakada mekonyum pasajı olan grup II'den (%5.3) anlamlı olarak farklı idi.

Sonuç: Vajinal misoprostol uygulamasının takip ettiği dinoproston kullanımı; oksitosin ilavesine daha az gerek duyulması ve daha kısa indüksiyon-doğum aralığı ile doğumun indüksiyonu için güvenilir ve etkilidir. (J Turkish-German Gynecol Assoc 2011; 12: 80-5)

Anahtar kelimeler: Doğum indüksiyonu, prostaglandin, intravajinal

Geliş Tarihi: 08 Aralık 2010 **Kabul Tarihi:** 27 Mart 2011

Introduction

Induction of labor is intended to achieve vaginal delivery by stimulating uterine contractions before its spontaneous onset, and is commonly performed in clinical practice. Generally, it is considered as a therapeutic option when the benefits of expeditious delivery outweigh the risks of continuing the pregnancy (1). Methods of induction of labor include administration of oxytocin, prostaglandin analogues and smooth muscle stimulants such as herbs or castor oil replacing the age-old mechanical methods such as digital stretching of the cervix and sweeping of the membranes, hygroscopic cervical dilators, extra-amniotic balloon catheters, artificial rupture of membranes and nipple stimulation (2).

Prostaglandins have been in use for cervical ripening and induction of labour since the 1970s and have been shown to be of benefit to reduce the need of caesarean section when the cervix is unfavorable (3). The goal of administration of prostaglandins in the process of induction of labour is to achieve cervical ripening before the onset of contractions (4).

Prostaglandin analogues, dinoprostone (PGE_2) and misoprostol (PGE_1), are widely used in induction of labor practice for ripening the cervix and stimulating uterine contractions in order to achieve vaginal delivery (5). Prostaglandin E_2 has now become

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the drug of choice in well-resourced settings for cervical ripening and induction of labor, but it is expensive, unstable and requires refrigerated storage. The advent of misoprostol was considered as a revolution in the field of labour induction, but concerns exist regarding an increased incidence of fetal distress (6), meconium-stained liquor and hyperstimulation (7).

This study, probably the first of its kind, was undertaken to compare the efficacy of vaginal misoprostol alone with dinoprostone followed by misoprostol introduced vaginally for third trimester cervical ripening and induction of labour.

Material and Methods

A study based on 111 primigravidae, ≥37 gestational weeks with singleton fetus in cephalic presentation admitted for labor induction, was conducted during the period between 19th Nov. 2009 to 5th Jun. 2010 in the Maharishi Markendeshwar Institute of Medical Sciences and Research, Mullana, (Ambala, India).

The institutional ethical committee approved the project and written informed consent was obtainedfrom each participant. Being a pilot study, sample size calculation was not required.

Those included were healthy primigravida patients aged 20-30 years with a singleton fetus in cephalic presentation ≥37 weeks, having average adequate gynaecoid pelvis and no clinical evidence of cephalo- pelvic disproportion. Other criteria for eligibility included a relaxed uterus, Bishop score ≤4, reactive non-stress test, estimated fetal weight between 2500-3500 grams. Exclusion criteria considered were women with a previously scarred uterus, poor fetal surveillance scores, contraindications for the application of prostaglandins (asthma, glaucoma, clinical evidence of cardiopulmonary, hepatic or renal disease), antepartum haemorrhage, significant maternal and fetal compromise, active genital herpes simplex infection or those who failed to supply the written informed consent.

One hundred and forty two women were enrolled for the study but 31 of these did not meet the inclusion criteria (non-cephalic presentation n=9, favorable Bishop score n=8, non-compliance to written informed consent n=7, severe intra-uterine growth restriction n=3, major degree placenta praevia n=2, signs of fetal compromise n=2). Allocation of the rest of the 111 patients was done into two groups by using a randomized table of numbers. Index cards with the random assignment were prepared and placed in sealed envelopes, a researcher who was blinded to the baseline examination findings opened the envelope, at the time of induction of labor, and the proceedings were done according to the group assignment.

All the patients were admitted to the hospital from the beginning of the labor induction process and were subjected to detailed history taking, a complete physical examination and investigations, including a complete haemogram, random blood sugar, blood urea, serum creatinine (as a preparation for emergent cesarean section, if needed), an obstetric ultrasonography and a cardiotocography.

The baby's condition was assessed by clinical assessment of growth and amniotic fluid volume and also the mother's report of fetal movements. Prior to starting the induction process, the woman was carefully assessed for evidence of fetal compromise by electronic fetal heart rate monitoring for 30 minutes. The non-stress test (NST) to ensure the well-being of the fetus was performed for each patient at the time of recruitment and admission to the hospital and one hour before the application of the prostaglandin. After the reassessment of the cervical Bishop score, either 25 mcg misoprostol, or 0.5 mg dinoprostone was administered in the posterior vaginal fornix as per the group allocation.

As depicted in Figure 1, two groups were assigned: group-I (n=55) where 25 mcg misoprostol was used intravaginally every 4 hours (six doses at the most) and group-II (n=56) in whom the process was initiated with intravaginal dinoprostone 0.5 mg followed eight hours later by 25 mcg misoprostol inserted in the same way every 4 hours (four doses maximum). In group-I, 25 mcg misoprostol tablet (available of late) was inserted in the posterior fornix following the vaginal examination; thereafter the patients were continuously monitored for one hour and then allowed to ambulate. Monitoring was continued intermittently until the next scheduled dose after 4 hours, unless the membranes ruptured or fetal heart rate tracing was not reactive. A maximum of six doses were given, until an adequate contraction pattern (three or more contractions within 10 min), cervical ripening (Bishop score ≥7 or dilation ≥3 cm), spontaneous membrane rupture occurred or 24 h had elapsed. Artificial rupture of membranes was generally performed when it was clinically safe-cervix was 80% effaced or 3 cm dilated or when dilation was ≥4 cm regardless of the effacement.

Once started, the woman was monitored closely for fetal heart rate (FHR) and uterine activity, as well as the mother's vital signs were constantly monitored for 60 min after each dose of misoprostol, and every 30 min from the onset of uterine contractions. Electronic fetal monitoring was used from the time at which regular contractions commenced after every 3 min or more.

At the time of each planned misoprostol dose, the woman was clinically reassessed. If there were 0-2 contractions every 10 minutes, then a further dose of misoprostol was given and if there were 3 or more uterine contractions in 10 minutes, then clinical judgement was used to assess the best way of continuing the induction to achieve optimal contractions (3 strong contractions in 10 minutes). Wherever required, intravenous oxytocin infusion was commenced, but not less than 4 hours after the last dose of vaginal misoprostol.

In group-II patients, 0.5 mg of dinoprostone in gel form was instilled intravaginally in the posterior fornix and continuous monitoring was done for one hour before allowing the patient to ambulate. Eight hours later, misoprostol protocol was started/followed in the same manner as in group-I, the difference being the number of doses reduced to four. In both the groups: oxytocin infusion was started following spontaneous rupture of membranes without an ensuing adequate contraction pattern or no change in cervical dilatation for 2 h at ≥4 cm. Oxytocin was administered according to the protocol: initiated at 4 mU/min and increased 4 mU/min every 30 min to a maximum of 16 mU/min until adequate effective uterine contractions were established. Once the patient was in active labor, further monitoring and conduction of delivery was done by a consultant obstetrician. If the Bishop score was unchanged and inadequate uterine contractions persisted 4 h after the last dose of misoprostol, the case was labelled as a failed induction and caesarean section was performed. Neonates were evaluated for Apgar scores.

To observe uniformity, right medio-lateral episiotomy was employed at the instance of crowning of the vertex in all the cases of vaginal delivery and the placenta was delivered by modified Brandt- Andrew's technique (controlled cord traction) at the clinical confirmation of its separation following delivery of the baby.

The vaginal administration of prostaglandins was performed by one of the resident doctors on duty, who was not involved

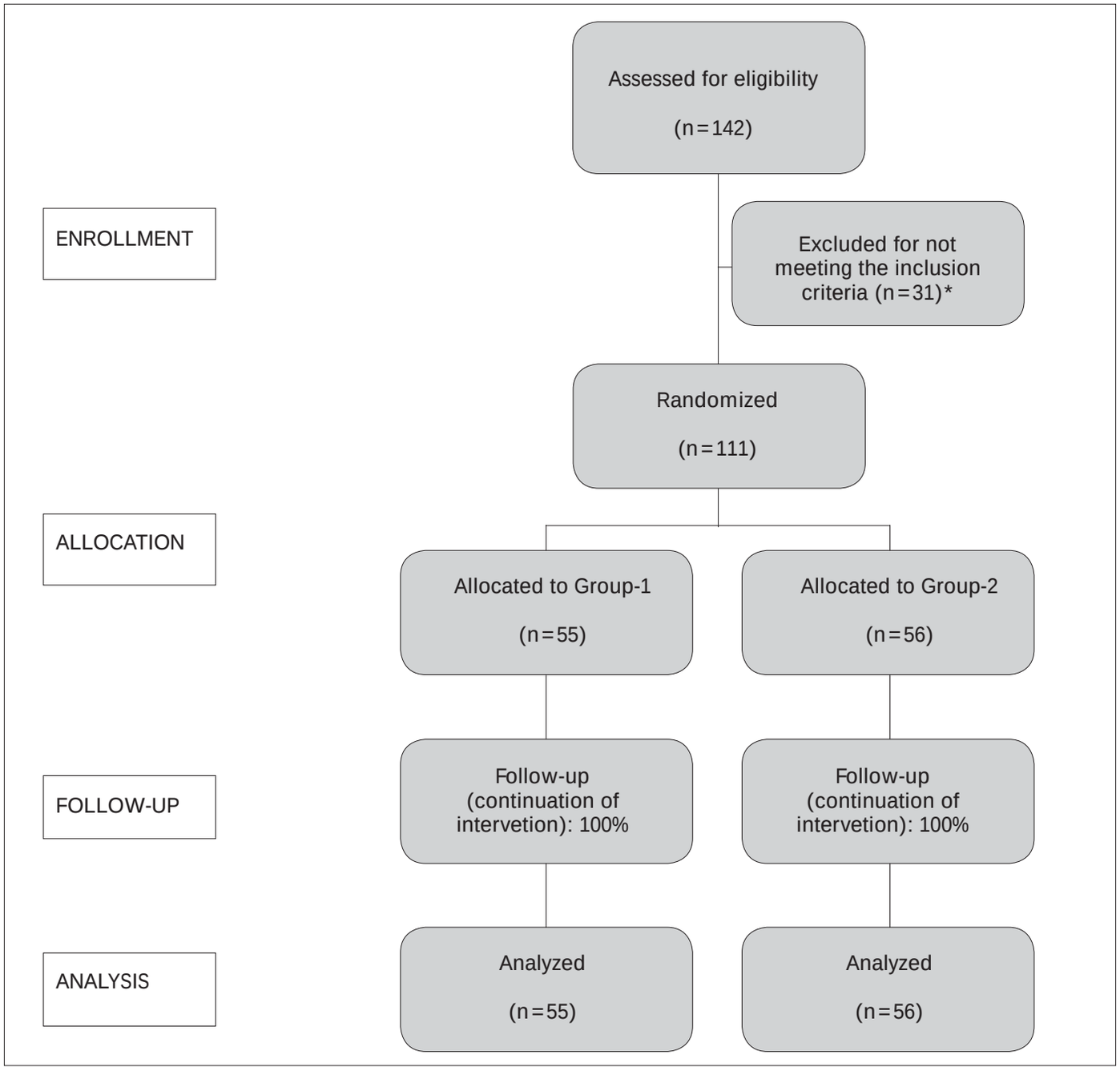

Figure 1. The CONSORT Flow Diagram showing the progress of participants at each stage of the study
***(non-cephalic presentation n=9, favorable Bishop score n=8, non-compliance to written informed consent n=7, severe intra-uterine growth restriction n=3, major degree placenta praevia n=2, signs of fetal compromise n=2)**

in managing these women in labor or delivery. The study was blind, since the patients were not aware of which type of medication was used, and the deliveries were then performed by a consultant obstetrician blinded to the induction regimen utilized. Uterine tachysystole was defined as more than five contractions per 10 minutes for at least 20 minutes, uterine hypersystole/hypertonus as when one contraction lasted more than 2 minutes and hyperstimulation syndrome as the presence of non-reassuring FHR tracing combined with either tachysystole or hypertonus (8). Non-reassuring FHR patterns were defined as persistent or recurring episodes of severe variable decelerations, late decelerations, prolonged fetal bradycardia or a combination of decreased beat-to-beat variability and a decelerative pattern (9). Tocolytic agents were kept on hand to manage such eventualities where possible and were to be given subcutaneous terbutaline 250 μg as a single dose.

All the observations were given consideration in both the groups. The data were tabulated and analyzed. Summary statistics such as mean, standard deviation were estimated. Chi-square and Fisher's exact tests were used for categorical data.

For continuous data such as age, weight, student's t-test was utilised. The significance was seen after applying log transformation and statistical significance was set at $p<0.05$.

Observations

The outcome measures considered were both obstetrical and neonatal. The primary outcome measures were time from induction to delivery and incidence of vaginal delivery within 12 and 24 hours and the Caesarean Section rate, the incidence of uterine tachysystole, abnormal (FHR) tracings. The secondary outcomes were the need for oxytocin augmentation, the incidence of meconium stained amniotic fluid, maternal morbidity, neonatal Apgar scores and the admission to neonatal intensive care within 24 hours.

As depicted in Table 1, the two groups were comparable in terms of patient age, weight, height and indication for induction (post-date pregnancy 40.0% and 39.3%, social 23.6% and 25.0%, oligohydramnios 16.4% and 14.3%, pregnancy induced hypertension 20.0% and 21.4% in group-I and group-II, respectively). Gestational age and the preinduction Bishop score in the misoprostol group-I were also comparable to the dinoprostone and misoprostol combination group-II.

There was a significant change in the Bishop score in the two groups after eight hours of initiating the process of induction, that is, after two doses of 25 mcg misoprostol in group-I and after 0.5 mg of dinoprostone in group-II, being 5.3 (range 4-7) compared to 7.4 (range 6-8), meaning thereby that the cervix became favourable in all cases in group-II.

The induction-delivery interval in the two groups was significantly shorter in group-II, with even less need for a second or third dose in the said group. A salient observation to be mentioned is that five of the patients in group-II had onset of active labor with dinoprostone alone, obviating the need for any misoprostol in these subjects. With the dinoprostone-misoprostol combination, more women delivered within 12 hours of induction (47.2%, n=26 vs 60.7%, n=34) and this difference even persisted at 24 h (80.0%, n=44 in group-I, in significant contrast to 91.1%, n=51 in group-II). Moreover, the need for oxytocin augmentation in labor was also different, being remarkably reduced in group-II. In addition, spontaneous rupture of the membranes occurred with almost the same frequency in both groups. Other significant findings, though adverse in nature, were observed more frequently in group-I: abnormal uterine actions-uterine tachysystole (10.9%, n=6 vs 3.6%, n=2), uterine hyperstimulation (1.8% vs 0.0%), and meconium stained amniotic fluid (18.2%, n=10 vs 5.3%, n=3) as did abnormal heart rate tracing (18.2%,n=10 vs 5.3%, n=3) (Table 2).

In both the groups, although the majority of women had a vaginal delivery (more in group-II), there was a statistically significant difference between the two groups with regard to the Caesarean Section rate, being 20.0%, n=11 in group-I as compared to 8.9%, n=5 in group-II (Table 3). There were no uterine ruptures or other major maternal complications resulting from the use of either of the prostaglandins- alone or in synergy. There was one episiotomy wound infection in group-I, one woman in each group had delayed discharge from the hospital due to puerperal pyrexia (which eventually turned out to be due to an urinary tract infection) and two women in the misoprostol group required bimanual uterine compression due to postpartum bleeding which did not amount to alarming proportions.

Table 1. Patient profile and indications of induction of labor

	Group-I (n=55) Mean (Range)	Group-II (n=56) Mean (Range)	p Value
Patient Profile			
Age (years)	21.6 (20.2-26.7)	22.3 (20.0-26.9)	>0.05
Weight (kg)	58.41 (43.4-76.4)	57.92 (42.5-77.0)	>0.05
Height (cm)	150.3 (148.2-154.2)	150.8 (147.6-153.8)	>0.05
Gestational age (wks)	38.2 (37.2-40.4)	38.2 (37.4-40.5)	
Bishop score at IOL	3.3 (2-4)	3.3 (2-4)	
Indications of Iol*	n (%)	n (%)	
Post-date pregnancy	22 (40.0%)	22 (39.3%)	
Social	13 (23.6%)	14 (25.0%)	
Oligohydramnios	9 (16.4%)	8 (14.3%)	
Term pregnancy with Pregnancy Induced Hypertension	11 (20.0%)	12 (21.4%)	

*IOL-Induction of labor

More neonates in group-I had first minute Apgar scores lower than 7, or needed neonatal resuscitation, but none of the babies had birth asphyxia. Meconium aspiration syndrome was not noticed in any of the neonates. (Table 4). There was a difference in the number of neonates admitted to the intensive care within 24 hours after delivery between the misoprostol and dinoprostone-misoprostol groups, being 5.4% (n=3) vs 1.8 (n=1).

In group-I, a salient adverse eventuality was confronted in the form of an unexplained stillbirth in a 26-year-old woman at 40 weeks of gestation after having received two doses of misoprostol. The sequence was that, for one hour of receiving the said dose of misoprostol, she continued to have normal FHR patterns with regular contractions of the uterus, but after the next half hour, there was no cardiac activity. Artificial rupture of the membranes was performed, which drained liquor clear of meconium and the vaginal delivery occurred within the next four hours and the baby had no cardio-respiratory activity. Gross examination of the newborn did not reveal any abnormality, but consent for autopsy could not be procured.

Discussion

Nowadays, induction of labor is more widely used than ever before (10, 11) and, according to Ventura et al. (12) the overall rate of induction of labor in the United States has more than doubled in a span of eight years. Recent studies have shown that this increase is mainly due to a rise in inductions for marginal or elective reasons. The most potent and acceptable methods of induction are the prostaglandins (1).

Misoprostol is a methyl ester of PGE_1 additionally methylated at C-16 and can be used orally, vaginally and sublingually (13). When introduced vaginally, absorbed serum levels are more prolonged (14). There are many studies which investigate the utilization of misoprostol in labor induction, but concerns still

exist regarding the increased incidence of fetal distress (15), meconium staining and hyperstimulation.

PGE_2 is an acceptable method of cervical ripening (15, 16) and is commercially available as a gel and vaginal insert, both of which are approved by the FDA for cervical ripening in women at or near term and both have been reported to increase the probability of successful initial induction by ripening the cervix (17). Dosage schedules used range from 6-12 hours (15, 18-20). In the current study we used eight hours for uniformity to coincide with the first two doses of misoprostol.

Lyons et al. (21) have shown in term pregnant rats that a higher dose of misoprostol is needed to induce PGE2 secretion in the cervix than in the myometrium, and furthermore that EP3 receptors (prostaglandin E2 receptors) are differentially expressed in the myometrium (increased) than in the cervix (unaltered) in response to misoprostol. The above findings indicate that misoprostol not only acts better on the myometrium than on the cervix, but an even higher dose is needed in order to ripen the cervix. Thus, it seems reasonable that initiating the labor induction process by cervical ripening with dinoprostone followed by repeated small misoprostol doses should reduce the risk of asynchrony between a well or even hyper-stimulated uterus and a still not efficiently ripened cervix.

Taking this explanation into consideration, the current study has been conducted and as per review of the literature, this appears to be the only comparison, up to date, between misoprostol alone and combination of dinoprostone followed by misoprostol in such well-homogenized groups. All of the women were nulliparous with intact membranes and at term, with no antenatal complications and all had an unfavorable cervix. In these carefully selected patients, dinoprostone was used initially to ripen the unfavorable cervix, followed by misoprostol to have a synergistic effect on the progress of labor. This combination, in the schedule mentioned, not only shortened the time between induction and delivery, but was also significantly more effective than misoprostol alone. The positive point was that this result was achieved with a comparatively low caesarean rate, although in the recent large meta-analysis (22) published by the Cochrane Library, the caesarean section rates were inconsistent. Even though misoprostol improves the kinetics of labor during induction in a more efficient way than dinoprostone used alone, concerns persist with respect to intrapartum fetal wellbeing. In order to avoid uterine hyperstimulation and abnormal FHR tracings, this study on the combination of dinoprostone and misoprostol resulted in a lower incidence of these complications. Our findings, in accordance with the previous Cochrane metanalysis, showed that, with only misoprostol, there was an increased probability of meconium staining of amniotic fluid as well as of uterine tachysystole and of abnormal FHR tracings. If neonatal outcomes such as neonatal resuscitation, low Apgar score in the first minute and admittance to the neonatal unit within the first 24 hours are taken into account, misoprostol may increase these complications in labor.

Table 2. Obstetrical outcome

Obstetrical Outcome	Group-I (n=55)	Group-II (n=56)	Statistical significance
Change in Bishop score after eight hours* Mean (Range)	5.3 (4- 7)	7.4 (6-8)	p<0.05
Time from induction to delivery (h) Mean (Range)	14.8 (9.8-22.4)	11.6 (8.8-19.2)	p<0.05
Delivery n (%)			
<12 h	26 (47.2%)	34 (60.7%)	p<0.05
<24 h	44 (80.0%)	51 (91.1%)	
Required oxytocin augmentation n (%)	34 (61.8%)	22 (39.3%)	p<0.05
Spontaneous rupture of membranes n (%)	19 (34.5%)	18 (32.1%)	NS
Meconium stained AF n (%)	10 (18.2%)	3 (5.3%)	p<0.05
Adverse Findings n (%)			
Abnormal FHR	10 (18.2%)	3 (5.3%)	p<0.05
Uterine Tachysystole	6 (10.9%)	2 (3.6%)	
Uterine Hyperstimulation	1 (1.8%)	- (0.0%)	

FHR=Fetal heart rate
AF=Amniotic fluid NS=not significant

Table 3. Mode of delivery

Mode of delivery	Group-I (n=55) n(%)	Group-II (n=56) n(%)	Statistical significance
Vaginal	44 (80.0%)	51 (91.1%)	p<0.05
Spontaneous	30 (68.2%)	44 (86.3%)	
Vacuum assisted	14 (31.8%)	7 (13.7%)	
Caesarean section	11 (20.0%)	5(8.9%)	p<0.05
Nonreassuring FHR	5 (9.0%)	2 (3.6%)	
Failed induction	4 (7.4%)	2 (3.6%)	
Lack of labor	2 (3.6%)	1 (1.8%)	
Progress*			

FHR=Fetal heart rate
*No progress in dilatation or descent of the presenting part for four hours in active phase of established labor

Table 4. Neonatal outcome

Neonatal Outcome	Group-I (n=55)	Group-II (n=56)	Statistical significance
Birth weight (g) Mean (Range)	28 (2520-3320)	2910 60 (2500-3480)	
Apgar score < 7	n (%)	n (%)	
1 min	7 (12.7%)	2 (3.6%)	
5 min	1 (1.8%)	- (0.0%)	
Neonatal resuscitation	7 (12.7%)	4 (7.1%)	
O_2 Supplementation	2	2	
Ambou ventilation	4	2	
Intubation in labor room	1	-	
Perinatal death	1 (1.8%)	- (0.0%)	
NICU admissions	3 (5.4%)	1 (1.8%)	

Attempting an explanation for the aforementioned side effects of misoprostol use, and taking into account other reports (23, 24), it appears that the increase in clinically relevant adverse effects is not only misoprostol related but it may be dose dependent. Misoprostol probably has a large inter-patient variability in terms of pharmacokinetics, but the more probable explanation could be that it may induce asynchrony between immature cervix effacement and uterine contractions. Based on these reviews and findings from the study under consideration, it is proposed that, in future, further randomized controlled studies be conducted to establish the regimen of dinoprostone followed by misoprostol in attempting to achieve priming of the cervix (with dinoprostone) before inducing effective uterine contractility (with subsequent misoprostol): this may reduce uterine hyperstimulation and neonatal complications. Merrell and coworkers (25) reported a series of 62 inductions of labor with vaginal misoprostol: there were two stillbirths, one apparently due to a tight nuchal cord, and one unexplained as reported in one of our cases. The exact cause of the stillbirth in this case remained unclear, thus emphasizing the need for continuous FHR monitoring during labor induction if regular uterine contractions persist (26). A significant observation worth mentioning is that five of the subjects delivered with dinoprostone only without the use of misoprostol.

According to Tan and Tay (27), dinoprostone improves the chances of successful ripening and shortens the interval from priming to induction, and priming to delivery. Combination with misoprostol reduces the need for repeated dinoprostone, thus cutting the cost. This combination may be more cost effective by not only shortening the period of hospital stay but also by reduced incidence of cesareans and neonatal resuscitation and the overall low incidence of adverse reactions in the mother and fetus.

Conclusion

Using dinoprostone for cervical priming followed by vaginal misoprostol not only hastened the progress of labor, with a greater percentage of women delivering vaginally and consequent reduction in caesarean section rate, but also reduced the adverse effects encountered with misoprostol when used alone, namely, tachysystole, uterine hyperstimulation and fetal heart abnormalities.

Conflict of interest

No conflict of interest was declared by the authors.

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Comparison of interval duration between single course antenatal corticosteroid administration and delivery on neonatal outcomes

Doğum öncesi tek kür kortikosteroid uygulaması ile doğum arasındaki sürenin yeni doğan akibeti üzerine etkisinin karşılaştırılması

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Abstract

Objective: This study was performed to determine the effect of antenatal corticosteroid the interval between administration and delivery affect on neonatal outcomes.

Material and Methods: An observational study was performed on all deliveries between 28-34 weeks gestation where delivery occurred vaginally after completing a single course of antenatal corticosteroid (dexamethasone). Women were divided into 3 groups on the basis of the interval from first corticosteroid dose to delivery (<2 days, 2-7 and >7 days). The primary outcome was the need for neonatal resuscitation and the secondary outcome was respiratory distress syndrome (RDS), which was described as "need for ventilation with positive pressure O2 during the first 24 hrs of life". P value <0.05 was significant.

Results: Of 104 neonates whose mothers received a full course of antenatal corticosteroid, 29 delivered <2 days, 41 delivered 2-7 days, and 34 delivered more than 7 days after the initial dose. Overall, those delivering within 2 days after the first injection of corticosteroid had more need for resuscitation and ventilation than those infants delivering between 2-7 days and after 7 days. Infants delivering between 2-7 days had a lower incidence of need for resuscitation and receiving respiratory support for more than 24 hours.

Conclusion: We found that the interval between corticosteroid administration and delivery influences the incidence of need for resuscitation and ventilation. Infants delivering less than 2 days of corticosteroid exposure have a higher frequency of need for resuscitation and ventilation than delivering between 2-7 days and after 7 days.

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Key words: Corticosteroids, ventilation, need to resuscitation, preterm delivery

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Özet

Amaç: Bu çalışma doğum öncesi kortikosteroid uygulaması ve doğum arasındaki intervalin yeni doğan akibeti üzerine etkisini belirlemek için gerçekleştirildi.

Gereç ve Yöntemler: 28-34 gebelik haftaları arasında ve doğum öncesi tek bir kortikosteroid (deksametazon) kürünün tamamlanmasından sonra görülen vajinal doğumlarda gözlemsel bir çalışma gerçekleştirildi. Kadınlar ilk kortikosteroid dozundan doğuma kadar geçen süreye dayalı olarak 3 gruba ayrıldı (<2 gün, 2-7 ve >7 gün). Birincil sonuç yenidoğan resüsitasyonuna gerek duyulması, ikincil sonuç respiratuar distres sendromu (RDS) idi; RDS "yaşamın ilk 24 saatinde pozitif basınçlı O2 ile ventilasyona gerek duyulması" olarak tanımlandı. P değeri <0.05 anlamlı idi.

Bulgular: Anneleri doğum öncesi tam kür kortikosteroid alan 104 yenidoğanın, başlangıç dozundan sonra 29'u <2 günde, 41'i 2- 7 günde ve 34'ü >7 günde doğdu. Toplamda, kortikosteroidin ilk enjeksiyonundan sonra 2 gün içinde doğanlar; 2-7 gün arasında ve 7 günden sonra doğanlardan daha fazla resüsitasyona ve ventilasyona gerek duydu. 2-7 gün arasında doğan bebeklerin resüsitasyona ve 24 saatten daha uzun süre solunum desteğine gereksinim insidansı daha düşüktü.

Sonuç: Kortikosteroid uygulaması ve doğum arasındaki intervalin resüsitasyon ve ventilasyon gereksinimi insidansını etkilediğini bulduk. Kortikosteroid uygulanmasından sonra 2 günden daha kısa sürede doğan bebekler 2-7 gün arasında ve 7 günden sonra doğanlara göre daha yüksek sıklıkla resüsitasyona ve ventilasyona gerek duymaktadır.

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Anahtar kelimeler: Kortikosteroidler, ventilasyon, resüsitasyon gereksinimi, preterm doğum

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Introduction

Preterm labor and preterm delivery are critical factors affecting neonatal morbidity and mortality (1). The most common complication of premature birth is respiratory distress syndrome (RDS), which is caused by insufficient surfactant production in the immature fetal lung (2). In 1972, Liggins reported that use of corticosteroids in the antenatal period improves the outcome of infants who deliver between 28 and 34 weeks of gestation (3).

During most of its gestational life, the fetus is exposed to very low levels of corticosteroids. A surge of corticosteroids in late pregnancy stimulates fetal lung surfactant production which is necessary for lung maturation. In preterm labor

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the neonate does not experience the late gestational steroid surge. These premature infants experience respiratory distress syndrome (RDS) and require mechanical lung ventilation (4). Synthetic corticosteroids exhibit transplacental passage when administered to pregnant women because of the low affinity for maternal corticosteroid binding globulin (CBG) and poor breakdown by placental steroid metabolizing enzymes (5). This treatment consists of corticosteroid administration for 48 hours, which is thought to stimulate innate surfactant production before delivery. Studies demonstrated improved respiratory outcomes among infants delivering within 7 days of corticosteroid therapy (5, 6).

Based on the original data of Liggins and Howie, where a positive effect of the corticosteroids was described up to 7 days, 2 R.N. Howie and G.C. Liggins, The New Zealand study of antepartum glucocorticoid treatment. In: P.M. Farrell, Editor, *Lung development: biological and clinical perspectives* Vol 2, Academic Press, New York (1982), pp. 255-265. the practice evolved to administer this treatment to undelivered at-risk patients on a weekly basis until delivery or maturity was reached (2). Whereas the positive effect of a single course of antenatal corticosteroids for fetal lung maturation is proven, the usefulness of repetitive administration of corticosteroids is still under investigation and unclear (7). Recent studies reported that the neonatal benefits do not diminish when delivery is beyond 7 days from the initial antenatal corticosteroids dose (2, 8). Some studies documented no beneficial or even adverse effects of multiple antenatal corticosteroids courses (9). In the controversy regarding some of these, some studies reported that a time interval of >14 days between the administration of antenatal corticosteroids and delivery is associated with an increased need for ventilatory support and surfactant use in neonates who deliver preterm (10, 11).

The objective of this study was to evaluate the effect of the time interval between the last corticosteroid treatment and delivery, and whether a prolonged interval (>7days) between antenatal corticosteroid therapy and delivery is associated with increased neonatal respiratory morbidity.

Materials and Methods

An observational study was designed and conducted on 104 preterm women who were between 28-34 weeks gestation and undergoing vaginal delivery after completing a single course of antenatal corticosteroid (Dexamethasone). The study was carried out in the obstetrics department of Shahid Sedughi hospital (University referral center) between 2006-2008. The adopted protocol was approved by the hospital research and ethics committee. The sample size estimation was based on studies with the hypothesis of need for respiratory support at gestational ages 26 to 34 weeks and using a Mann-Whitney test with a 0.05 two-sided significance level (n Query Advisor, Version 5.0). All women were interviewed individually by the researcher. Written informed consent was obtained from all the patients. The questionnaires were filled out for each patient at the beginning of the study and detailed information was collected on all deliveries by an obstetrics first year resident. Gestational age was based on the last menstrual period and/or ultrasonographic measurement of crown-rump length in early pregnancy and after 12 weeks gestation by ultrasonic measurement of fetal biparietal diameter and femur length. Individuals who reviewed the maternal charts were blinded to neonatal outcome data. Neonatal data were collected prospectively for infants admitted to the neonatal intensive care unit.

Only neonates of mothers who received a complete course of antenatal corticosteroids were included. A single course was defined as Dexamethasone in a dosage of 6 mg given intramuscularly every 12 hours for two days (12). Patients were grouped on the basis of the time interval between the administration of the first corticosteroid dose and delivery, < 2 days, 2-7 days and >7 days. Multiple gestations and neonates with congenital anomalies were excluded. Also the women with preeclampsia, severe maternal disease, receiving tocolytic agent, women with a diagnosis of preterm premature rupture of membranes, chorioamnionitis and cases in which delivery occurred after 34 weeks gestation were excluded. Neonates delivering within 7 days and after 7 days of initiation of corticosteroid treatment were compared with those delivering <2 days from treatment initiation in regard to the need for resuscitation and respiratory morbidities (including need for respiratory support by mechanical ventilation or continuous positive airway pressure for >24 hours).

Data was analyzed with SPSS 11.5 software using the Student t test, Mann Whitney U test and χ^2 testing. P value <0.05 was considered statistically significant.

Results

One hundred and four women were identified who delivered between 2006 and 2008 at 28 - 34 weeks gestation and had received a single course of antenatal corticosteroid therapy for the purpose of advancing pulmonary maturity. Of 104 neonates whose mothers received antenatal corticosteroid, 29 delivered <2 days, 41 delivered 2-7 days, and 34 delivered more than 7 days after the initial dose. The groups were similar in gestational age at delivery, maternal age, gender, and birth weight (Table 1). The groups differed only in the interval from treatment to delivery, as expected by group assignment. Neonates delivering between 2-7 days of treatment had lower rates of resuscitation and ventilation. Infants delivering in under 2 days had more resuscitation and ventilation than those who delivered 2-7 days of treatment (20 (68.7%) vs 18 (43.9%) and 25 (83.1%) vs 21 (51.2%), p=0.01 and p=0.001 respectively) (Table 2). Table 3 shows that both resuscitation and ventilation were significantly less in deliveries after 7 days compared with infants delivering <2 days of treatment (20 (68.7%) vs 17 (50%) and 25 (83.1%) vs 25 (73.5%) p<0.05), but the need to resuscitate was similar. Of 104 neonates whose mothers received a full course of antenatal corticosteroid, 35 (33.7%) were <32 weeks of gestation. Common odds ratios are presented for analyses that were stratified by gestational age. Of these, 10 neonates delivered <2 days, 17 neonates delivered 2-7 days and 8 neonates delivered after 7 days of antenatal corticosteroid exposure. Table 4 shows

that differences between groups in respiratory outcomes were most evident for neonates at <32 weeks of gestational age. APGAR score in neonates delivering between 2-7 days of treatment was higher than the others, but this was not significant.
Comparison of neonatal respiratory outcomes of <32 weeks of gestation who delivered after 7 days and 2-7 days after antenatal corticosteroid administration is given in Table 5. Delivering after 7 days of corticosteroid administration have significantly better outcome.

Discussion

The most common complication of premature birth is respiratory distress syndrome (RDS), which is caused by insufficient surfactant production in the immature fetal lung (2). Studies showed that antenatal corticosteroid therapy for fetal maturation is effective and reduces perinatal mortality and pulmonary and cerebral morbidity in preterm infants (7, 13). The most effective timing for administration has not been established. Clinical evidence suggests that antenatal steroids are most effective in deliveries between 24 hours and 7 days after treatment, but that the benefits of treatment may begin to decrease after 7 days (2). It is unclear whether the beneficial effects of antenatal corticosteroid therapy diminish with time. Therefore, many clinicians prefer to repetitively administer corticosteroids

Table 1. Baseline demographic characteristics between groups

Characteristics	Delivery ≤2 days (n=29)	Delivery within 7 days (n=41)	Delivery >7 days (n=34)	P value
Maternal age (year) (Mean±SD)	28.4±6.1	27.8±6.4	28.0±6.7	0.4
Parity (N (%))				
Nullipara	12 (41.4)	22 (53.7)	16 (47.1)	0.2
Multipara	17 (58.6)	19 (46.3)	18 (52.9)	
Gestational age at initiation of treatment (week) (Mean±SD)	30.1±2.5	30.8±2.8	31.5±2.2	0.4
Gestational age at delivery (week) (Mean±SD)	31.7±2.3	31.5±2.8	31.7±2.0	0.3
Mode of delivery				
Vaginal	16 (55.2)	21 (51.2)	15 (44.1)	0.1
Cesarean section	13 (44.8)	20 (48.8)	19 (55.9)	
Neonatal gender (N (%))				
Male	18 (62.1)	23 (56.1)	14 (41.2)	0.1
Female	11 (37.9)	18 (43.9)	20 (58.8)	
5 minutes APGAR score (Median)	5	8	6	0.02
Birth weight (g) (Mean±SD)	1451±385	1424±349	1460±361	0.3

Table 2. Comparison of respiratory outcomes among neonates who delivered <2 days and 2-7 days after antenatal corticosteroid administration

Variable	Corticosteroid exposure <2 days (n=29)	Corticosteroid exposure 2-7 Days (n=41)	P value
Need to resuscitation (N (%))	20 (68.7)	18 (43.9)	0.001
Ventilation for >24 h (N (%))	25 (83.1)	21 (51.2)	0.001
Total days of NICU (Mean±SD)	9±3.21	12±4.885	0.001

Table 3. Comparison of respiratory outcomes among neonates who delivered 2 - 7 days and after 7 days after antenatal corticosteroid administration

Variable	Corticosteroid exposure <2 days (n=29)	Corticosteroid exposure >7 days (n=34)	P value
Need for resuscitation (N (%))	20 (68.7)	17 (50)	0. 01
Ventilation for >24 h (N (%))	25 (83.1)	25 (73.5)	0.001

Table 4. Comparison of neonatal respiratory outcomes of <32 weeks of gestation who delivered <2 days and 2-7 days after antenatal corticosteroid administration

Variable	Corticosteroid exposure <2 days (n=10)	Corticosteroid exposure 2-7 days (n=17)	Odds ratio (95% CI)	P value
Need for resuscitation (N (%))	7 (70)	7 (41.2)	2.1 (1.9-3.7)	0. 001
Ventilation for >24 h (N (%))	9 (90)	10 (58.8)	2.8 (1.9-3.9)	0.001

Table 5. Comparison of neonatal respiratory outcomes of <32 weeks of gestation who delivered after 7 days and 2-7 days after antenatal corticosteroid administration

Variable	Corticosteroid exposure <2 days (n=10)	Corticosteroid exposure >7 days (n=8)	Odds ratio (95% CI)	P value
Need for resuscitation (N (%))	7 (70)	4 (50)	2.3 (1.8-3.7)	0. 001
Ventilation for >24 h (N (%))	9 (90)	6 (75)	2.1 (1.1-3.4)	0.001

to pregnant women who have not been delivered within 7 days of initial treatment and are still at high risk for preterm delivery. Some studies reported no beneficial or even adverse effects after multiple courses of antenatal corticosteroids (9, 14).

Our studies evaluated the effect of interval between a single dose of antenatal corticosteroid and delivery on neonatal outcome (need for resuscitation and ventilation for more than 24 hours), and showed that, when delivery occurred between 2 -7 after a single dose of corticosteroid, treatment can be more effective than <2 and >7 days. In this study, when delivery occurred in less than 2 days after the beginning of treatment, the need for resuscitation and ventilation were increased. Although in vitro experiments with human fetal lung explants have shown that the biochemical effects of steroid treatment also begins to decrease after 7 days (15), in our study only an increased need for ventilation for more than 24 hours was seen in those neonates delivering more than 7 days from treatment, but immediate resuscitation after birth was not similar to those delivering between 2-7 days.

The results provide more evidence for other studies. Peaceman et al, reported similar results in 2005 (2). However, they used both dexamethasone or betamethasone in their study and these drugs can effect with different intervals. Waters in 2008 also reported similar results (11). In contrast, Ring et al reported an increased risk for ventilatory support and surfactant use in neonates who delivered after 14 days following the administration of antenatal corticosteroids (10). This may be prolonged more than 2 weeks between administration and deliveries in this study. Another study performed by Ay in Turkey, showed there was no statistically significant difference between groups of single and two courses of antenatal steroid therapy regarding the incidence of RDS and mechanical ventilator treatment (16).

We used a single course of corticosteroid treatment in our study because of controversy in other studies (in multiple or single course of corticosteroid treatment and their results). Banks et al reported that multiple courses of antenatal corticosteroids did not improve neonatal outcome, especially no reduction of the RDS was found. The outcome of infants delivered 1-6 days or 7-13 days after receiving antenatal corticosteroids was similar (14); and conversely, Throp et al could show that prolonged antenatal betamethasone therapy improved neonatal outcome and is not associated with higher risks of maternal adverse effects (18). This study compared 28-34 week gestational age infants. Although Ring et al reported an increased need for ventilatory support and surfactant use in neonates who deliver at >28 weeks of gestation in their study (H), our results showed that there were no difference in analyses <32 week of gestation and 32-34 weeks.

Conclusion

We found that theinterval between corticosteroid administration and delivery influences the incidence of need for resuscitation and ventilation. Infants delivering less than 2 days after corticosteroid exposure have a higher frequency of need for resuscitation and ventilation than those delivering between 2-7 days and after 7 days.

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Conflict of interest

No conflict of interest was declared by the authors.

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Comparison of perinatal and maternal outcomes of severe preeclampsia, eclampsia, and HELLP syndrome

Preeklampsi, eklampsi ve HELLP sendromu olgularında perinatal ve maternal sonuçların karşılaştırılması

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Abstract

Objective: To compare maternal and perinatal outcomes in pregnancies complicated by severe preeclampsia, eclampsia, and HELLP (hemolysis, elevated liver enzyme levels, and low platelets) syndrome.

Materials and Methods: Maternal and neonatal charts of 1,222 consecutive pregnancies complicated by severe preeclampsia, eclampsia, or HELLP syndrome at our maternal-perinatal unit were reviewed. Patients were divided into three groups: 903 (73.9%) with severe preeclampsia, 123 (10.1%) with eclampsia, and 196 (16.0%) with HELLP syndrome.

Results: The overall incidence of adverse maternal outcome was 5.9%. The rates of adverse maternal outcomes for women with HELLP syndrome and eclampsia were higher than for severe preeclampsia (13.8% vs. 11.4% vs. 3.4%, respectively) (p=0.000). Birth weight was lower in patients with HELLP syndrome than in patients with eclampsia and severe preeclampsia (p=0.005). No significant difference in neonatal morbidity was found among the three groups. Perinatal mortality tended to be higher in the severe preeclampsia group than in the HELLP syndrome and eclampsia groups (p=0.231).

Conclusion: Pregnancies complicated by HELLP syndrome had significantly higher maternal morbidity than those with severe preeclampsia and eclampsia. Perinatal and neonatal outcomes in pregnancies complicated by severe preeclampsia, eclampsia, and HELLP syndrome were dependent on gestational age rather than being disease dependent. (J Turkish-German Gynecol Assoc 2011; 12: 90-6)

Key words: Severe preeclampsia, eclampsia, HELLP syndrome, perinatal and maternal outcome

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Özet

Amaç: Şiddetli preeklampsi, eklamsi ve HELLP sendromu ile komplike olmuş gebelerdeki maternal ve perinatal sonuçları karşılaştırmak.

Gereç ve Yöntemler: Maternal-perinatal ünitemizde tedavi olan şiddetli preeklampsi, eklamsi ve HELLP sendromu ile komplike olmuş 1222 gebe incelendi. Olgular üç guruba ayrıldı; 903 (%73.9) şiddetli preeklampsi, 123 (%10.1) eklampsi ve 196 (%16.0) HELLP sendromu.

Bulgular: Toplam maternal komplikasyon oranı % 5.9 olarak bulundu. Maternal komplikasyon oranı HELLP sendromu ve eklampsi ile komplike olmuş geblerde şiddetli preeklampsi ile komplike olmuş geblerden daha yüksek bulundu (%13.8, %11.4, %3.4,) (p=0.000). Doğum ağırlığı HELLP semdromu gurubunda eklampsi ve şiddetli preeklampsi gurubuna gore daha düşüktü (p=0.005). Guruplar arasında neonatal morbitide yönünden istatistiksel anlamda fark bulunamadı (p>0.05). Peritanal mortalite oranı HELLP sendormu ve eklampsi gurubu ile karşılaştırıldığında şiddetli preeklampsi gurubunda daha yüksek oranda meydana gelsede istatistiksel anlamda fark bulunamadı (p=0.231).

Sonuç: HELLP sendromu ile komplike olmuş gebeler şiddetli preeklamapsi ve eklampsi ile komplike olmuş gebelere oranla daha fazla maternal morbiditeye sahiptir. Perinatal ve neonatal sonuçlar ise şiddetli preeklampsi, eklampsi ve HELLP sendromundan çok doğumdaki gestasyonel yaşa bağlıdır.

(J Turkish-German Gynecol Assoc 2011; 12: 90-6)

Anahtar kelimeler: Şiddetli preeklampsi, eklampsi, HELLP sendromu, perinatal ve maternal sonuçlar

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Introduction

Hypertensive disorders during pregnancy represent a significant public health problem throughout the world, and preeclampsia is the most common of these disorders (1). Villar et al. reviewed available information on the incidence and prevalence of preeclampsia/eclampsia utilizing large epidemiological studies (2). They estimated that hypertension complicates approximately 5% of all pregnancies. Of these, approximately half are due to or associated with preeclampsia. Based on these estimates and case-fatality rates, they calculated that up to 40,000 women, mostly in developing countries, may die due to preeclampsia or eclampsia each year.

The clinical course of severe preeclampsia results in progressive deterioration of both maternal and fetal conditions. Traditional management of severe preeclampsia has focused on maternal safety, with expedited delivery. Because these pregnancies are associated with high rates of maternal mor-

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bidity and mortality and with potential risks for the fetus, it is generally agreed that such patients should be delivered if the disease develops at >34 weeks of gestation (3, 4). In patients with severe preeclampsia at <34 weeks of gestation, several authors have suggested some form of expectant management in an attempt to prolong gestation and improve perinatal outcome (3-7). For patients with severe fetal growth restriction (FGR) with or without oligohydramios or evidence of maternal organ dysfunction (eclampsia, HELLP syndrome), some authors have recommended steroids to enhance lung maturation, with delivery 48 hours after initiating steroid administration (3-9).

The main objective when managing severe preeclampsia, eclampsia, and HELLP syndrome is to reduce maternal mortality and morbidity rates. Current literature emphasizes an increased risk of adverse outcomes for patients with severe preeclampsia, eclampsia, and HELLP syndrome. However, few studies have compared perinatal and maternal outcomes in pregnancies complicated by severe preeclampsia, eclampsia, and HELLP syndrome.

Materials and Methods

The Bakırkoy Women and Children Education and Research Hospital in Istanbul, Turkey serves as a tertiary care facility for western Turkey, where there are approximately 14,000 deliveries annually. During the 6-year period from January 1, 2002 through December 2007, 1,222 of these pregnancies antenatally managed at this hospital were complicated by severe preeclampsia, eclampsia, and HELLP syndrome. This study was approved by the hospital's Ethics Committee.

Women had severe preeclampsia if they met one or more of the following criteria of The American College of Obstetricians and Gynecologists (10): systolic blood pressure >160 mm/Hg or diastolic blood pressure >110 mm/Hg, headache, visual disturbances, epigastric or right-upper-quadrant pain, pulmonary edema, and proteinuria (urinary protein level >5 g/24 h). Women with severe preeclampsia selected for analysis also met all of the following laboratory criteria: platelet count ≥150,000/mm^3, serum lactate dehydrogenase <600 U/L, serum total bilirubin <1.2 mg/dL, and serum aspartate aminotransferase <70 U/L. Eclampsia was defined as tonic-clonic seizures occurring during a hypertensive pregnancy. Patients with any causes for convulsion other than eclampsia were excluded.

HELLP syndrome was divided into three classes by Martin et al. (11). They defined class 1 HELLP syndrome as a platelet nadir below 50,000/mm^3, whereas those with platelet nadirs between 51,000 and 100,000/mm^3 were defined as class 2. Class 3 HELLP syndrome represented patients with hepatocyte death but a higher platelet count nadir of 101,000-150,000/mm^3.

Gestational age was determined using the best obstetric criteria, including either the last menstrual period or ultrasonography (where available) at <20 weeks gestation or both. On admission, hematocrit, hemoglobin, platelet count, and liver enzymes were determined, and 24-h urine collection was started. A hemogram and blood biochemistry tests were repeated daily or at a 2-day intervals. Fetal heart rate monitoring (FHR) was performed at least two to three times per day. Nifedipine and/or alfa-methyldopa were used as antihypertensive agents. An intravenous infusion of magnesium sulfate at a rate of 2 g/h was started, after a loading dose of 4.5 g for 10 min, in patients with persistently elevated systolic or diastolic blood pressure and/or prodromal symptoms and was continued postpartum for 24 h in severe preeclampsia and 48 h in eclampsia. Two doses of betamethasone (12 mg intramuscularly) were also given at 24 h intervals.

Maternal indications for delivery included persistent elevated blood pressure >160/110 mm Hg despite treatment with antihypertensive drugs, persistent or worsening symptoms, deteriorating renal function, severe ascites, abruptio placenta, oliguria, pulmonary edema, preterm labor, preterm rupture of membranes, evidence of class 1-2 HELLP syndrome, and eclampsia. Fetal indications for delivery included a non-reassuring fetal status as determined by the fetal heart rate tracing (decreased variability, repetitive late decelerations, or severe variable decelerations), FGR, oligohydramnios, severe abnormal umbilical artery Doppler findings such as absent or reverse end diastolic flow, and attainment of the 34th or ≥34th week of gestation on admission.

Oligohydramnios was diagnosed with an amniotic fluid index ≤5 cm or a two-diameter pocket <15 cm^2. FGR was defined as a birth weight below the 10th percentile for gestational age.

The maternal variables studied included age, gravity, parity, gestational age at delivery, timing of eclampsia onset, and adverse maternal outcome. Maternal outcomes included abruptio placentae, acute renal failure (ARF), pulmonary edema, ascites, acute respiratory distress syndrome (ARDS), neurological deficits, visual changes, intracranial hemorrhage, and maternal death. ARF was diagnosed when oliguria or anuria in association with a creatinine clearance ≤20 mL/min was present with an elevated serum creatinine level ≥2 mg/dL. Pulmonary edema was assessed based on clinical findings and chest radiography. A diagnosis of severe ascites was made by estimation during an ultrasonographic examination, cesarean delivery, or laparotomy (≥1000 mL of fluid measured by a suction apparatus).

Neonatal and fetal medical records were reviewed for the following outcomes: FGR, oligohydramnios, mode of delivery, intrauterine fetal death, perinatal (fetal death and early neonatal death at <7 postnatal days) and neonatal (postnatal 0-28 days) mortality, ARDS, grade 3-4 intraventricular hemorrhage (IVH), stage 2-3 necrotizing enterocolitis (NEC), sepsis, admission to the intensive care unit (ICU), duration of stay in the ICU, and duration of hospitalization. ARDS was defined by the presence of characteristic radiographic findings and oxygen requirements at 24 h. Grade 3 intraventricular hemorrhage was defined as hemorrhage with ventricular dilation, and grade 4 as hemorrhage with parenchymal involvement. NEC was defined by radiographic findings of grade 2 pneumatosis cystoides intestinalis and a grade 3 pneumoperitoneum. Intrauterine death and intrapartum loss of nonviable fetuses during pregnancy termination due to maternal indications were accepted as fetal death.

Data are presented as rates, percentiles, medians (interquartile ranges), and means±SDs. Statistical comparisons were performed using analysis of variance, Kruskal-Wallis test, and χ^2 test, as appropriate. P-values < 0.05 were considered significant. Because double comparisons cannot be performed with the Kruskal-Wallis test, the groups significant in this analysis were compared as groups of two using the Mann-Whitney U-test. Levels of significance were evaluated with the Bonferroni correction (n/a=0.05/3=0.017), and p<0.017 was considered significant.

Results

During the study period, 1222 cases of severe preeclampsia, eclampsia, or HELLP syndrome were treated at our institute. Among these, 123 (10.1%) had eclampsia, 903 (73.9%) had severe preeclampsia, and 196 (16.0%) had HELLP syndrome (Fig. 1). This classification was determined based on the patients' initial presentation. Patients with HELLP syndrome and eclampsia were evaluated as the eclampsia group. Forty-one cases of eclampsia experienced HELLP syndrome. A total of 734 (60.0%) patients (601 with severe preeclampsia, 57 with eclampsia, and 76 with HELLP syndrome) were referred from surrounding hospitals and clinics, 297 (24.4%) were given antenatal care at our hospital (213 with preeclampsia, 35 with eclampsia, 49 with HELLP syndrome), and 191 (15.6%) patients received no antenatal care prior to admission (89 patients with severe preeclampsia, 31 with eclampsia, 71 with HELLP syndrome).

Table 1 compares the clinical characteristics of the cases. Maternal age, gravity, and parity were higher in patients with HELLP syndrome than in those with severe preeclampsia or eclampsia, and women with eclampsia were more likely to deliver by cesarean section than were those with HELLP syndrome or severe preeclampsia (p=0.005).

Major maternal complications are presented in Table 2. The overall incidence of adverse maternal outcome was 5.9%. Statistical differences were found among the three groups for all adverse maternal outcomes studied. The rate of adverse

Table 1. Clinical characteristics in 3 study groups

	Severe Preeclampsia (n=903)	Eclampsia (n=123)	HELLP syndrome (n=196)	P	P1[b]	P2[b]	P3[b]
Age (year, median, Q1-Q3)	27 (24-32)	25 (22-29)	28 (25-33)	<0.0001[a]	<0.0001	0.144	<0.0001
Gravidity (median, Q1-Q3)	2 (1-3)	1 (1-2)	2 (1-3)	0.0002[a]	0.0003	0.355	0.0003
Parity (median, Q1- Q3)	0 (0-2)	0 (0-1)	1 (0-2)	0.0006[a]	0.0012	0.358	0.0009
Nulliparity, %	49.8	35	53.1	0.004*			

[a]According to the Kruskal–Wallis test (triple comparison) P<0.05
[b]Mann-Whitney U test P1=Severe Preeclampsia versus Eclampsia, P2=Severe preeclampsia versus HELLP Syndrome, P3=Eclampsia versus HELLP Syndrome
Significant p value after Bonferroni correction <0.017
*According to χ^2 test for trend

Table 2. Clinical characteristics in 3 study groups

Maternal outcomes	Severe Preeclampsia (n=903)	Eclampsia (n=123)	HELLP syndrome (n=196)	p
Maternal morbidity	35 (3.9%)	15 (12.2%)	29 (14.8%)	p<0.0001
ARDS	4 (0.4%)	3 (2.4%)	1 (0.5%)	0.038
Acute renal failure	14 (1.6%)	5 (4.1%)	21 (10.7%)	p<0.0001
Neurologic deficits	1 (0.8%)	3 (0.3%)	2 (1%)	0.0015
Visual change	6 (0.7%)	6 (4.9%)	1 (0.5%)	p<0.0001
Pulmonary edema	5 (0.6%)	0	2 (1%)	0.496
Ascites	3 (0.3%)	0	1 (0.5%)	0.738
Intracranial hemorrhage	0	0	1 (0.5%)	0.072
Abruptio placentae	64 (7.1%)	10 (8.1%)	18 (9.2%)	0.581
Cesarean delivery	617 (68.3%)	105 (86.3%)	150 (76.5%)	0.005
Maternal death	1 (0.1%)	0	1 (0.5%)	0.407

According to the χ^2 test for trend

Table 3. Fetal findings of study groups

Fetal findings	Severe Preeclampsia (n=903)	Eclampsia (n=123)	HELLP syndrome (n=196)	P
Gestational age at delivery (wk, median, Q1-Q3)	34.4 (31.7-36.7)	34 (30.7-36.9)	34 (31.05-35.9)	0.009[a] P1[b] 0.574 P2[b] 0.001 P3[b] 0.165
Birth weight, (g, median, Q1-Q3)	975 (835-1315)	1145 (760-1320)	940 (700-1200)	0.005[a] P1[b] 0.308 P2[b] 0.003 P3[b] 0.006
Fetal growth restriction, %	53.4	39.2	50.8	0.013[t]
Oligohydramnios, %	23.9	21.7	25.4	0.755[t]
Absent or reverse end diastolic flow, %	11.3	8.3	12.4	0.521[t]
5-min Apgar score <7, %	6.1	11.9	4.8	0.048[t]
Female infant, %	52.9	50.0	52	0.829[t]
Placental weight (g, median, Q1-Q3)	230 (150-310)	265 (195-320)	180 (140-250)	0.591
Fetal mortality (IUFD), %	10.5	5.1	11.6	0.153[t]
≤32 weeks, %	29.4	7.7	29.6	0.016[t]
>32 weeks, %	3.8	3.8	3.4	0.979[t]

IUFD: Intrauterine fetal death
[a]According to the Kruskal–Wallis test (triple comparison) P<0.05
[b]Mann–Whitney U test P1=Severe Preeclampsia versus Eclampsia, P2=Severe preeclampsia versus HELLP Syndrome, P3=Eclampsia versus HELLP Syndrome. Significant p value after Bonferroni correction <0.017
[t]According to the χ^2 test for trend

maternal outcomes for women with HELLP syndrome and eclampsia were higher than those for women with severe preeclampsia (14.8% vs. 12.2% vs. 3.9%, respectively) (p=0.000). The rate of abruptio placentae tended to be higher among women with HELLP syndrome than among women with severe preeclampsia and eclampsia, but the difference was not significant (9.1% vs. 8.1% vs. 7.1%; p=0.581). Two maternal deaths occurred, producing a case fatality rate of 0.2% (one in the HELLP syndrome group and another in the severe preeclampsia group). Death was related to intracranial hemorrhage in one case and to an ARDS complication in another case.

Fetal findings are presented in Table 3. Birth weights were lower in infants of patients with HELLP syndrome than in infants of those with eclampsia or severe preeclampsia (p=0.005). Infant gestational age at delivery was lower for patients with HELLP syndrome than for those with eclampsia or severe preeclampsia (p=0.036). The percentage of oligohydramnios and of absent or reversed end diastolic flow, and the 5-min Apgar score, except FGR, were not different among women with eclampsia, severe preeclampsia, or HELLP syndrome. FGR was higher in patients with severe preeclampsia than in those with eclampsia and HELLP syndrome (p=0.013). A total of 105 intrauterine fetal deaths occurred, but no significant difference in fetal mortality was found among the three groups (p=0.153). No significant differences in neonatal morbidity (admission to ICU, RDS, grades 3 and 4 IVH, grades 2 and 3 NEC, sepsis, duration in the neonatal ICU) were found among the three groups (Table 4). The duration of hospitalization was similar for patients with severe preeclampsia and eclampsia, but was significantly shorter for those with severe preeclampsia than for those with HELLP syndrome. Perinatal mortality tended to be higher in the severe preeclampsia group than in the HELLP syndrome and eclampsia groups, but the difference was not significant (p=0.231).

Discussion

We examined maternal and perinatal outcomes for severe preeclampsia, eclampsia, and HELLP syndrome and found the following. 1) The mean gestational age and birth weight at delivery in the HELLP syndrome group were lower than those in the severe preeclampsia and eclampsia groups. 2) No significant differences were found for neonatal mortality and morbidity. 3) Abruptio placentae tended to be higher among women with HELLP syndrome than in the other groups, but the difference was not significant. 4) Perinatal and fetal mortality tended to be higher in the severe preeclampsia and HELLP syndrome groups than in the eclampsia group, but the difference was not significant. 5) When an analysis was performed according to gestational age before and after the 32nd week, perinatal mor-

Table 4. Perinatal and neonatal outcomes of study groups

Perinatal and neonatal outcomes	Severe Preeclampsia (n=903)	Eclampsia (n=123)	HELLP syndrome (n=196)	P
Admission to ICU, %	14.8	21.6	17.3	0.169[a]
Duration of ICU (d, median, Q1-Q3)	13.5 (6-23.7)	8 (3.2-26.7)	15 (9-32.5)	0.345[a]
RDS, %	13.2	19.5	16.0	0.169*
IVH grades 3 and 4, %	1.3	2.7	2.6	0.379*
NEC grades 2 and 3, %	4.1	4.4	7.7	0.159*
Sepsis, %	6.7	6.2	8.3	0.735*
Duration of hospitalization (d, median, Q1-Q3)	4 (2-12)	4 (3-15)	6 (3-15)	0.0001[a] P1[b] 0.025 P2[b] 0.0001 P3[b] 0.182
Perinatal mortality, %	13.1	7.7	13.7	0.231*
≤32 weeks, %	36.5	15.4	36.4	0.034*
>32 weeks, %	4.9	3.8	3.3	0.706*
Neonatal mortality, %	5.6	4.6	5.9	0.890*
≤32 weeks, %	21	11.4	21.6	0.415*
>32 weeks, %	1.7	1.4	0.9	0.815*
>28 days mortality, %	1.1	1.9	4.8	0.009*

[a]According to Kruskal–Wallis test (triple comparison) P<0.05
[b]Mann-Whitney U test P1=Severe Preeclampsia versus Eclampsia, P2= Severe preeclampsia versus HELLP Syndrome, P3=Eclampsia versus HELLP Syndrome. Significant p value after Bonferroni correction <0.017
*According to χ^2 test for trend

tality at ≤32 weeks for patients with severe preeclampsia and HELLP syndrome was higher than that in the eclampsia group. 6) Significantly higher maternal morbidity was observed in the HELLP syndrome group than that in the other groups.

Primaparous young women with low socioeconomic status are the most typical preeclamptic and eclamptic cases. Our study also revealed clinical and sociodemographic data similar to the patient profiles in the extant literature. Most of these patients had had no regular antenatal visits, were primiparous, and young. HELLP syndrome has classically been described as a disease process that occurs more often in older, multigravid women than in younger nulliparous women with typical preeclampsia (13, 14). In our study, maternal age, gravity, and parity were higher in patients in HELLP syndrome than in those with severe preeclampsia and eclampsia, which agreed with a previous study (12).

Pregnancies complicated by severe preeclampsia/eclampsia or HELLP syndrome are associated with an increased risk for maternal morbidity and mortality. Complications leading to maternal morbidity include severe bleeding from abruptio placentae, pulmonary edema, ARF, cerebrovascular hemorrhage, and liver rupture. These complications are usually seen in women who develop severe preeclampsia, eclampsia, or HELLP syndrome before 32 weeks gestation (14). Women with HELLP syndrome have an increased risk for adverse maternal outcome compared with those who have severe preeclampsia/eclampsia (10, 15). The differences in the outcomes observed in these studies may be related to the earlier gestation in women with HELLP syndrome than in women with severe preeclampsia/eclampsia. In this study, the overall adverse maternal outcome rate was 5.9%. Statistical differences were found among the three groups for all adverse maternal outcomes studied. As expected, HELLP syndrome was associated with an increased risk for adverse maternal outcome (14.8%) compared with the risk associated with severe preeclampsia/eclampsia.

ARF, the most common adverse outcome in our study, was noted in 0.33% of patients. HELLP syndrome is the most frequent cause leading to ARF during pregnancy (16, 17). As expected, we found that the most frequent cause of ARF during pregnancy was HELLP syndrome (10.7%), which agreed with previous studies (16, 17). Maternal deaths associated with hypertensive disorders of pregnancy assumed greater importance than etiologies that were previously more frequently encountered, such as infection and hemorrhage. In our study, maternal deaths were due to intracranial hemorrhage and ARDS.

It is generally accepted that perinatal and neonatal morbidity and mortality rates increase in pregnancies complicated by severe preeclampsia, eclampsia, or HELLP syndrome. Different perinatal morbidity and mortality rates are presented in the literature (18-20). Although neonatal morbidity was generally

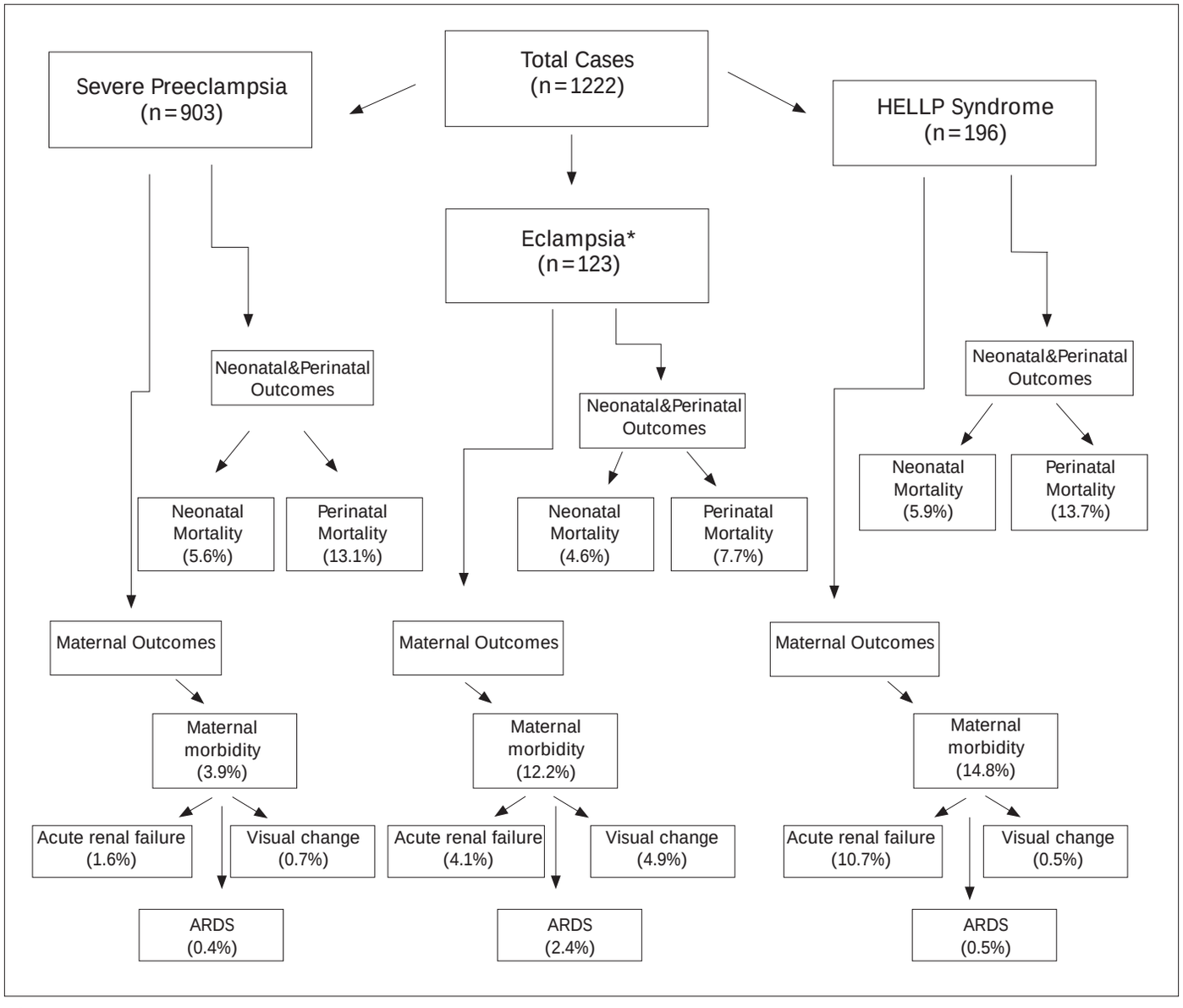

Figure 1. **Flow diagram of the study**

(*Patients with HELLP syndrome and eclampsia were evaluated in the eclampsia group. 41 cases of eclampsia experienced HELLP syndrome.)

similar across groups in our study, perinatal mortality was higher in pregnancies complicated by HELLP syndrome or severe preeclampsia before 32 weeks of gestation due to an increased number of stillbirths. Interestingly, the eclampsia group performed somewhat better considering early (<32 weeks) fetal losses. This was probably due to more abdominal deliveries and fewer growth-restricted fetuses in the eclampsia group. The high number of patients in the study population who did not have regular prenatal follow-ups may explain the high fetal and perinatal mortality. These findings suggest that perinatal morbidity and mortality are gestational-age dependent rather than disease dependent in cases with severe preeclampsia, eclampsia, or HELLP syndrome. Magann et al. determined that fetal morbidity and mortality are dependent on gestational age and reported similar and nonsignificant relationships between HELLP syndrome, severe preeclampsia, and eclampsia (21). Romero et al. showed that the majority of neonatal complications are due to prematurity (22).

Severe preeclampsia, eclampsia, or HELLP syndrome manifest on average between 32 and 34 weeks of gestation. It is common clinical practice that a 32-34-week pregnancy should be delivered immediately. Before 32-34 weeks, expectant management is generally possible for selected patients in a perinatal center (23). Steroids are administered to induce fetal lung maturity. We prefer aggressive management in cases involving HELLP syndrome and eclampsia. In our study, we determined a median gestational age of 34 weeks (range, 31.05-35.9 weeks) for the appearance of HELLP syndrome, which was lower than that for severe preeclampsia and eclampsia.

The only effective treatment for severe preeclampsia/eclampsia and HELLP syndrome is delivery, but no randomized trial has been conducted to determine the optimal method of delivery.

The rate of cesarean delivery increases with increased occurrence of hypertensive disorders during pregnancy (24). Vaginal delivery is recommended for severely preeclamptic cases in the absence of obstetric indications for a cesarean section. In our study, women with eclampsia were more likely to deliver by cesarean section than were those with HELLP syndrome or severe preeclampsia. Fetal distress was the most frequent indication for cesarean section.

In summary, as expected, this study demonstrated that pregnancies complicated by HELLP syndrome have significantly higher maternal morbidity than do those with severe preeclampsia and eclampsia. Perinatal and neonatal mortality as neonatal morbidity were similar among the groups. When an analysis was performed according to gestational age, a significant difference was observed in perinatal and fetal mortality before compared with after 32 weeks of gestation. These findings indicate that perinatal and neonatal outcomes of severe preeclampsia, eclampsia, and HELLP syndrome are dependent on the gestational age of delivery and are not diagnosis dependent.

Conflict of interest

No conflict of interest was declared by the authors.

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The technique of robotic assisted laparoscopic surgery in gynaecology, its introduction into the clinical routine of a gynaecological department and the analysis of the perioperative courses - a German experience

Uterus hastalıklarında robot yardımlı laparoskopik jinekolojik cerrahi tekniği; bir jinekoloji departmanının klinik rutinine girişi ve perioperatif sürecin analizi - bir Alman deneyimi

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Abstract

Objective: Robotic assisted surgery is an advancement on conventional laparoscopy. The first and single FDA-approved device is the daVinci™ system, which provides means to overcome the limitations of conventional laparoscopy. In Germany the use of the robotic system in gynaecology is at the threshold of a promising development. There is a wide spectrum of indications, such as simple and radical hysterectomies, including pelvic and paraaortic lymph node dissection. The introduction of the robotic system into the clinical routine is demonstrated.

Material and Methods: Robotic assisted laparoscopic interventions have been performed in the reporting hospital since April 2008. In the course of treatment of 172 cases, an increasing rise of complexity of surgical procedure has been achieved. The daVinci™ system is well adaptable in clinical routine. Hitherto, the clinical outcome has been favourable, higher-grade specific complications occurred very rarely. The short time advantages are a decrease of postoperative length of stay, a reduction of postinterventional need of analgetics and an overall accelerated period of recovery has been demonstrated compared to conventional abdominal procedures. It also shows that a drastic decrease of open conventional abdominal procedures concerning uterine pathologies appeared in the reporting department.

Results: Perioperative advantages of robotic assisted laparoscopic interventions are, above all, the decrease of morbidity (concerning blood loss, need of analgetics, length of stay, etc.). Surgical advantages are the more complex applicability, improved precision, dexterity and vision (3D), a greater autonomy of the surgeon, a smaller learning curve and an increase of preparation consistent with the anatomical structures. In contrast, disadvantages concern an initial greater time investment, the potentially different management of complications, the limited applicability in multiquadrant surgery and the difficulty regarding cost coverage respective to recovery.

Conclusions: In conclusion, robotic assisted minimal invasive surgery has an enormous potential in gynaecology; by simplifying the essential surgical procedure. The advantages of this technique will be approachability for a majority of gynaecological patients. The feasibility of a multitude of gynaecological surgical interventions has already been approved partially in a small number of cases. The upcoming challenge

Özet

Amaç: Robot yardımlı cerrahi konvansiyonel laparoskopinin bir ilerlemesidir. FDA'nın onayladığı ilk ve tek araç daVinci™ sistemidir; bu sistem konvansiyonel laparoskopinin sınırlamalarının aşılmasını sağlamaktadır. Almanya'da robotik cerrahinin jinekolojideki kullanımı başarı vadeden bir gelişmenin eşiğindedir. Geniş bir endikasyon aralığı vardır; basit ve radikal histerektomiler (pelvik ve paraaortik lenf nodlarının diseksiyonunu içeren) gibi. Burada robot sisteminin klinik rutine girişi sunulmaktadır.

Gereç ve Yöntemler: Hastanemizde Nisan 2008'den bu yana robot yardımlı laparoskopik girişimler yapılmaktadır. 172 olguluk bir seride kompleksitesi giderek artan cerrahi işlemler başarıyla tamamlanmıştır. daVinci™ sistemi klinik rutine iyi adapte edilebilmektedir. Şu ana kadar istenir klinik sonuçlar elde edilmiştir ve yüksek dereceli özgün komplikasyonlar çok ender görülmüştür. Konvansiyonel abdominal işlemlerle kıyaslandığında, kısa sürede ortaya çıkan avantajlar olarak ameliyat sonrası yatış süresinde kısalma, işlem sonrası analjezik gereksinmesinde azalma ve toplam olarak bakıldığında daha hızlı bir toparlanma gösterilebilmiştir. Raporlayan departmanda görülen uterus patolojileriyle ilgili açık konvansiyonel abdominal işlemlerde belirgin bir azalma da görülmüştür.

Bulgular: Robot yardımlı laparoskopik girişimin perioperatif en büyük avantajı morbiditenin azalmasıdır (kan kaybı, analjezik gereksinimi, yatış süresi vb.). Cerrahi avantajları, daha kompleks olgularda uygulanabilirlik, kesinlik, beceriklilik ve üç boyutlu görüşte iyileşme, cerraha daha büyük bir otonomi sağlama, öğrenme kolaylığı ve anatomik yapılara uygun yaklaşımlarda artıştır. Buna karşın, dezavantajları başlangıçta daha fazla zaman harcanması, komplikasyonların potansiyel olarak farklı tedavi edilmesi, birden çok kadranı ilgilendiren girişimlerde sınırlı uygulanabilirlik ve maliyetin yeterince karşılanamaması ile alakalı sorunlar.

Sonuçlar: Sonuç olarak, robot yardımlı minimal invaziv cerrahi jinekolojide büyük bir potansiyele sahiptir. Temel cerrahi işlemlerin basitleşmesiyle bu yöntemin avantajları jinekolojik hastaların büyük bir çoğunluğunca ulaşılabilir olacaktır. Birçok jinekolojik cerrahi girişimin fizibilitesi şu ana kadar kısmen az sayıda olguda onaylanmıştır. Şu anki

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now is to verify the short and long term advantages of robotic surgery in prospective trials, especially concerning gynaecological oncology.
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hedef robot yardımlı cerrahinin kısa ve uzun süreli avantajlarının özellikle jinekolojik onkolojiyle ilgili prospektif çalışmalarda doğrulanmasıdır.
(J Turkish-German Gynecol Assoc 2011; 12: 97-103)
Anahtar kelimeler: Robotik cerrahi, jinekoloji, daVinci tekniği, onkolojik jinekoloji
Geliş Tarihi: 16 Mayıs 2011 **Kabul Tarihi:** 21 Mayıs 2011

Introduction

The robotic assisted surgery represents an advancement of the traditional laparoscopic technique and has to be subsumed under the minimally invasive procedures. The exclusively available and FDA-approved device is the daVinci™ system by Intuitive Surgical (Sunnyvale, CA) which is widely used in divers surgical disciplines, mainly in urology, and in numerous countries, particularly in the United States. The system provides the perspective to overcome the limitations of conventional laparascopy, i. e. 2D-view, rigidity of the instruments, restricted dexterity, precision and control, rapid physical fatique of the surgeon, etc. (2, 6, 17, 24, 29, 39, 43, 45, 56). In Germany the use of the robotic system in gynaecological care is only at the beginning of a promising development. The spectrum of indications includes simple hysterectomies, above all in patients with uterine fibroids, with history of several previous abdominal operations, salpingoophorectomy in larger adnexal masses, interventions in extensive endometriosis, sacrocolpopexies, myomectomies (1, 47, 61), tubal reanastomosis and mainly the modified radical and radical hysterectomies (10, 20-22, 33, 41, 48, 49, 51, 53) in early stages of endometrial (16, 25, 26, 28, 31, 32, 36, 37, 50, 58-69) and cervical cancer (12, 42, 65), especially nerve sparing radical hysterectomy, including pelvic and paraaortic, respectively upper paraaortic lymph node dissection, but also with less frequency trachelectomy, parametranectomy (3, 11, 14, 52, 54, 55), interventions in early ovarian cancer (30, 40), pelvic exenteration (15, 35), colposuspension and the lateral colpopexy via transperitoneal approach (19, 63, 64), interventions in uterine malformations, and others (23). Many of these indications for robotic assisted surgery are validated only in a small number of cases respectively in small randomized trials (12). Concerning non-oncological and oncological gynaecological treatment increasing patient data is published showing the superiority of the daVinci™ technique compared with conventional laparoscopy.

Technique and method

The daVinci™ system consists of three components. 1. The surgeon console, which is located several meters distant to the operating table; the robotic arms, the camera and the energy source by means of stereoscopic sight, hand manipulators and pedals individually adjusted to the surgeon are controlled by him from the surgeon console. 2. The InSite Vision® system, which allows the generation of a 3D sight by using a 12 mm wide angled endoscope containing two 5 mm cameras. 3. The so-called “patient-side cart“ with the robotic arms and the attached trocars with the fixed special instruments (EndoWrist® instruments). This results in tremor elimination, graduated grasps, more degrees of freedom in the flexibility of the surgical instruments and a tremendous improvement of the surgical field vision by stereoscopic sight and the attainable magnification (Fig. 1). Some limitations such as the prolongated preparation time (positioning, setup, docking manoeuvre, etc.), the restricted haptic perception, the reduced tactility as well as the barrier in the multiquadrant surgery have to be considered (2, 6, 17, 29, 39, 43, 45, 56).

Since April 2008 robotic assisted laparoscopic gynaecological interventions with the daVinci™ system have been performed in the reporting institution (18). The implementation of this technique has been encouraged by favourable institutional conditions (availability of the daVinci™ system, specially trained OR staff). Subsequent to a two-day lab training (IRCAD [Research Institute against Digestive Cancer], Strasbourg, France) the first robotic surgical procedures took place, whereas initially only benign gynaecological disorders has been chosen in terms of exercising, basically simple total and supracervical hysterectomies with and without salpingoophorectomy (Fig. 2). In the further course an increasing rise of complexity of the surgical procedures has been carried out (increasing uterine weight, multiple myomectomies (Fig. 3, 4), applications of the system in patients with multiple previous abdominal operations); a continuing expansion of the spectrum of indication is done, i. e. radical hysterectomies (Fig. 5) with and without pelvic and paraaortic lymph node dissection (Fig. 6), treatment of extensive endometriosis, sacrocolpopexy (Fig. 7), lateral colpopexy via a transperitoneal approach. It should not be remissed that only patients have been selected which would have undergone an open abdominal operation otherwise, or patients requiring

Figure 1. Components of the daVinci™ system: A. patient side cart, B. surgical console, C. stereoscopic endoscope, D. stereoscopic viewer of the console, E. robotic camera arm, F. degrees of freedom of the EndoWrist™ instruments (source: Intuitive Surgical, Sunnyvale, CA)

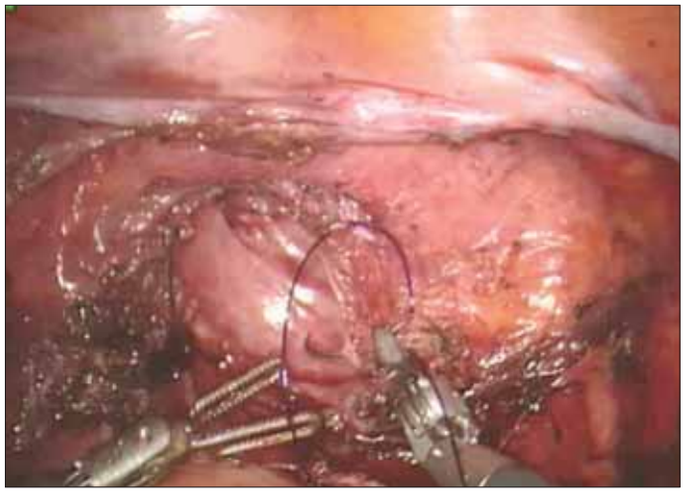

Figure 2. Vaginal cuff closure (after simple hysterectomy)

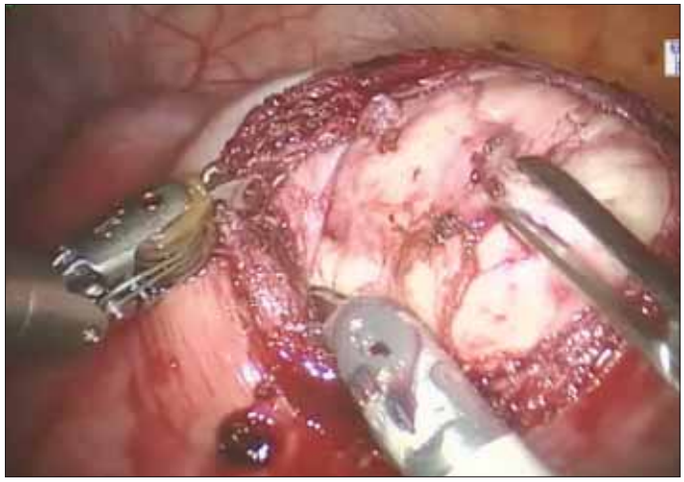

Figure 3. Myomectomy (large intramural fibroid)

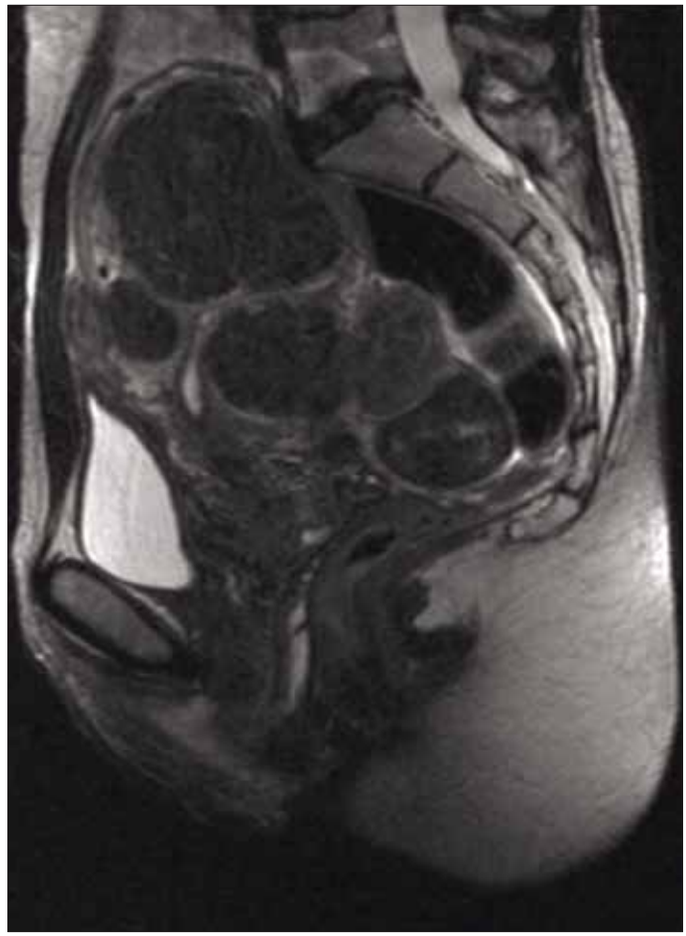

Figure 4. MRI image of a 36 years old, nonparous woman with the desire of having children with multiple fibroids with an overall weight of 800g; all fibroids could be resected; in the postoperative controll an almost normal uterine size has been documented

a protracted traditional laparoscopy with high risk of conversion to laparotomy.

The clinical data of the surgical and postoperative courses from the patients which have been operated with the assistance of the robotic system are registered systematically (data of the patient histories, surgical times, length of time of the console performance, postoperative length of stay, intraoperative and postoperative complications, course of hemoglobin concentration, length of time requiring analgetics, etc.).

Results

So far 172 patients have been undergone robotic assisted surgery with the daVinci™ system (Fig. 8); in 50% of the cases total hysterectomy, in 9.9% supracervical hysterectomy, in 23.8% single or multiple myomectomies, in 11.6% (20 cases) radical hysterectomies with pelvic lymph node dissection +/- paraaortic lymph node dissection, in 3.5% Cervicosacropexies, and 1.1% isolated pelvic lymph node dissections have been performed (Fig. 9). Up to now in these cases it could be demonstrated that the use of the daVinci™ robotic system can be implemented rapidly into the clinical routine of the department after a short period of time of initial training phase. Hitherto, the clinical outcome was favorable, higher-grade specific complications did occur very rare. In one case a partial bowel resection followed secondary because of suspicion of accidental bowel injury undetected in primary surgery (which is in the range of normal bowel complication rate in endoscopic surgery; finally a bowel injury could not be confirmed histopthologically), in 1 case (0.6%) secondary urinoma due to intraoperative right ureter lesion (likely by reason of HF surgery and consecutive thermal injury), in 1.7% (3 cases) bladder injuries occurred, which could be treated by simple double layer suturing intraoperative, in 1 case (0.6%) a lesion of the right external iliacal vein developed during pelvic lymph node dissection (handled with clipping without need for laparotomy), the conversion rate to abdominal laparotomy amounts to date 1.2% (2 cases); in 2.3% (4 cases) transient peripheral neurological disorders such as radial nerve palsy appeared induced by suboptimal patient positioning; subsequent to optimizing the patient positioning no further neurological disorders have been observed (Table 1). As short time advantages of the robotic assisted surgical procedure a decrease of postoperative length of stay, a reduction of

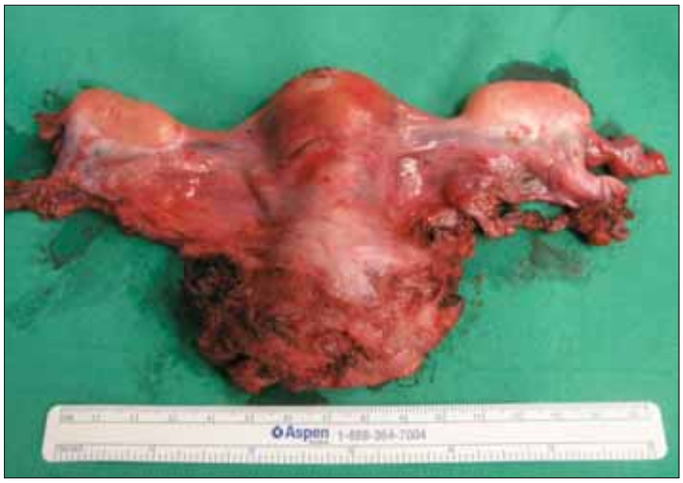

Figure 5. Surgical specimen of a 43 years old woman with a stage IB2 cervical cancer; uterine specimen of a type C radical hysterectomy with bilateral salpingo-oophorectomy (dorsal view)

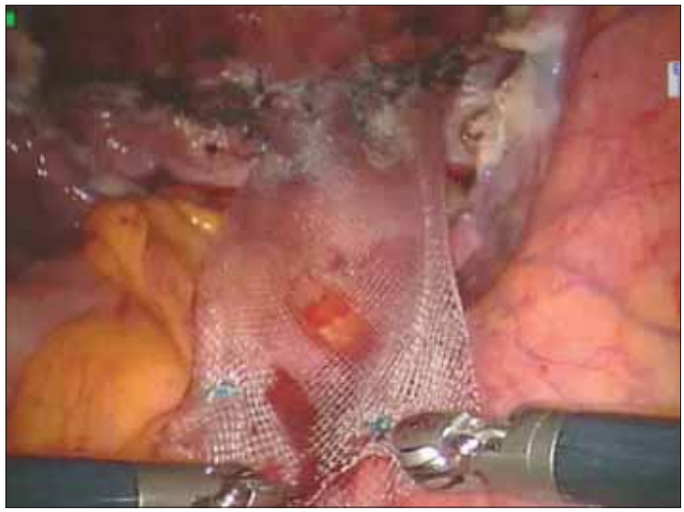

Figure 7. Cervicosacropexy (the mesh is sutured at the cervix and will now fixed at the promontorium)

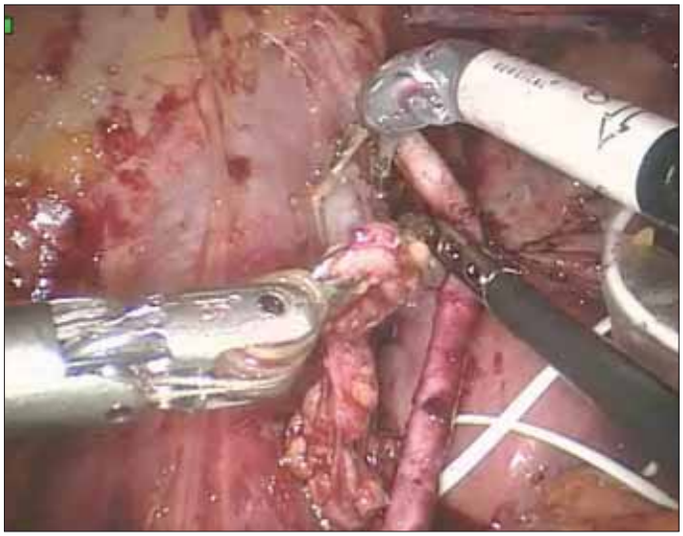

Figure 6. Pelvic lymph node dissection (left external iliacal vein)

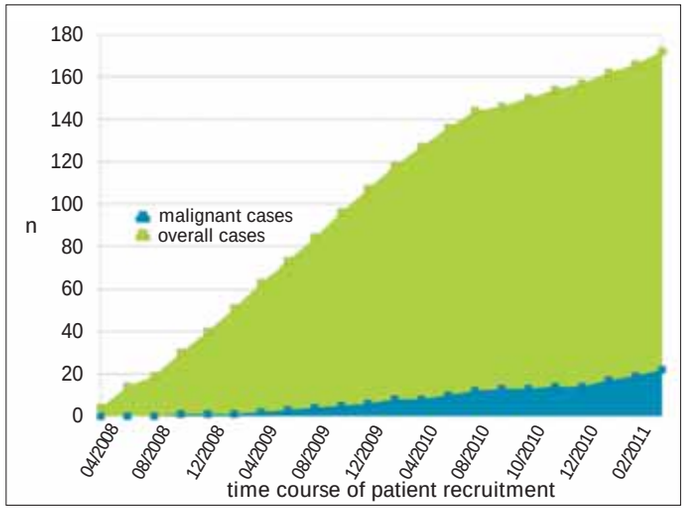

Figure 8. Time course of patient recruitment (April 2008-March 2011)

postinterventional need of analgetics and an overall quickened period of recovery could be demonstrated compared to conventional surgical procedures. As well it shows that a drastic descent of open conventional abdominal procedures concerning uterine pathologies appeared in the reporting department of the municipal hospital (Fig. 10). Furthermore the recruitment of robotic cases decelerates by reason of exclusive selection of more complex cases (such as radical hysterectomies and complex myomectomies [Fig. 8 and 11]), showing that robotic surgery advocates inversely traditional laparoscopy by making the surgeons more confident with minimal invasive procedures even in more complicated cases.

Discussion

In the field of gynaecological surgery and gynaecological oncology elderly and aged patients are frequently affected; this cohort of patients shows commonly an associated relevant comorbidity such as cardiovascular disease and metabolic syndrome. Therefore continuous efforts to reduce surgical morbidity and mortality are necessary, so to improve overall surgical outcome (7-10). On the other hand there is also a great number of young women in the reproductive age who needs gynaecological interventions such as myomectomy or complex surgical restoration in case of extended endometriosis; in this group the preservation of the physical integrity (i. e. less scars) and a quick convalescence respectively a maximal abbreviation of absence in professional and recreational life due to illness have not to be underestimated. From the employment of the daVinci™ robotic system immediate benefits such as reduced intraoperative blood loss respectively transfusion rate, reduced percentage of intraoperative and postoperative complications, shorter OR times, less postoperative need for analgetic medication and shorter duration of hospital stay would be expected,

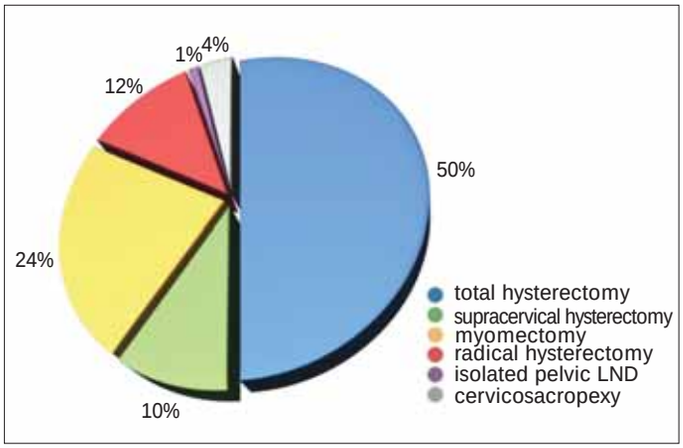

Figure 9. Spectrum of performed cases

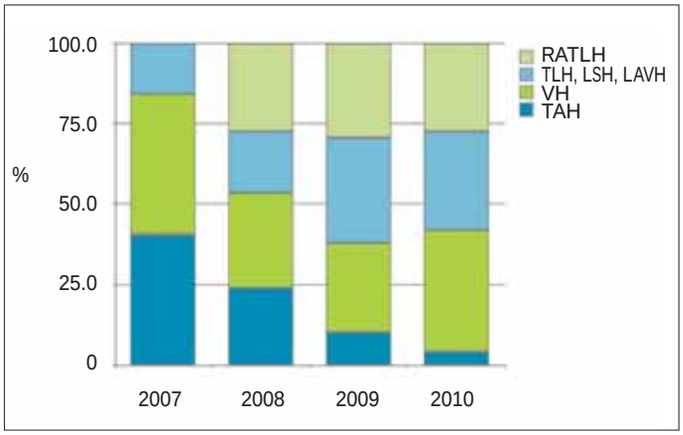

Figure 10. Shifting of the surgical approach in benign hysterectomy from 2007-2010; excluding hysterectomy for pelvic organ prolapse; shows a drastic descent of open abdominal hysterectomies, intraduction of the daVinci™ system in 2008 (RATLH = robot assisted total laparoscopic hysterectomy, TLH = total laparoscopic hysterectomy, LSH = laparoscopic supracervical hysterectomy, LAVH = laparoscopic assisted vaginal hysterectomy, VH = vaginal hysterectomy, TAH = total abdominal hysterectomy

all with the objective to decrease morbidity and subsequently the long-term costs of public health efforts (1, 2, 6, 17, 27, 29, 43, 45, 56).

Surgical advantages are the more complex applicability of minimal invasive procedures, the distinct improved precision, dexterity and surgical sight guaranteed by stereoscopic view, resulting in a diminished prostration, a better adaptation to obese patients, a smaller learning curve due to a natural surgical feeling, a greater autonomy towards difficult controllable factors (e. g. camera guidance), an increase of preparation true to the anatomical structures, and finally the expected and from the mentioned factors resulting enlargement of the possible applications, which is only limited predictable at present.

In contrast, disadvantages concern at least initially a greater time investment, a potentially different management of complications, even in relation to major hemorrhage in the surgical field, the limited applicability in the multiquadrant surgery, the

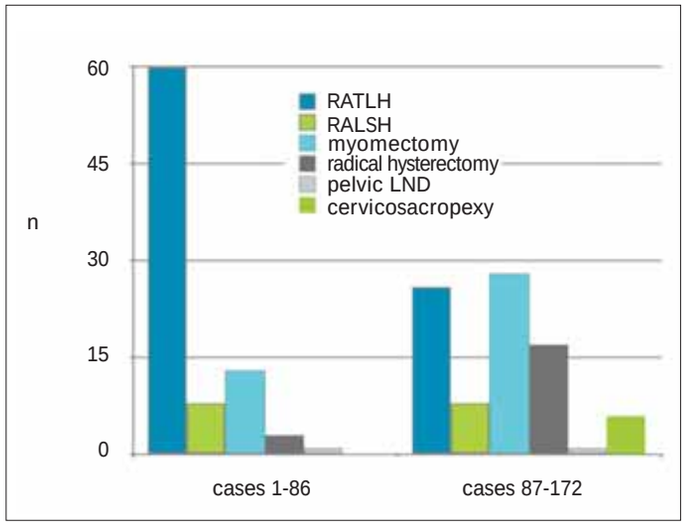

Figure 11. Change of diversity of cases; shows the trend to more complex cases (RATLH = robot assisted total laparoscopic hysterectomy, RALSH = robot assisted laparoscopic supracervical hysterectomy, LND = lymph node dissection)

Table 1. Intra and postoperative complications

Type of complication	n	%
Conversion to laparotomy	2	1.2
Bladder injury[1] with intraoperative treatment	3	1.7
Injury of the right external iliacal vein	1	0.6
Transient peripheral neurological disorders	4	2.3
Suspicion of accidental bowel injury (not confirmed)	1	0.6
Pelveoperitonitis with re-laparoscopy	1	0.6
Thermal injury of the right ureter with following urinoma[2]	1	0.6
Intraoperative Transfusion	1	0.6
Vaginal cuff dehiscence	0	0
Postoperative hemorrhage	0	0

[1]: No bladder lesion in the area of the trigonum, [2]: Conservative treatment with DJ splint and drainage of the urinoma

indispensable reliability of the program support on the part of the hospital administration and management, and the not expected amelioration regarding cost coverage respectively recovery (1, 5, 27, 31, 57).

Conclusion

The establishment of the daVinci™ surgical system for robotic assisted laparoscopy in the field of gynaecology means ultimately the participation in the next generation of minimal invasive surgical procedures as a consequence of the medical and technological progress. Because of this, numerous advances will be started as a sequel of approvement of endoscopic techniques.

The robotic assisted minimal invasive surgery has the potential to revolutionize the existing standards of the gynaecological surgical procedures, especially the oncological interventions, both by a largely elimination of postoperative morbidity and by preservation of the radicality and principles of oncological surgery (4, 5, 7-9, 13, 27, 34, 38, 44, 46). Concurrently by simplification of the essential surgical procedure these advantages will be approachable by the majority of oncologic-gynaecological patients (9). Even nerve-sparing surgical procedures in extended radical hysterectomies for what particularly the robotic assisted surgery has the potential have a promising perspective. A number of pending questions has to be answered, e. g. the insurance of the advantages of the robotic system by means of prospective controlled and randomized trials both regarding short-term parameters such as postoperative morbidity and amelioration of oncological outcome in long-term follow-up [12] and improved quality of life by reduced prolonged or chronic surgery associated morbidity, and arising costs (1, 5, 27, 31, 57). The feasibility of a multitude of gynaecological surgical interventions has been already approved partially in a small number of cases. The upcoming challenge now is to verify the short and long term advantages of robotic surgery in prospective trials, especially concerning gynaecological oncology. Otherwise, due to a broad adoption of robot-assisted surgery in industrial nations the willingness to disclaim the advantages and the comfort of robotic surgery in order to have an adequate number of control cases could be minor.

Conflict of interest

No conflict of interest was declared by the authors.

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Malignant intraperitoneal mesothelioma-Başkent University experience

Malign intraperitoneal mezotelioma – Başkent Üniversitesi deneyimi

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Abstract

Objective: To evaluate diagnostic and treatment results of malignant intraperitoneal mesothelioma in one setting.

Materials and Method: 12 patients treated for malignant peritoneal mesothelioma from January 2007 to June 2009 in Başkent University Ankara Hospital, Department of Gynaecology and Obstetrics were evaluated. In a retrospective observational study design tumour stage, grade, differentiation, time from first symptoms, pleural involvement, peritoneal cancer index, surgical cytoreduction, chemotherapeutic regimen, number of cycles, disease free survival and overall survival were evaluated. Disease free survival, overall survival, time until first symptoms were researched.

Results: The main presenting symptom was abdominal distension. Primary cytoreductive surgery followed by chemotherapy was performed in 9 patients. In 6 patients completeness of cytoreductive score below 2 was achieved. As a first line chemotherapy the most often used was cisplatin in combination with pemetrexed. Themean time from first symptoms until the diagnosis was 1.9 months. Disease free survival of 4.4±1.0 months after completing particular treatment and overall 1-year survival of 85.7 % was observed. No correlations between first symptoms (0.27, p=0.52), time until the diagnosis (-0.29, p=0.44) and overall survival were observed. Similarly, correlations between peritoneal cancer index (0.25, p=0.67), prior surgical score (-.45, p=0.37), completeness of cytoreduction score (0.61, p=0.27) and overall survival were not observed.

Conclusions: Because of the low number of patients and different treatment approaches data from a particular patient setting are inconclusive, but from the literature there is evidence that patients with malignant intraperitoneal mesothelioma should undergo optimal cytoreduction and receive a combination of cisplatin and pemetrexed as a first line chemotherapy for intravenous or cisplatin in different chemotherapy regimens using the intraperitoneal administration route, if accessible, with even higher overall survival rates.

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Özet

Amaç: Tek bir merkezde malign intraperitoneal mezoteliomanın tanı ve tedavi sonuçlarını değerlendirmek.

Gereç ve Yöntemler: Başkent Üniversitesi Ankara Hastanesi, Kadın Hastalıkları ve Doğum Departmanında Ocak 2007 - Haziran 2009 arasında malign peritoneal mezotelioma için tedavi edilmiş 12 hasta çalışmaya alındı. Retrospektif gözlemsel çalışma dizaynında tümör evresi, derecesi, farklılaşması, ilk semptomlardan beri geçen süre, plevral tutulum, peritoneal kanser indeksi, cerrahi hücre azaltımı, kemoterapötik rejim, tedavi döngülerinin sayısı, hastalıksız sağkalım ve toplam sağkalım değerlendirildi. Hastalıksız sağkalım, toplam sağkalım, ilk semptomlara kadar geçen süre araştırıldı.

Bulgular: Başlıca başvuru semptomu karında şişkinlikti. Kemoterapinin izlediği birincil sitoredüktif cerrahi 9 hastada gerçekleştirildi. 6 hastada 2'nin altında sitoredüktif tamlığı skoruna ulaşıldı. İlk seçenek kemoterapi olarak en sık kullanılan pemetreksed ile kombinasyonda sisplatin idi. İlk semptomlardan tanıya kadar geçen ortalama süre 1.9 aydı. Belirli tedavinin tamamlanmasından sonra hastalıksız sağkalım 4.4±1.0 ay ve toplam 1-yıllık sağkalım %85.7 olarak gözlendi. Toplam sağ kalım ile ilk semptomlar (0.27, p=0.52) ve tanıya kadar geçen süre (-0.29, p=0.44) arasında korelasyon gözlenmedi. Benzer şekilde, toplam sağ kalım ile peritoneal kanser indeksi (0.25, p=0.67), önceki cerrahi skoru (-.45, p=0.37), sitoredüksiyon tamlığı skoru (0.61, p=0.27) arasında korelasyon gözlenmedi.

Sonuçlar: Düşük hasta sayısı ve farklı tedavi yaklaşımları nedeniyle bu özel hasta grubundan gelen veriler bir sonuca ulaşmamıştır, fakat literatürde malign intraperitoneal mezoteliomalı hastaların optimal sitoredüksiyon geçirmesi ve ilk seçenek kemoterapi olarak intravenöz sisplatin ve pemetreksed alması veya eğer mümkünse, daha yüksek toplam sağ kalım oranları ile, intraperitoneal uygulama yolunu kullanan farklı kemoterapi rejimlerinde sisplatin alması gerektiğine dair kanıtlar bulunmaktadır

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Introduction

Primary malignant peritoneal mesothelioma is a rare tumour with a poor prognosis. Mesotheliomas are strongly associated to asbestos exposure, but only 50% of patients having peritoneal mesotheliomas have been exposed to asbestos (1, 2). In some parts of Europe, the processing of asbestos reached its peak in the middle of the 1980s, therefore a rising number of cases is expected until 2020. The highest incidence of mesotheliomas is observed in Australia, The Netherlands, United Kingdom and Italy, varying from 33-22 cases per million (3). The overall prevalence is 1-2 cases per million. Mostly the tumour arises from mesothelial cells in the pleura, while primary malignant mesotheliomas in the abdominal cavity comprise between 10 to 40% (4-8).

Regardless of the site of origin, the prognosis is usually poor, with a median survival of 4-12 months for pleural tumours and less than 1 year for peritoneal tumours (9, 10). Successful treatment is based on early diagnosis and appropriate treatment which embraces optimal tumour debulking procedure, especially from surfaces of the parietal peritoneum, and chemotherapy. It is thought that completeness on the cytoreduction score is one of the most important prognostic factors for the treatment of malignant peritoneal mesothelioma. The overall response rate reported with a single agent chemotherapy, combined chemotherapy, intraperitoneal chemotherapy, continuous hyperthermic peritoneal perfusion are 13.1%, 20.5%, 47.4%, and 84.6%, respectively (11). Cisplatin is the most studied agent, with activity in 25% of patients (12).

The present paper reports 12 cases of malignant primary peritoneal mesothelioma who were treated by debulking surgery and systemic chemotherapy in one institution.

Material and Methods

The electronic data base at the Baskent University Hospital from January 2005 to June 2009 was reviewed retrospectively for malignant peritoneal mesothelioma and included in this study. All consecutive patients with intraperitoneal mesothelioma were included in the study. Only cases with a definitive diagnosis of peritoneal malignant mesothelioma were included. Cases were accepted as mesothelioma if the light microscopy, imunohistochemistry, and clinical/surgical findings were fully consistent with the diagnosis. Benign mesothelial lesions, such as adenomatoid tumour, well-differentiated papillary mesothelioma, localized fibrous tumours, and multicystic mesothelioma were not included the study. The staging system for malignant peritoneal mesotheliomas proposed by Sebbag and Sugarbaker was selected and in most cases tumour differentiation was reported as belonging to one of three-adonomucious, epithelial and biphasic or sarcomatous type (13). Cases with uncertain diagnosis and indistinct imunohistochemistry profile were re-evaluated by a pathologist. Finally, a total of 12 peritoneal malignant mesothelioma cases were found to be eligible to enter the study. For tumour spread and completeness of cytoreductive surgery, patients were divided as having peritoneal cancer index (PCI) above or below 28 and completeness of cytoreduction (CC) denoted with a single score from 0 to 3. The completeness of cytoreduction score is defined as follows: score “0” indicates that no visible peritoneal carcinomatosis remains after cytoreduction; score “1” indicates that tumour nodules persisting after cytoreduction are less than 2.5 mm; score “2” indicates tumour nodules between 2.5 mm and 2.5 cm and score “3” indicates tumour nodules greater than 2.5 cm or a confluence of unresected tumour nodules at any site within the abdomen or pelvis.

Presence or absence of disease involving the pleural cavity was determined by computer tomography. Thickened pleura above 10 mm or pleural effusion cytologicaly approved for malignancy were considered as having concomitant pleural disease. Overall survival (OS) was considered as a primary endpoint of the study, as secondary endpoints were disease free survival (DFS) and time from first symptoms. In some cases, it was impossible to assess disease free survival, because patients were followed up in other institutions and departments in Turkey.

Prior surgery score was assessed as a complete count of surgeries for a particular patient and evaluated for correlations with survival parameters.

All of the patients included in the analysis received only systemic chemotherapy.

For data collection and calculations SPSS 17.0 was used. Correlation analysis between patients with peritoneal cancer index above and below 28, cytoreductive score, disease free, overall survival and surgical procedures was analyzed with the nonparametric Spearman’s correlation test. For correct nonparametric correlation analysis, overall survival of patients was ranged according to those who survived more and less than one year, similarly ranging was performed for correct application of disease free survival-patients were divided in those who had disease free survival more or less than 5 months. Correlation among time from first symptoms, time to diagnosis and overall survival was assessed with parametrical Pearson’s correlation test. Statistically significant difference was accepted at level of 0.05.

Results

The age range for the patients was 26-69 years with a mean age of 57 years.

Abdominal distension was the first and most often observed symptom when patients presented to hospital or outpatient unit. Mean time from first symptoms until the diagnosis was 1.9 ± 0.6 months with a range from 0-4.5 months. No correlations between first symptoms (0.27, $p = 0.52$), time until the diagnosis (-0.29, $p = 0.44$) and overall survival were observed.

Histologically, the majority of mesotheliomas were epitheloid (tubulopapillary) (10/12) with only one patient having mesothelioma of mixed subtype and one patient with biphasic subtype.

For completeness of optimal tumour debulking, such procedures as colostomy and splenectomy were performed for several patients. Parietal stripping of the peritoneum was carried out on only one patient (Table 1). There was no correlation observed between survival parameters and splenectomies (0.17, $p = 0.72$). Correlation for colostomies was not possible to assess because

Table 1. Characteristics of serum biomarker levels, tumor dissemination, management and survival for patients included in the study

Patient number	Age	Ca-125, U/ml	PCI	CC	Surgery	Histology	Pleural disease	First line Chemotherapy	DFS, months	Alive or Dead	OS, months
1	60	106.0	> 28	1	Hysterectomy + BSO + BPPLND + Omentectomy	Epitheloid	Yes	Cisplatin + Pemetrexed	4.0	Dead	13.5
2	54	398.0	> 28	2	Hysterectomy + BSO + BPPLND + Omentectomy + Appendectomy	Epitheloid	Yes	Cisplatin + Pemetrexed	7.0	Alive	23.0
3	67	696.2	> 28	2	Hysterectomy + BSO + BPPLND + Omentectomy + Appendectomy	Epitheloid	No	Cisplatin + Gemcitabine	0.0	Dead	3.0
4	26	40.2	< 28	1	BPPLND + Omentectomy*	NA	NA	Cisplatin + Gemcitabine	7.0	Alive	13.5
5	54	55.0	> 28	2	Hysterectomy + BSO + BPPLND + Omentectomy + Appendectomy + Splenectomy	Epitheloid	Yes	NA	NA	NA	NA
6	59	NA	NA		Hysterectomy + BSO + BPPLND + Omentectomy	Mixed	Yes	Carboplatin + Paclitaxel	7.0	Dead	60.0
7	64	35.6	> 28	2	Hysterectomy + BSO + Omentectomy + Appendectomy	Epitheloid	Yes	NA	NA	NA	NA
8	66	24.0	> 28	2	Hysterectomy + BSO + BPPLND + Omentectomy + Appendectomy + Colostomy	Epitheloid	No	Gemcitabine + Carboplatin	NA	NA	NA
9	52	74.0	> 28	1	Hysterectomy + BSO + BPPLND + Omentectomy + Appendectomy + Peritonectomy	Epitheloid	No	Refused	3.0	Alive	20.0
10	68	4.4	> 28	1	Hysterectomy + BSO + BPPLND + Omentectomy + Appendectomy + Splenectomy	NA	NA	Carboplatin + Paclitaxel	NA	Alive	3.0
11	56	6.5	> 28	1	Hysterectomy + BSO + Omentectomy + Appendectomy + Splenectomy	Epitheloid	No	Capecitebine + Oxaliplatin	3.0	Alive	12.0
12	69	99.5	> 28	1	Hysterectomy + BSO + Omentectomy + Colostomy + Splenectomy	Biphasic	No	Cisplatin + Pemetrexed	NA	Alive	0.5

PCI: Peritoneal cancer index; DFS: Disease free survival; OS: Overall survival; BSO: Bilateral salpingoophorectomy; BPPLND: Bilateral pelvic and para aortic lymphnode dissection; *patient who had hysterectomy with bilateral salpingoophorectomy before for benign condition; NA: Not available

patients who had colostomies had a too short follow-up period. To evaluate the result of chemotherapy for one patient explorative laparotomy was performed and for three patients secondary tumour mass debulking surgery was done.

Most patients admitted to the hospital were late stage with wide tumour dissemination and a peritoneal cancer index above 28. Only one patient had a peritoneal cancer index below 28. According to the TGM staging system proposed by Sebbag and Sugarbaker patients were staged as follows -1 patient stage II, 4 with stage III, 5 patients stage IV and for two patients there was unknown lymph node status, but regarding the extent of the disease, they were both stage III or IV. In 6 patients completeness of cytoreductive score below 2 was achieved. After completing of surgery, 5 patients did not have any evidence of metastases, for 2 patients it was not possible to asses the presence of metastases, 2 patients had parenchymal liver metastases, 1 patient had pelvic lymphnode metastases and 2 patients had paraaortic lymphnode metastases. There were 4 patients with a prior surgery score of two; all other patients had surgery only once. Correlations between peritoneal cancer index (0.25, $p=0.67$), prior surgical score (-0.45, $p=0.37$), completeness of cytoreduction score (0.61, $p=0.27$) and overall survival were not observed.

For three patients neoadjuvant chemotherapy was given and tumour debulking surgery was performed after the third cycle For three patients cisplatin in combination with Pemetrexed (ALIMTA, manufactured by Eli Lilly and Company, Indianapolis, United States) was given as a first line chemotherapy. For two

patients cisplatin in combination with gemcitabine was given as a first line chemotherapy. For three patients chemotherapy was not completed - one discontinued because of poor performance status, one patient refused and one died after the fifth cycle of gemcitabine and carboplatin. One patient received second line chemotherapy of cisplatin and gemcitabine following the first line chemotherapy of cisplatin and pemetrexed and for one patient chemotherapy was repeated six times with 6 cycles each time. For the last patient, cisplatin with paclitaxel was given as a first line chemotherapy, six cycles of topotecan was received in a second line chemotherapy; etoposide, docetaxel and liposomal doxorubicin were applied as third line chemotherapy agents. Then chemotherapy was continued with carboplatin and liposomal doxorubicin, then gemcitabine as a single agent and after that cisplatin with pemetrexed. As a palliative chemotherapy cyclophosphamide 50 mg a day with metotrexate 2.5mg 2 days a week was ordinated. Overall survival of a particular patient was 100 months.

Information regarding the clinical outcome was available for 9 of our 12 cases, with a mean disease free survival of 4.4±1.0 months after completing a particular treatment and 1-year overall survival of 85.7%.

By the end of the study three patients were dead and six were still alive.

Discussion

Malignant mesothelioma of the peritoneum is a rare disease. Despite the fact that there are no specific symptoms for malignant peritoneal mesothelioma, in the literature similar data for occurrence of abdominal distension in 56% of patients suffering from malignant peritoneal mesothelioma have been reported (14). Abdominal distension was also the most commonly observed symptom, accounting for 75.0% in our study. Manzini reported patients complaining most often about abdominal pain, comprising 35% of patients (15). We observed abdominal pain in 58.3% of our patients and for one third of patients abdominal pain was observed together with abdominal distension. Ascitis was observed in a very high proportion of patients -81.8%, whereas in the literature there are reports of ascitic collection in 36-90% of cases (16-18). This difference may be explained by an investigation method and the amount of abdominal fluid to be considered as pathologic. According to our data, mean time from first symptoms to diagnosis was 1.9 months. Other authors have reported a time interval of 122-180 days from first symptoms (15, 16). Those data indicate a rather large time interval between first symptoms and diagnosis, therefore there is still a place to improve diagnostic techniques that would lead to faster diagnostic and better cure rates.

The small number of cases precludes a uniform therapeutic approach (19). The most common treatment strategy for peritoneal mesothelioma involves a multimodality approach with surgical debulking followed by systemic and/or intraperitoneal chemotherapy. It has been observed that completeness of cytoreductive score below 2 is associated with improved survival and it is the most significant prognostic factors (13). In our study completeness of cytoreduction score below 2 was achieved for 6 patients, but no benefit or improved survival was observed over these who had cytoreductive score for completeness of 2 or higher (0.61, $p=0.27$ for OS and -0.25, $p=0.63$ for DFS). There are reports that all patients with lymph node metastases die within 2 years (20). In our study 5 patients had lymphatic or parenchymal metastases. Two of them died, two patients are alive after 20 and 23 months following the diagnosis and there is no information about one other patient who had lymph node metastases.

Regardless of improved survival rates of hyperthermic intraperitoneal chemotherapy, systemic chemotherapy is still given in most oncogynaecologic centres. For three patients neoadjuvant chemotherapy was given and tumour debulking surgery was performed after the third cycle. For one patient neoadjuvant chemotherapy of carboplatin and paclitaxel was given because this case was misdiagnosed as bulky ovarian cancer. Cisplatin in combination with pemetrexed was applied as a first line chemotherapy for three patients, nonetheless no particular chemotherapy regimen correlated with prolonged disease free survival (0.09, $p=0.85$) or overall survival (0.26, $p=0.58$) when compared to other chemotherapy regimens. In the literature, the response rates are significantly higher for patients treated with the pemetrexed in combination than for patients treated with cisplatin alone (41% versus 17%, $p<0.0001$). Patients treated with pemetrexed and cisplatin have also a significantly longer progression free survival (5.7 versus 3.9 months, $p=0.001$) and overall survival (12.1 versus 9.3 months, $p=0.02$) when compared to cisplatin alone (21).

Several studies have observed tumour response rates of between 16-48% for gemcitabine used in combination with cisplatin (23-25). Three patients from our study received cisplatin in combination with gemcitabine. There was a trend for shorter overall survival for these patients receiving cisplatin with gemcitabine when compared to other regimens, although the difference was not statistically significant (mean 13.2 vs. 21.2 months, $p=0.59$).

Another chemotherapy regimen described in the literature discloses a response rate of 26% when gemcitabine is used in combination with carboplatin (26). According to available data, this combination has lower response rates than a combination of gemcitabine and cisplatin.

In the literature there are few articles about topotecan administration for patients with mesothelioma. In a study of patients evaluated with unresectable tumours, the topotecan administered for palliative purposes reported no objective responses with 18 patients having stable disease for a median of 74 days. The median survival for all patients was 230 days, with 23% alive at 1 year (27).

One patient from our study received six cycles of topotecan as a second line treatment, after which chemotherapy was continued in a third line with liposomal doxorubicin, docetaxel and etoposide. In a study of 33 patients receiving lyposomal doxorubicin, 31 patients were evaluable for response and only two patients had a partial response with a median survival of 13 months for all the study patients (28). One patient with a variety of repeated chemotherapy cycles had an overall survival of 100 months. That may indicate the efficacy of repeated

chemotherapy cycles despite the tumour progression. A variety of chemotherapeutic regimens and administration routes with corresponding survival rates are displayed in Table 2.
Of the five patients with a cytoreductive score for completeness of 2 or higher there was available information regarding only two patients, of whom one was dead and another was still alive after 23 months from diagnosis.

Conclusion

Because of the low number of patients and different treatment approaches, data from a particular patient setting are inconclusive, but from the literature there is evidence that patients with malignant intraperitoneal mesothelioma should undergo optimal cytoreduction and receive a combination of cisplatin and pemetrexed as a first line chemotherapy for intravenous or cisplatin in different chemotherapy regimens using the intraperitoneal administration route, if accessible, with even higher overall survival rates.

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

Table 2. Summary of trials reflecting different chemotherapeutic agents, route of administration, overall (OS), disease free survival (DFS) and ongoing trials

Study	Patients n	Route of administration	Chemotherapeutic agents and patients in study arms	Median OS, months	Median DFS, months	1-year survival	2-year survival	3-year survival	5-year survival
Vogelzang et al., 2003 (21)	456	I.V.	Pemetrexed + Cisplatin (226) Cisplatin (222)	12.1 9.3	5.7 3.9	50.3% 38.0%	- -	- -	- -
Feldman et al., 2003 (28)	49	HIPEC	Cisplatin + Fluorouracil + Paclitaxel	92.0	17.0	86.0%	77.0%	59.0%	59.0%
Jänne et al., 2005 (29)	73	I.V.	Pemetrexed (32) Pemetrexed + Cisplatin (66)	8.7 13.1	- -	0.0% 66.0%	- -	- -	- -
Yan et al., 2006 (30)	62	HIPEC + EPIC	Cisplatin + Doxorubicin + Paclitaxel	79.0	- -	84.0%	-	58.0%	50.0%
Yan et al., 2006 (20)	100	HIPEC + EPIC	Cisplatin + Doxorubicin + Paclitaxel	52.0		78.0%	64.0%	55.0%	46.0%
Elias et al., 2007 (31)	22	HIPEC	Oxaliplatin (10) Oxaliplatin + Irinotecan (12)	100.0	40.0	88.0%	83.0%	68.0%	63.0%
Simon et al., 2008 (22)	20	I.V.	Pemetrexed + Gemcitabine	26.8	-	67.5%	50.0%	-	-
Hesdorffer et al., 2008 (32)*	27	HIPEC + I.V.	Cisplatin + Mitomicin + Doxorubicin	70.0	-	-	-	67.0%	-
Carteni et al., 2009 (33)	109	I.V.	Pemetrexed (38) Pemetrexed + Cisplatin (37) Pemetrexed + Carboplatin (34)	10.3 - -	6.2 - -	41.5% 57.4% -	- - -	- - -	- - -
Blackham et al., 2010 (34)	34	HIPEC	Mitomycin (19) Cisplatin (15)	10.8 40.8	8.3 10.6	47.0% 80.0%	47.0% 80.0%	42.0% 80.0%	16.0% -

I.V.: Intravenous, HIPEC: Hyperthermic intraperitoneal chemotherapy, EPIC: Early postoperative intraperitoneal chemotherapy, *: Additionally receiving radiation therapy

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Embryo culture media for human IVF: which possibilities exist?

İnsan İVF'i için embriyo kültür ortamı: hangi olasılıklar mevcut?

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Abstract

The last three decades have seen considerable progress in the development of culture media for ART and infertility treatment. Basic research on the metabolism of mammalian preimplantation embryos demonstrated the specific needs in the evolving stage of the embryo growing in vitro. Two different philosophies led to two different culture strategies for human preimplantation embryos: the 'back-to-nature' or sequential culture principle, and 'let-the-embryo-choose' or one-step culture principle. Both systems are commercially available and the discussion between the different groups of scientists is ongoing. As a matter of fact, all ART culture media currently used are not optimal for the growing human preimplantation embryo. However, further research is needed to reduce stress to the human preimplantation embryo and determine how many embryos from a treatment cycle are capable of producing a live birth.

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Key words: Culture media, back-to-nature, 'let-the-embryo-choose', media components

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Özet

Son 3 yılda ART ve infertilite tedavisi için kültür ortamı geliştirilmesinde önemli ilerlemeler görülmektedir. İmplantasyon öncesi memeli embriyosu metabolizması üzerine temel araştırma, in vitro gelişen embriyonun gelişim aşamalarındaki özgün gereksinimlerini gösterdi. İki farklı düşünce şekli implantasyon öncesi insan embriyoları için iki farklı kültür stratejisine yol açtı: 'doğaya dönüş' veya ardışık kültür ilkesi ve 'bırak embriyo seçsin' veya tek-adım kültür ilkesi. Her iki sistem de ticari olarak mevcuttur ve farklı gruplardaki bilim adamları arasındaki tartışma devam etmektedir. Gerçekte, şu an kullanımda olan ART kültür ortamlarının hiç biri implantasyon öncesi insan embriyoları için optimum değildir. Bununla beraber, implantasyon öncesi insan embriyosuna stresi azaltmak ve bir tedavi döngüsünden elde edilen embriyoların kaç tanesinin canlı bir doğum oluşturma becerisine sahip olduğunu belirlemek için daha fazla araştırmaya gerek vardır. (J Turkish-German Gynecol Assoc 2011; 12: 110-7)

Anahtar kelimeler: Kültür ortamı, 'doğaya dönüş', 'bırak embriyo seçsin', besiyeri bileşenleri

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Introduction

The goal of embryo culture in an assisted reproductive (ART) programme is to improve the quality of embryos developing in the laboratory and the chances of successful delivery of a healthy baby. Culture conditions for human embryos have evolved over the past thirty years (1-4).

Cleaving embryos normally develop in the fallopian tube, whereas the natural environment for morulae and blastocysts is the uterine cavity. Previously, it was conventional to use media permitting culture of human in-vitro fertilized embryos for 2 to 3 days to reach the four-to-eight cell stage, with additional embryo transfer to the patient (5). Premature replacement of the human embryo to the uterus may in part account for the low implantation rates associated with human IVF, with only approximately 10% of embryos transferred leading to a live birth. Further basic research on the metabolism of in-vitro fertilized embryos revealed that there are specific needs, depending on the developmental stage of the preimplantation embryo. In addition, improvements in culture media resulted from an increased understanding of the environment of the oviduct and uterus (6-8). Since 1997, the extended culture in sequential serum-free culture media has attracted more attention. The ability to culture zygotes to the blastocyst stage should help to synchronize the embryo with the female reproductive tract, and to help to identify those embryos with little development potential (3).

Evolution of embryo culture media

The idea introduced by Bernard in the late 1800s that the immediate environment surrounding living tissues is an active one led, in turn, to the notion that organs and tissues could be studied outside their setting in a suitable fluid formulated to facilitate these studies (9). Less than 10 years later, Ringer devised a solution of salts still used today in surgical treatments. It is an interesting aspect that the culture media evolved and used in the clinical setting were construed to support the development of somatic cell culture applications. The first success of fertilization of the human oocyte in vitro by Robert Edwards was accomplished in a simple, chemically defined media. These commercially available media were a modified Earle's balanced salt solution, and a modified Ham's

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F10 or T6. They were supplemented with maternal serum thus converting them into biological media (10, 11). Menezo et al. (12) broke with the tradition of using balanced salt solution and produced a medium containing amino acids without the need of a serum supplement. Another medium specifically designed for human IVF was human tubal fluid (HTF) (13). Human tubal fluid, supplemented with either whole serum or with serum albumin, gained great popularity for the use of day 2 or day 3 human embryo cultures, and has remained in use ever since.

A culture medium is a foreign environment for the human embryo. Hence, the design of media is complicated, because the components must be selected, and their concentrations determined in order to minimize stress for the cultured embryo (2, 14, 15). It also became clear that early embryos show an evolving need for energy substrates, moving from a pyruvate-lactate preference - while the embryos are under maternal genetic control - to glucose-based metabolism after activation of the embryonic genome (7, 16). Two investigators responded to these findings by modifying the HTF media. Quinn (17) removed glucose and inorganic phosphate, QB 11, for obtaining the first glucose-free medium. Pool (18) also generated a HTF variant, called Preimplantation Stage 1 or P-1 medium, a glucose- and phosphate-free medium, but additionally containing the amino acid taurine. The improved understanding of both the physiological changes in oviduct and uterus (7) and the different metabolic needs of the cleavage-stage and blastocyst-stage embryo led to the development of stage-specific or "sequential" complex media G1/G2 (3). Barnes et al. (19) used this combination to produce pregnancy and live birth after the transfer of a single viable human blastocyst, and the media were first slightly modified and marked as the GIII/G5 series of media. Other popular sequential systems, such as Quinn's series in the United States and the MediCult/Origio media from Europe and Cook from Australia, are also in widespread use. Lawitts and Biggers (20, 21) broke new ground to design a chemically defined media. They applied the principle of simplex optimization to determine the optimal concentration of each media component. This resulted in the formulation of Simplex Optimizition Medium (SOM). SOM has been modified in several ways to mKSOM^{AA}. Henceforth, it is possible to provide a one-step protocol (so-called global medium) to culture human zygotes to the blastocyst stage (2, 5). Now, there exist three commercially available one-step human embryo culture media (global®, LifeGlobal, U.S.; Gynemed GM501®, Lensahn, Germany; SSM™, Irvine Scientific, U.S.). At least nine companies now advertise media for the culture of human preimplantation embryos to the blastocyst stage (Table 1).

The recovery of immature oocytes followed by in-vitro maturation (IVM) of these oocytes led to the development of specific conventional available IVM culture media.

Two philosophies for human embryo culture media

The design of media for the culture of preimplantation embryos has been influenced by two fundamentally different philosophies (2, 22). However, growth of ART embryos is inferior to that of in vivo embryos, indicating that ART procedures invoke cellular and metabolic stress situations and the ART embryo is forced to spend energy to adapt to this foreign environment. In particular, the culture media is an important factor in successful in vitro interactions between gametes and subsequent embryo development (3). Manufacturers of human embryo culture media follow either the "back-to-nature" (sequential media) or the "let-the-embryo-choose" (global media) philosophy (23). The key components of both modern media are shown in Table 2.

The sequential culture-"back-to-nature" principle

The "back-to-nature" attempts to mimic the changing needs of the developing zygote and embryo in a media should approximate the concentration to which the embryo is naturally exposed (3, 23). The embryo is capable of actively controlling ionic gradients etc, and is able to regulate its internal environment. Therefore, with regard to embryo physiology, it is appropriate to consider the preimplantation period in at least two phases: pre- and post-compaction (3). Such a breakdown of the preimplantation period is of importance when one considers changes to medium formulations. Other considerations include the time at which the embryonic genome is activated (3).

The monoculture "let-the-embryo-choose" principle

The design of a culture medium involves the simultaneous use of all the concentrations in a mixture because the effects of each component in the medium may depend on the concentrations of the other components (23). As long as concentrations are within 'tolerable ranges', the embryo itself will adapt and utilize whatever it requires (2, 23, 24). This philosophy led to a family of media in which all of the substances necessary to early embryological development are provided, and there is no need for a media change. One-step formulation is applied throughout the entire in-vitro development from fertilization to the blastocyst stage of the embryo.

Four protocols can be used for the culture from fertilization to the blastocyst stage in an ART laboratory: [a] sequential media protocol, with an interrupted culture where two media of different compositions are used sequentially, change of medium occurs on day 3 of embryo culture, [b] sequential media protocol with fresh medium change every day, [c] monoculture, uninterrupted culture using one medium throughout the 5 days of embryo culture, [d] interrupted culture where a monoculture medium is used throughout but is renewed on day 3 of embryo culture.

Key components of ART culture media

Studies using the development of mammalian preimplantation embryos in-vitro have played a major role in the understanding of pre-embryo physiology (for reviews, see 3, 24-30). As a result, this is also the limitation in the development of culture media for the human embryo. The most widely used models for the human embryo have been the mouse and the cow.

Carbohydrates

In brief, the early embryo shows a rather simplistic physiology and maintains only low levels of oxidative metabolism, whereas it exhibits a somatic-cell like physiology after compaction

Table 1. Available commercial systems for human IVF culture

One media system			
Company	**Medium**	**Culture period**	**Website**
LifeGlobal	global®	day-1 to day-5/6	www.lifeglobal.com
Gynemed	GM501	day-0 to day-5/6	www.gynemed.de
IrvineScientific	SSM™	day-0 to day-5/6	www.irvinesci.com
Sequential media system			
Company	**Medium**	**Culture period**	**Website**
Cook Medical	Cleavage K-SICM	day-1 to day-3	www.cookmedical.com
	Blastocyst K-SIBM	day-3 to day-5/6	
CooperSurgical	Quinns Advantage®Cleavage	day-1 to day-3	www.coopersurgical.com
	Quinns Advantage®Blastocyst	day-3 to day-5/6	
FertiPro	FERTICULT™IVF Medium	day-1 to day-2	www.fertipro.com
	FERTICULT™ G3 Medium	day-3 to day-4	
InVitroCare	IVC-TWO™	day-0 to day-3	www.invitrocare.com
	IVC-THREE™	day-3 to day-5	
Irvine Scientific	ECM®	day-0 to day-3	www.irvinesci.com
	MultiBlast®	day-3 to day-5	
Origio	EmbryoAssist™	day-0 to day-3	www.origio.com
	BlastAssist™	day-3 to day-5	
	ISM1	day-0 to day-3	
	ISM2	day-3 to day-5	
Vitrolife	G-1™PLUS	day-1 to day-3	www.vitrolife.com
	G-2™PLUS	day-3 to day-5	
	IVF™	day-0 to day-3	
	CCM™	day-3 to day-5	

utilizing a wider spectrum of nutrients, biosynthetic rates are increasing, along with an increased respiratory capacity and an ability to utilize glucose (8, 28). This involves a shift in the energy requirements at the time at which the embryonic genome is activated or at the post-compaction stage. Zygotes and subsequent cleavage stages prefer pyruvate as the primary source of energy, while the eight-cell-stage embryo uses glucose (31-33). Glucose is a key anabolic precursor and is required for the synthesis of triacylglycerols and phospholipids, and as a precursor for complex sugars and glycoproteins. Glucose also metabolized by the pentose phosphate pathway (PPP) generates ribose moieties required for nucleic acid synthesis (34).

Amino acids

It has been proposed that “amino acids-(AA)”, a term which includes all 20 common and naturally occurring amino acids, are important regulators of mammalian preimplantation development (for reviews, see 8, 29, 35). Prior to embryonic genome expression, the embryo utilizes carboxylic acids and AA as energy sources (29). In addition, certain AA are known to function as biosynthetic precursor molecules (36), osmolytes (37), buffers of internal pH (38), antioxidants (39) and chelators, especially for heavy metals (40). It is important to note that there are also specific changes in the nitrogen requirements of the embryo (27, 28). The seven non-essential AA and glutamine stimulate the growth of the early cleavage embryo (41). In contrast, an inhibitory effect was seen on blastocyst development and viability if the thirteen essential AAs are presented at an early stage (42). At the post-compaction stage, both groups of AAs act stimulatory to the inner cell mass of blastocysts, while the non-essential AAs and glutamine lead to stimulation of the throphectoderm and hatching from the zona pellucida (43, 44). Leese at al. (45-47) have described AA turnover studies on the mammalian embryo and argued for “quiet” embryo metabolism during preimplantation embryo culture and development to produce the most viable embryos.

However, AAs in culture media also spontaneously undergo breakdown to release ammonium into the culture medium with concentration being time dependent. Ammonium is toxic to the embryo and reduces viability (48). Especially L-glutamine (Gln) is highly unstable in solution, where it breaks down fairly rapidly into equimolecular amounts of ammonium and pyrrolidine-5-carboxylic acid (for review see, 49). Therefore, Lane and Gardner (42) introduced a two-step (sequential) cul-

Table 2. Key components of modern media

Components	One media system Gynemed GM501®	Sequential media G-1™PLUS	Sequential media G-2™PLUS
Salts	Sodium chloride	Sodium chloride	Sodium chloride
	Potassium chloride	Potassium chloride	Potassium chloride
	Calcium chloride	Calcium chloride	Calcium chloride
	Monopotassium phosphate	Sodium citrate	Sodium citrate
	Magnesium sulphate	Magnesium sulphate	Magnesium sulphate
		Sodium dihydrogen phosphate	Sodium dihydrogen phosphate
Buffer	Sodium bicarbonat	Sodium bicarbonate	Sodium bicarbonate
Energy Substrates	Glucose	Glucose	Glucose
	Sodium lactate	Sodium lactate	Sodium lactate
	Sodium pyruvate	Sodium pyruvate	Sodium pyruvate
Non-Essential AA's	NEAA's	8 NEAA's	9 NEAA's
Glutamine Dipeptide	Alanyl-Glutamine		
Essential AA's	EAA's	2 EAA's	11 EAA's
Chelator	EDTA	EDTA	none
Macromolecules	none	Hyaluronan, HSA	Hyaluronan, HSA
Fatty acid	none	Lipoic acid	none
Vitamins	none	none	4 Vitamins
Indicator	Phenol Red optional	none	none
Antibiotic	Gentamicin	Gentamicin	Genamicin
Water	yes	yes	yes

ture media protocol to remove the accumulated ammonium. Another possibility is replacing Gln with a stable dipeptide of Gln (24). It must be noted that culture media should include sufficient levels of sulphur containing amino acids to minimize apoptosis leading to monozygote twinning (50).

EDTA (Ethylenediaminetetraacetic acid)

Its usefulness is based on its role as a ligand and chelating agent, i.e. its ability to "sequester" metal ions. After being bound by EDTA, metal ions remain in solution but exhibit diminished reactivity. The addition of EDTA to culture media alleviates the 2-cell block in mice embryos (51) and inhibits premature utilization of glycolysis by cleavage stage embryos, thereby preventing any Crabtree-like effect that is associated with arrest in culture (48). However, EDTA at a concentration of 0.1mmol/L reduces blastocyst development and cell number (52). Other investigators indicated that an EDTA concentration of 0.005-0.01 mmol/L did not have a deleterious effect on murine preimplantation or postimplantation development (53).

Regulation of cell volume-osmolytes

Maintenance of a constant volume in the face of extracellular and intracellular osmotic perturbations is a critical problem faced by all cells. Most cells respond to swelling or shrinkage by activating specific metabolic or membrane-transport processes that return cell volume to its normal resting state. These processes are essential for the normal function and survival of cells (54). The osmotic pressure of oviduct fluid is >360 mOsmol (55). However, the osmolarity of most commercially available ART culture media is lower-at about 250-300 mOsmol. When the NaCl concentration is forced up to 290 mOsmol, the development of the embryo is severely impaired (56). Addition of extracellular AA, such as glycine, betaine, proline, alanine and hypotaurin which act as organic osmolytes, protects the preimplantation embryo against hypertonicity and increases embryo development (37, 56, 57).

Impact of pH and buffers

The pH only refers to hydrogen ion concentration and is only meaningful when applied to aqueous (water-based) solutions. When water dissociates it yields a hydrogen ion and a hydroxide ion, H_2O <--> $H^+ + OH^-$ (for review see, 58, 59). It must be noted that pH is dynamic. The balance of pH depends on the association or dissociation of compounds. The most important ions are sodium, potassium, magnesium, chloride and lactate and also the AA glycine which acts as an intracellular zwitterionic buffer (60). An acceptable pH range for embryo culture media may be set between pH 7.4 and 7.2. Culture media pH is regulated by the balance of CO_2 concentration, supplied by the media and by the concentration of bicarbonate in the media. However, the intracellular

pH in human cleavage embryonic cells is pH=7.2 (61) and pH is an important cellular function which is necessary to maintain intracellular homeostasis. Moreover, after the compaction stage the preimplantation embryos appear to have more control over their intracellular pH, because of the formation of tight junctions between cells (38, 62). Hence, there is a trend to culture cleavage stage embryos in a slightly lower pH and morulae and blastocysts in a slightly higher pH (low-high paradigm). Table 3 provides information about the recommended pH of commercial available media.

In the past, handling media were used with phosphate-buffered saline solutions (PBS) or different "Good's" buffers (63). Nowadays, especially two "Good's" puffers are used in commercially IVF handling media. The most commonly used buffer is 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES at 21 mmol/L), whereas some companies include 3-(N-morpholino)-propanesulphonic acid (MOPS). Both buffers have a pK_a value of 7.2, it is the closest of the zwitterionic buffers to the pH_i of embryos of 7.12.

Although both buffers have been widely used in IVF handling, studies indicated there may be species specific sensitivities to HEPES (64, 65). Results demonstrated that, in the presence of optimal culture conditions, such as pH, gas concentration, osmolarity etc., HEPES is able to support mammalian embryo development and can also act as a chelator of heavy metals such as copper (66). If using MOPS for IVF handling at 37°C the pK_a for this buffer is actually 7.02 (59), which is low, because most IVF laboratories target their media pH at 7.3. Yet, MOPS can interact with DNA in cellular preparations (67). Currently, it has not yet been defined whether both buffers used have an impact on embryo osmotic regulation (59).

Table 3. Recommended pH-ranges of IVF culture media (adapted from Swain, 2010)

Company	Medium	pH-range
LifeGlobal	global®	7.2-7.4
Gynemed	GM501	7.2-7.4
Irvine Scientific	SSM™	7.28-7.32
	ECM®	7.2-7.25
	MultiBlast®	7.3-7.4
Cook Medical	K-SICM	7.3-7.5
	K-SICB	7.3-7.5
Cooper Surgical	Quinns Advantage®Cleavage	7.1-7.3
	Quinns Advantage®Blastocyst	7.2-7.4
FertiPro	FERTICULT™IVF Medium	7.2-7.6
	FERTICULT™G3 Medium	7.3-7.6
InVitroCare	IVC-TWO™	7.25-7.45
	IVC-THREE™	7.25-7.45
Origio	EmbryoAssist™	7.3-7.5
	BlastAssist™	7.3-7.5
	ISM1	7.2-7.4
	ISM2	7.2-7.4
Vitrolife	G-1™PLUS	7.27±0.07
	G-2™PLUS	7.27±0.07
	IVF™	7.35±0.10
	CCM™	7.35±0.10

Macromolecules

Common sources for macromolecules are proteins for culture media such as human serum albumin or synthetic serum. Both are added at concentrations of 5 to 20%. Today, most commercial media include synthetic serum in which the composition is well known. Protein in the form of albumin is thought to maintain the stability of cell membranes and chelate trace amounts of toxic components presented in culture water, media components and culture dishes. Other functions include capillary membrane permeability and osmoregulation. The presence of macromolecules in embryo culture media serves to facilitate manipulation of gametes and embryos (8). However, the uses of any blood products involve the risk of potential contamination and infection of preimplantation embryos.

Some investigators have used synthetic polymers such as polyvinyl alcohol (PVA) and polyvinyl pyrrolidone (PVP) in ART (29) but neither can be considered a physiological alternative to protein (68).

Another physiological alternative to albumin is the glycosaminoglycan hyaluronate (also called hyaluronic acid or hyaluronan). The human embryo expresses the receptor for it throughout preimplantation development (69). While hyaluronate could not only replace serum albumin in culture, it increased the implantation rate of resultant mouse embryo blastocysts (70). Therefore, hyaluronate can replace albumin as a sole macromolecule in an embryo transfer medium and in some infertile patients it can improve ongoing pregnancy rates (71).

Vitamins

The addition of vitamins as antioxidants to the culture media containing glucose and phosphate helped to prevent a loss in respiration and metabolic control (72). The following possible vitamins are components of different ART culture media: ascorbic acid, cyanocobalamin, folic acid and tocopherol. Their optimum concentrations were determined using mouse zygote assays. Moderate dosages of vitamins C and E were seen to reduce oxidative damage in mouse embryo culture and improve their blastocyst development rate (73).

Growth factors

Mammalian embryos are naturally exposed to a complex mixture of growth factors that play a key role in growth and differentiation from the time of morula to blastocyst transition. However, defining their role and potential for improving in-vitro preimlantation development is complicated by factors such as gene expression of both the factors and their receptors. The blastocyst expresses ligands and receptors for several growth factors, many of which can cross-react thus making it difficult to interpret the effect of single factors added to a culture media (74, 75).

Antibiotics

Embryo culture media are routinely supplemented with antibiotics to prevent bacterial contamination (76). Nowadays, commonly used antibiotics are penicillin (β-lactam; 100U/ml), streptomycin (aminoglycoside; 100 μg/ml) and gentamycin (aminoglycoside; 50 μg/ml). The anti-bacterial effect of penicillin is attributed to its disturbance of cell wall integrity through the inhibition of the synthesis of peptidoglycan. Penicillin has no direct toxic effects on the preimplantation embryo. Streptomycin and gentamycin disturb bacterial protein synthesis. However, the aminoglycosides show more toxic effects (76).

Literature review for comparison of media types

A number of recent studies have been conducted to compare the effectiveness of commercially available ART culture media types. A search was conducted on published literature. Interestingly, most studies prefer 3-day human IVF embryo culture and embryo transfer for comparison of different media types (77-81). Differences in embryo quality were observed in studies that used modern formulated media versus standard media, but no differences in pregnancy rates were reported (77, 78). Moreover, no differences between a single or one-step defined medium versus a cleavage-stage media with regard to fertilization, pregnancy implantation rates, and ongoing pregnancy were found by following studies (78, 81). Ebert et al. (79) reported similar results. Only the rate of pregnancy losses was significantly lower in patients with the one-step medium GM501 as compared to the Universal IVF medium.

Three studies assess pregnancy outcomes and embryo morphology after transfer of day-3, day-5 or -6 embryos (82-84). Van Langendonckt et al. (82) matched two sequential media, G1.2/G2.2 and Sydney IVF cleavage/blastocyst media. Both media yielded similar outcomes in the blastocyst transfer programme, but a lower day-3 embryo quality in the G1.2 media. The other two studies compared a single-step medium versus a sequential medium. Reed et al. (83) reported no significant difference for results on day-3 transfer. However, for day-5 transfer, a greater number of blastocysts were available from the single medium. Paternot et al. (84) described similar positive results using GM501.

Two other studies compared a single-step versus a sequential media system for the development of the human embryos to the blastocyst stage (85, 86). Biggers and Racowsky (85) found that significantly more IVF-embryos cultured in the single-step medium showed cytoplasmic pitting. IVF-blastocyst formation rates were not significantly different between the two media systems. Sepulveda et al. (86) referred to donor cycles, and had better development rates on days 3, 4 and 5 as well as significantly higher implantation rates for embryos cultured in the single medium.

Furthermore, three other studies used the mouse model for comparison of commercially available media (53, 87, 88). Biggers and colleagues (53) observed no significant differences in the proportion of the blastocysts, rates of hatching, numbers of cells in the inner cell mass and trophectoderm between KSOM^{AA} and G1.2/G2.2. However, Perin et al. (87) reported a higher blastocyst formation, higher cell numbers in the inner cell mass and higher hatching rates after culture in the single-step medium KSOM^{AA}. Hentemann and Bertheussen (88) compared two sequential media, BlastAssist M1 and M2 versus G1/G2, in a mouse model and achieved similar results.

Concluding remarks

In this review, two different types, 'back-to-nature' and 'let-the-embryo-choose', of culture media were presented with the recent literature. Both media philosophies are part of worldwide practice in the ART laboratories. Based on recent literature, it can be concluded that global one step media are at least as useful as sequential media.

Human embryos can develop in vitro in rather different types of media from basic systems to sequential complex culture media. ART culture media contain only a subset of parts which are found under in vivo conditions. Hence, embryos cultured in-vitro was exposed to constant stress. Suboptimal culture conditions force the embryo to undergo adaptations, and thus lead to lower pregnancy and higher abortion rates.

It is evident that all necessary steps in ART as part of the treatment of infertility can influence the epigenetic programming during early development (89). Therefore, it is essential that a high level of quality control exists in the laboratory, and it is suggested that further investigations are necessary to optimize environmental conditions in which the preimplantation embryo can evolve.

Conflict of interest

No conflict of interest was declared by the authors.

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H1N1 infection in Pregnancy: clinical course in two women

Gebelikte H1N1 enfeksiyonu: İki hastada klinik seyir

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Abstract

Pregnant women are one of the major risk groups for disease related morbidity and mortality from influenza A (H1N1, swine flu) pandemic. Healthy pregnant women are supposed to have 4 to 5 fold increased rate of serious illness and hospitalization compared to non-pregnant subjects. Herein, the clinical course of novel influenza A (H1N1) infection in two pregnant women was presented. One woman expired due to delay in treatment, while the other one was discharged on day six after prompt treatment. We would like to emphasize that obstetricians should be aware of the clinical and radiological manifestations of influenza A for prompt diagnosis and treatment. Obstetricians also should prepare themselves to provide adequate care for pregnancy related complications encountered by pregnant women with H1N1 infection. (J Turkish-German Gynecol Assoc 2011; 12: 118-20)

Key words: H1N1, influenza, pregnancy, oseltamivir

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Özet

Gebe kadınlar influenza A (H1N1) pandemisinde hastalığa bağlı morbidite ve mortalitede major risk grubudur. Sağlıklı gebe kadınların gebe olmayanlara göre ciddi hastalık geçirme ve hasteneye yatırılma oranlanın 4-5 kat daha fazla olduğu tahmin edilmektedir. Bu çalışmada, influenza A virüsünün iki gebe kadında gösterdiği klinik seyrin sunulması amaçlandı. Bir kadın tedavideki geçikmeye bağlı kaybedilirken, diğeri tedavinin hemen başlanması sayesinde altıncı gün taburcu edildi. Çalışmada obstetrisyenlerin influenza A'nın hızlı tanı ve tedavisi için hastalığın klinik ve radyolojik bulgularının farkında olmalarının önemi vurgulamak istedik. Obstetrisyenler tanının yanısıra, H1N1 enfekte gebede gelişebilecek gebeliğe bağlı komplikasyonların tedavisi için de hazırlıklı olmalıdır.

(J Turkish-German Gynecol Assoc 2011; 12: 118-20)

Anahtar kelimeler: H1N1, influenza, gebelik, oseltamivir

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Introduction

Pregnant women are one of the major risk groups for disease related morbidity and mortality from the 2009 influenza A (H1N1, swine flu) pandemic (1). Healthy pregnant women are supposed to have 4 to 5 fold increased rate of serious illness and hospitalization with influenza (2-5).

In April 2009, the first cases of a severe pneumonia like illness affecting mostly healthy people began to be reported from Mexico (1). In May 2009, the centers of Disease Control and Prevention in the United States released a report documenting severe complications of influenza A in pregnant women including 20 confirmed cases and one death. To our knowledge there are a few case series of pregnant women infected with H1N1 reported previously in the literature (6-8) and there were no cases reported from Turkey. In this report, we present the different clinical courses of novel influenza A (H1N1) infection in two pregnant women with emphasis on the prompt treatment.

Case Reports

Case 1

A 36-year-old female with a 29 weeks twin pregnancy was admitted to the obstetric clinic with the complaint of vaginal bleeding during coughing. A productive cough had been present for two days. Her vital signs at admission were: pulse rate 84 per minute, blood pressure 110/70mm Hg, temperature 36.5°C. She had irregular contractions of the uterus, at 5 to 10 minutes intervals. On ultrasonography, a di-amniotic and mono-chorionic twin pregnancy with low lying placenta near the internal cervical os was present. There was no cervical dilatation. Intravenous ritodrin therapy 0.3 mg/min was given for 24 hours. The next day she had no contractions, no fever, babies were well on sonography. On hospital day three, the cough progressively worsened, and the patient became dyspneic and had a fever of 38°C. She was sent for consultation to the department of internal medicine and infectious diseases. Physical examination was remarkable for bi-basilar pulmonary crackles, and third degree systolic murmur on auscultation. Her chest X-ray revealed bilateral multifocal areas of consolidation consistent with pneumonia (Fig. 1). Second degree mitral and third degree tricuspid insufficiency were detected on echocardiography. On the suspicion of influenza A, a nasal swab was obtained and oseltamivir was recommended after explaining the risks and benefits. She rejected the treatment with oseltamivir. Ceftazidime pentahydrate and budesonide were started. On admission day four, heavy vaginal bleeding occurred suddenly and the fetal heart sounds of one of the

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babies could not be detected. Sonography confirmed that one of the fetuses was dead, and the other was alive. Emergency cesarean section was performed. A female infant weighing 1100 gr with Apgar scores of 5 and 6 (1 and 5 minutes respectively) and a dead female fetus of 1020 gr were delivered. A broad hematoma extending from the prevesical area to the ileocecal region was detected. It was thought to be due to excessive heavy coughing. After operation, her condition continued to deteriorate and she was transferred to the intensive care unit. Mechanical ventilation with high FiO_2 and high PEEP was applied to maintain proper oxygenation. The diagnosis of H1N1 influenza was confirmed by polymerase-chain-reaction. Oseltamivir phosphate and levofloxacin were added to the treatment protocol. However, the patient expired on the sixth day after admission due to sepsis and progressive Adult respiratory distress syndrome (ARDS).

Case 2

A 30-year-old pregnant woman at 32 weeks gestation was admitted to the infectious disease outpatient clinic with fever, sore throat and productive cough that had been present for 2 days. On admission, vital signs included a pulse rate of 112 per minute, blood pressure of 110/80mm Hg, temperature of 38.5°C. Physical examination was notable for hyperemia of the pharynx and bilateral pulmonary crackles. Bilateral multifocal air space consolidation was detected on chest X-ray (Fig. 2). Because of the ongoing epidemic of influenza A, a nasal swab was obtained and the patient was isolated. Oseltamivir phosphate and ampicilin clavulanic acid were started for treatment, after the patient was informed of the risks and benefits of the drugs. The diagnosis was confirmed by polymerase-chain-reaction two days later. On admission day 4, cough and production of sputum were decreased, and fever disappeared. On day 5, she was discharged on her own demand. No problem was detected for the patient and her baby on the follow up one week after discharge. She was uneventfully delivered at 38 weeks of pregnancy.

Discussion

Two cases presented in this report demonstrate variability in the course of the novel influenza A (H1N1) infection in pregnant women. In the first case, antiviral therapy was delayed due to rejection of the patient. ARDS developed in a short time, leading to a catastrophic outcome. In the second case, treatment was started early, and the disease was resolved in five days. The 2009 H1N1 influenza can cause severe illness and death in pregnant women. Preexisting co-morbidities may further increase the morbidity and mortality. In our first case, there was nothing remarkable in the previous history, but cardiac valvular insufficiency was detected during the work up. It might have contributed to the rapid deterioration of the patient. In our second case, the treatment was started early, just after admission, whereas in the first case the treatment was delayed until the 4th day of admission. Obstetricians should be aware of the clinical and radiological manifestations of novel H1N1 influenza. Sore throat, fever, myalgia, cough, shortness of breath and history of household contact are the alarming signs, but some of these signs may be attributable to pregnancy. It causes delays in the initiation of therapy. Therefore, novel H1N1 influenza must be kept in mind in pregnant women with suggestive clinical findings, and treatment must be started promptly in suspected cases. Prompt diagnosis with early initiation of antiviral treatment is critical in pregnant patients with H1N1 influenza. The centers for Disease Control and Prevention (CDC) recommends prompt antiviral therapy in suspected and confirmed cases, preferably in the first 48 hours (3). In a recent series of 94 pregnant women reported from California, USA, it was stated that pregnant women

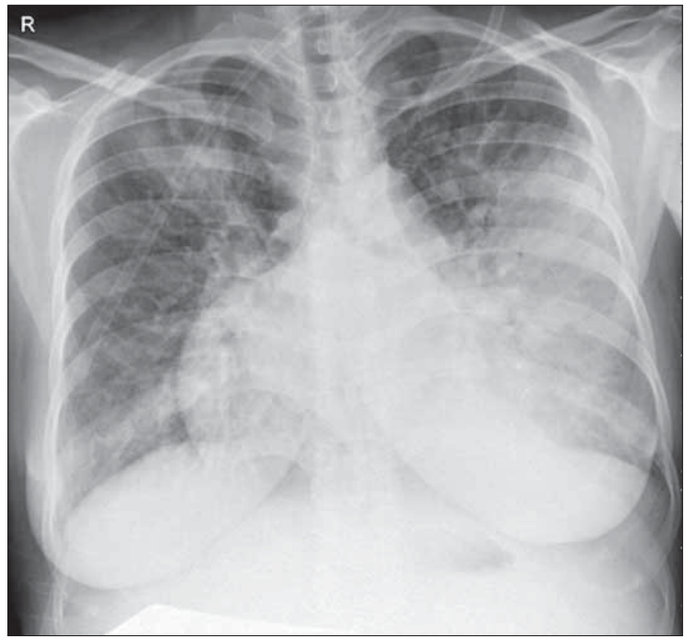

Figure 1. **PA Chest X-ray shows bilateral multifocal areas of consolidation, with left middle and lower lung zones predominant**

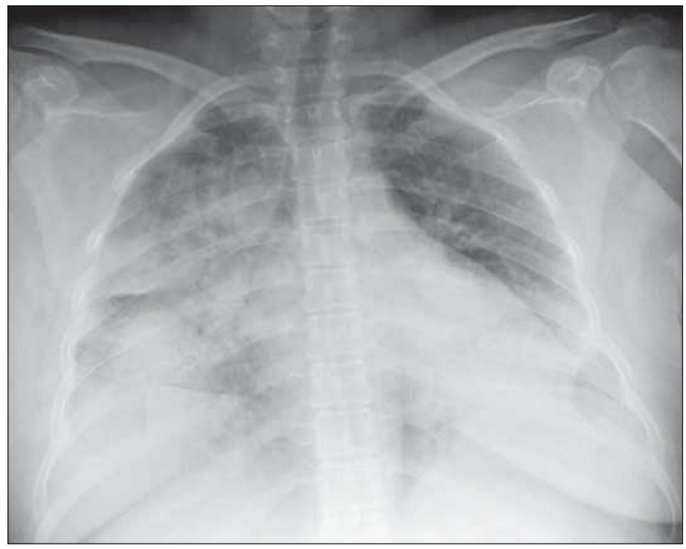

Figure 2. **PA chest X-ray shows bilateral consolidation particularly in the middle and lower zones of the right lung, and lower zone of the left lung**

who received treatment after 48 hours had approximately four fold increased risk of admission to intensive care unit or death compared to those who received treatment earlier (9). Therefore, antiviral therapy should be started as soon as possible on clinical presentation of fever, sore throat and cough without waiting for the laboratory results and the patient should be persuaded to prevent delays as in our case (9). Oseltamivir and zanamivir can both be used in pregnancy and neither of them are teratogenic (9). Since more data was available for oseltamivir, it was usually preferred in pregnant women. The subject of teratogenicity must be clarified to convince patients. The benefit of the treatment overweighs the risk for the fetus (10). The treatment should preferably be started in first 48 hours and continued for five days (75 mg Oseltamivir 2x1). For post-exposure chemo-prophylaxis, 10 days of 75 mg Oseltamivir once a day was recommended by CDC (10).

According to the January 2010 report of Turkish Ministry of Health, forty women died during pregnancy and puerperal period due to H1N1 influenza (11). Immunization is the only means of protection against H1N1 infection. Because pregnant women form one of the high risk groups, they should be encouraged to be vaccinated. Maternal vaccination generates an immune response and decreases the risk of infection in the mother and the infant up to the six month after delivery. Vaccination for pregnant women was introduced to the public in early December 2009 in our country, with a strong recommendation to all pregnant women for the sake of both mother and fetus (1).

In conclusion, pregnant women are a risk group for influenza A. The disease may progress rapidly with high morbidity and mortality in pregnant patients. Prompt diagnosis with early treatment is critical for a better outcome.

Conflict of interest

No conflict of interest was declared by the authors.

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Simple trachelectomy of early invasive cervix carcinoma in the second trimester

İkinci trimesterde, erken invaziv serviks karsinomanın basit trakelektomisi

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Abstract

Although cervical carcinoma is among the most frequently encountered malignancies during pregnancy only a small number of cases during pregnancy have been reported. Usually, the patients have been treated by radical trachelectomy with or without chemotherapy during the pregnancy.

Laparoscopic pelvic lymph node dissection with frozen section, simple trachelectomy and cerclage were performed in the 22nd week of pregnancy. The histologic examination confirmed a squamous cell carcinoma of the cervix of 35mm diameter, lymphangioinvasion (L1), low grade, clear surgical margin, negative pelvic lymph nodes according to stage Figo IB. Adjuvant chemotherapy with three cycles of cisplatin was performed after surgery. Delivery was performed by cesarean section followed by radical hysterectomy in the the 32nd week of pregnancy. Recurrent adjuvant chemotherapy with three cycles of cisplatin and local vaginal iridium radiation were performed after surgery. Patient had no surgery related complications. No relapse of cancer has been diagnosed during the following 16 months.

Simple trachelectomy may be alternative treatment option to radical trachelectomy for pregnant women with early stage cervical cancer without lymph node metastasis.

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Key words: Cervical cancer, pregnancy, trachelectomy, chemotherapy

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Özet

Servikal karsinoma gebelik süresince en sık karşılaşılan maligniteler arasında olmasına rağmen gebelik süresince olguların sadece küçük bir kısmı rapor edilmektedir. Genellikle, hastalar gebelik sırasında kemoterapi ile birlikte ya da kemoterapi olmaksızın radikal trakelektomi ile tedavi edilmektedir.

Donmuş kesit (frozen section) ile laparoskopik pelvik lenf nodu diseksiyonu, basit trakelektomi ve serklaj gebeliğin 22. haftasında gerçekleştirildi. Histolojik inceleme; lenfanjiyoinvazyon (L1), düşük derece, temiz cerrahi sınır, negatif pelvik lenf nodları ile FİGO IB evresine uyan, 35 mm çapında serviksin yassı hücreli karsinomunu doğruladı. Cerrahiden sonra üç döngü sisplatin ile adjuvan kemoterapi uygulandı. Doğum gebeliğin 32. haftasında sezaryen ile ve takibinde radikal histerektomi ile gerçekleştirildi. Cerrahiden sonra üç döngü sisplatin ile yinelenen adjuvan kemoterapi ve lokal vajinal iridyum ışınlaması yapıldı. Hastada cerrahi ile ilişkili komplikasyonlar olmadı. İzleyen 16 ay süresince kanser tekrarı tanısı konmadı.

Basit trakelektomi lenf nodu metastazı olmayan erken evre serviks kanserli gebe kadınlar için radikal trakelektomiye alternatif tedavi seçeneği olabilir.

(J Turkish-German Gynecol Assoc 2011; 12: 121-3)

Anahtar kelimeler: Servikal kanser, gebelik, trakelektomi, kemoterapi

Geliş Tarihi: 20 Temmuz 2010 **Kabul Tarihi:** 24 Ağustos 2010

Introduction

Cervical cancer is the most common gynecologic malignancy associated with pregnancy. Approximately 15% of all cervical cancers and 45 % of surgically treated stage IB cancers occur in woman under the age of 40 (1).

Radical hysterectomy terminates the pregnancy and results in the loss of future fertility. Abdominal or vaginal radical trachelectomy is a fertility-preserving alternative to radical hysterectomy for young women with cervical cancer (2-9). However, there is no evidence that a radical trachelectomy is required for all early invasive cancers (10).

This case report presents the treatment of a pregnant patient in the second trimester, with squamous carcinoma of the cervix Figo IB, by simple trachelectomy. A brief review of the literature is also presented.

Case Report

High grade lesion was diagnosed in the 20nd week of pregnancy by routine check of the cervix. The biopsy revealed invasive cervical cancer. The clinical examination revealed Figo Ib stage disease.

Fetal malformations were ruled out by sonography. Laparoscopic pelvic lymph node dissection (Fig. 1 a,b,c,d) with frozen section, simple trachelectomy and cerclage were performed in the 22 nd week of pregnancy. The histologic examination confirmed a squamous cell carcinoma of the cervix with a 35mm transverse diameter,17 mm depth, lymphangioinvasion (L1), low grade, clear surgical margin, negative pelvic lymph nodes (7+8) pT1b1 G3 N0.

Adjuvant chemotherapy with three cycles of cisplatin was performed after surgery.

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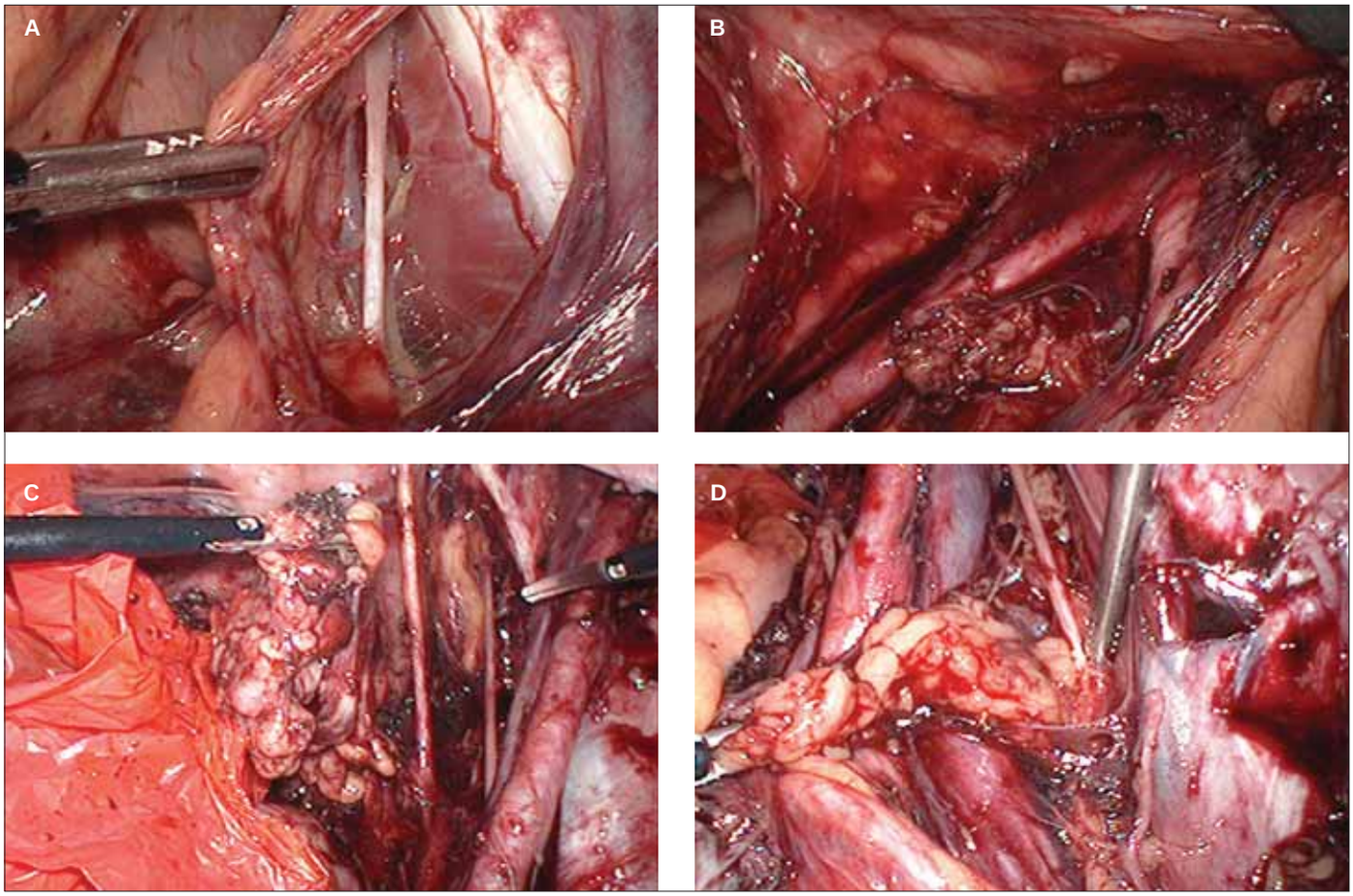

Figure 1. A) Lymphonodectomy in regio lumbosacralis, B) N. obturatorius, C) "en bloc" resection of lymph nodes in regio obturatoria, D) Lymphonodectomy in regio presacralis

Monthly examinations showed no sign of fetal or maternal complications. Because of the cortisone therapy given along with the cisplatin,lung priming was not necessary. A longitudinal laparotomy has been chosen as an approach for the C-section with following piver III hysterectomy, paraaortal, presacral lymphadenectomy and ovarian transposition. All eleven sampled lymph nodes were not infiltrated by cancer cells. The final staging was Figo I b, pT1b1,N0 (0/26), M0, G3, L1. Recurrent adjuvant chemotherapy with three cycles of cisplatin and local vaginal iridiumradiation were performed after surgery.

The newborn developed normally and showed no chemotherapically related side effects. Apgar score was 8/9/9. Post partum, a persistent ductus arteriosus with slight enlargement of the left ventricle, which was closed by conservative treatment, was diagnosed. Post partum, the patient underwent further three cycles of radio-chemotherapy. No cancer reccurence was diagnosed for the following 16 months.

Discussion

Management of cervical cancer during pregnancy depends on several factors, such as stage of the disease, nodal status, histological subtype of the tumor, term of the pregnancy, and whether the patient wishes to continue her pregnancy. However, the review of the literature showed that, in patients with early-stage disease diagnosed during the first two trimesters of pregnancy, there is an increasing tendency to delay pregnancy in order to achieve fetal lung maturity.

The largest data on fertility-sparing procedures in early stage cervical cancer has been reported with radical trachelectomy in non pregnant women (2-6). Characteristic of this method, which was first described by Dargent et al. (2), is the removal of parametrium inferior to the upper vagina. To avoid pregnancy termination by radical hysterectomy, radical trachelectomy is also used in pregnant women with early cervical cancer (7, 8). However, there is no evidence that a radical trachelectomy is required for all early invasive cancers (9). The question is whether less aggressive surgery provides similar effectiveness to radical trachelectomy. Rob et al. (10) determined the feasibility and safety of using less-radical fertility-preserving surgery in non-pregnant women in a comparative study, and suggested that large cone or simple trachelectomy combined with laparoscopic pelvic lymphadenectomy can be a feasible method that yields a high, successful pregnancy rate.

Laparoscopic lymph node dissection seems to be a safe procedure in pregnancy (11-13).

To evaluate the feasibility, toxicity, and pharmacokinetics in the maternal and fetal compartments during chemotherapy

in pregnancy patients with cervical cancer, Marnitz et al. (13) examined cisplatin levels in the amniotic fluid and umbilical cord. Amniocentesis was performed at the time of the second cisplatin cycle. They found that the concentration in the amniotic fluid samples reached 10% of the maternal blood levels. At the time of delivery, the corresponding concentration in the amniotic fluid was approximately one-third of the umbilical cord levels. However, teratogenic effects of cisplatin used in the second and third trimester of pregnancy are not described (14-17).

When the clinical stage of the disease has been determined, the family and oncologists have to make the decision either to terminate the pregnancy or perform surgery. The presence of nodal metastasis is the most important predictive factor, and its assessment is crucial in deciding whether the pregnancy can safely continue. The poor prognosis for patients with lymph node metastasis should be taken into consideration.

Treatment of stage IB cervical cancer in pregnancy by simple trachelectomy, cerclage, and laparoscopic pelvic lymph node dissection with following neoadjuvant chemotherapy as a first step therapy may improve fetal and maternal short-terrm outcome in second trimester pregnancy. Continued research in this area will determine the long term outcome of the young women.

Conflict of interest

No conflict of interest was declared by the authors.

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Prolonged usage of intravaginal clindamycin cream combined with ampicillin for the management of PPROM - a case report

PPROM yönetiminde uzamış intravajinal klindamisin kremin ampisilinle birlikte kullanımı - bir olgu sunumu

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Abstract

Prolonged PPROM may be catastrophic both for the mother and fetus due to ascending infections. The decision was expectant management in the setting of a spontaneous preterm premature rupture of membranes (PPROM) case and the prevention of chorioamnionitis was essential. We aimed to describe maternal and neonatal outcomes in expectant management of PPROM beginning from the 24th gestational week (GW) of pregnancy up to the 34th week under treatment with continuous usage of intravaginal clindamycin cream. We concluded that expectant active management of PPROM with antibiotics may be a suitable treatment option in carefully selected patients after receiving the patient's approval. Intravaginal clindamycin cream may be combined with systemic antibiotics (ampicillin and erythromycin) and may be a maintenance single drug for the prophylaxis of ascending vaginal infections.

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Key words: PPROM, Clindamycin vaginal cream, chorioamninitis

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Özet

Uzamış PPROM asendan enfeksiyonlara bağlı anne ve bebek için oldukça kötü sonuçlar doğurabilmektedir. Spontan preterm ve prematür membran rüptürü (PPROM) ile müracaat eden olgumuzda, gelişebilecek koryoamnionit riskine karşı korunma zorunlu olup tedavi yaklaşımı ekspektan yönetim olarak belirlenmiştir. Amacımız 24. gebelik haftasında PPROM ile müracaat eden olgunun, 34. gebelik haftasına kadar intravajinal klindamisin kremin sürekli kullanımı ile takibi ve maternal, neonatal sonuçlarını tariflemektir. Sonuç olarak, PPROM olgularında antibiyotikler ile ekspektan aktif yönetim, dikkatli seçilmiş ve onamı alınmış olgularda uygun tedavi yaklaşımı olabilir. Asendan enfeksiyonlardan korunmada vajinal klindamisin sistemik antibiyotiklerle (ampisilin ve eritromisin) kombine tercih edilebileceği gibi idame tedavide tek ajan olarakta kullanılabilir.

(J Turkish-German Gynecol Assoc 2011; 12: 124-6)

Anahtar kelimeler: PPROM, Klindamisin vajinal krem, koryoamnionit

Geliş Tarihi: 01 Haziran 2010 **Kabul Tarihi:** 03 Ağustos 2010

Introduction

Preterm births account for 75% of perinatal mortality and more than half the long-term morbidity. 25-30% of preterm deliveries follow preterm premature rupture of membranes (PPROM) which is defined as the spontaneous rupture of membranes before 37 completed weeks of gestation and before labor onset. It occurs in approximately 3% of pregnancies and is associated with significant

Maternal and neonatal infection, abruptio placenta, preterm delivery and cord prolapse are potential complications of PPROM. One-third of women with PPROM develop potentially serious infections, such as chorioamnionitis, endometritis or septicemia (1). The fetus and neonate are at maternal, fetal and neonatal risks. greater risk of PPROM-related morbidity and mortality than the mother, such as hyaline membrane disease, intraventricular hemorrhage, periventricular leukomalacia, infection (eg, sepsis, pneumonia, meningitis), and necrotizing enterocolitis. The rates of these morbidities vary with gestational age and are higher in the setting of chorioamnionitis (1).

Bacterial vaginosis is the overgrowth of vaginal mixed anaerobic flora over normal lactobacillary bacteria and is associated with serious perinatal complications such as miscarriage, preterm delivery and PPROM (2). The precise pathophysiology is peculiar, but ascending infection leading to endometrial inflammation is suspected. Here we would like to report a PPROM case in the setting of bacterial vaginosis in the previability zone which was managed successfully and yielded a healthy newborn.

Case Report

A 34-year old G2P1 patient was referred to Gulhane Military Medical Academy, Obstetrics and Gynecology department

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due to rupture of the membranes at 24 weeks of pregnancy. Her first pregnancy was uneventful except for a cesarean section which was done due to a breech presentation. Until about 24 weeks, she was followed up in a primary health care unit. She had her last appointment 1 month previously for second trimester anomaly screening, which was reported as normal. Painless rupture of the membranes occurred 12 hours before her admission to our unit without any history of trauma. Obstetric ultrasonographic examination (Siemens Sonoline Antares™) revealed a live, 24 week-old, severe oligohydramniotic fetus with 30mm amniotic fluid index (AFI) and cervical length was verified by transvaginal probe as 35 mm. The fetal umbilical artery, ductus venosus and middle cerebral artery doppler indices were within normal limits. On sterile speculum examination, clear amniotic fluid passing from the external cervical os was seen by Valsalva maneuver and also thin homogenous vaginal discharge with a fishy odor was noticed. On wet mount of vaginal discharge, typical 'clue cells' were seen. No uterine contractions were identified by the tocometer probe. There was no sign of chorioamnionitis confirmed by her physical exam and laboratory findings. (Body temperature 36.8°C, blood pressure 110/70 mmHg, pulse rate 82/min, leukocyte count 8800/mm^3 with a sedimentation rate of 8mm/hr).

The therapy options and fetal-neonatal complications were discussed in detail with the family. Despite the fact that expectant management can lead to poor perinatal outcomes, the family decided to continue the pregnancy. After receiving a consent form from the family she was hospitalized. Due to PPROM, sulbactam sodium-ampicillin sodium IV 1 gr, q12hr was started and used for the first 48 hours and then erythromycin p.o. 500 mg, q8hr was used until the 10^{th} day. Clindamycin vaginal cream 2% q24hr was started concomitantly with Ampicillin therapy. Betamethasone in two divided doses of 12 mg was administered at twelve hour intervals. As there were no uterine contractions or sign of cervical dilatation, no tocolytic therapy was indicated.

One week later, in her examination; the discharge associated with bacterial vaginosis had disappeared but there was still some clear amniotic fluid oozing from the cervix. As the rupture of membranes was accompanied by active vaginosis, in order to minimize intrauterine infection risk, clindamycin therapy was continued once a day. After this initial treatment, she was followed-up bi-weekly by ultrasonography and her laboratory findings. Fetal growth indices were appropriate on her 28^{th} GW, and AFI was 52 mm. Since there was no sign of chorioamnionitis and the fetal status was reassuring, close follow-up was continued. AFI measurement at 30 and 32 weeks gestation were 54 and 58 mm respectively. Amniotic fluid oozing went on until the end of her pregnancy and so the clindamycin therapywas continued. Her pregnancy remained stable until 34 weeks of gestation when irregular contractions started and cervical dilation occurred. She underwent a repeat cesarean section; a 1840-gram healthy male fetus was delivered. The 5-minute Apgar score was 9. Postoperative course was uneventful both for the mother and newborn. The newborn did not develop respiratory distress syndrome and there was no sign of pulmonary hypoplasia, skeletal deformity or systemic infection as expected. He was followed up in the neonatal intensive care unit (NICU) for 1 week and finally discharged in a healthy condition with a follow-up appointment for developmental assessment.

Discussion

In the etiology and consequences of Premature Rupture of Membranes (PPROM), the gestational age and fetal status at membrane rupture have significant implications, thus maintaining and prolonging pregnancy has been the mainstay of the treatment. Starting at 23 weeks gestation, one week increments in gestational age are associated with substantial improvements in survival when delivery occurs between 23 and 32 weeks gestation (3). Nevertheless, in such conditions, if the clinician's decision is expectant, life-threatening risks such as stillbirth, maternal and perinatal infection-sepsis, abruptio placenta and oligohydramnios related conditions such as skeletal deformities, pulmonary hypoplasia and cord compression are probable complications and should always be kept in mind. These complications should always be discussed with the family before giving expectant management decision. In the present case we gained approximately 10 weeks by expectant management and this period is invaluable for a 24 week fetus.

Oligohydramnios was found to be associated with a shorter latency period in PPROM cases (4). Low amniotic fluid volume at admission has also been associated with adverse pregnancy outcomes. The pathophysiology still remains unclear. In the present case the patient was severely oligohydramniotic on admission but in her follow-up the amniotic fluid volume increased over time and it prevented the infant from worse complications such as pulmonary hypoplasia, facial deformation, and orthopedic abnormalities. So, the amniotic fluid volume is an important prognostic indicator for the assessment of neonatal outcome of the PPROM fetuses (5).

All women with PPROM should be monitored for signs of infection; these should include, routine clinical parameters; maternal temperature, uterine tenderness and contractions, maternal and fetal heart rate. Antibiotic therapy for PPROM is now routinely used and it is associated with prolonged time to delivery and reduced neonatal morbidity (6). The goal of antibiotic therapy is to reduce the frequency of maternal and fetal infection and delay the onset of preterm labor. The type and duration of antibiotic therapy vary in different studies (7) and there is no general consensus yet. Mine S. et al. concluded that continuous antibiotic prophylaxis in PPROM does not improve the outcome and its cost is ineffective. They suggest intermittent and short-term antibiotic prophylaxis as preferable to the continuous antibiotic prophylaxis in PPROM patients (8). In a recent Cochrane review, 14 placebo-controlled randomized trials involving over 6000 women evaluated the use of antibiotics following PPROM before 37 weeks of gestation (9). Compared to placebo/no treatment, antibiotic use was associated with a significant reduction in chorioamnionitis (RR 0.57, 95% CI 0.37-0.86), There was inadequate data to determine whether any antibiotic regimen (drug, dose, duration) was better than another, but macrolide antibiotics (eg, erythromycin) appeared to be safer than beta-lactam antibiotics (eg, amoxicillin-clavulanate),

as the latter were associated with an increased risk of necrotizing enterocolitis. In a review, ampicillin 2 g IV every 6 hours and erythromycin 250 mg IV every 6 hours for 48 hours followed by amoxicillin 250 mg orally every 8 hours and erythromycin 333 mg orally every 8 hours for 5 days was suggested (10).

We administered sulbactam-ampicillin IV 1 gr. bid as a starting regimen for the first 48 hours and then erythromycin p.o 500 mg bid was used until the 10th day. On the other hand clindamycin vaginal cream 2% once a day was started concomitantly with Ampicillin and used until delivery. This antibiotic regimen also provided adequate prophylaxis for Group B streptococcus (GBS), which is indicated in women whose GBS test results are positive or unknown.

An important infectious condition leading to PPROM and preterm birth is bacterial vaginosis. Bacterial vaginosis leads to a reduction in normal lactobacillar bacteria in vaginal flora and is caused by mixed anaerobic flora. Bacterial vaginosis is present in up to 20% of women during pregnancy (11) and the majority of these patients are asymptomatic. Endometrial inflammation and direct ascending bacterial invasion of the membranes are suspected, but the exact mechanism of how bacterial vaginosis leads to preterm birth is still unclear. The classical diagnosis of bacterial vaginosis is confirmed by the criteria of Amsel (12) and our case fulfills three out of four. The evidence to date does not suggest any benefit in screening and treating all pregnant women for asymptomatic bacterial vaginosis to prevent preterm birth (13). However, when bacterial vaginosis accompanies PPROM, in order to end this vicious circle, we usually prefer using antibiotics. The most widely used antibiotics for bacterial vaginosis are metronidazole and clindamycin, and we preferred clindamycin by vaginal route.

Clindamycin is categorized as Group B by FDA pregnancy risk categories. Fetal tissue levels increase following multiple dosing, with the drug concentrating in the fetal liver, but no reports linking the use of clindamycin with congenital defects have been reported (14). Usually one week of therapy is sufficient in pregnancy, but in association with PPROM, we used it as long as the passing of amniotic fluid continued for prophylaxis of ascending infections. This administration was not only for bacterial vaginosis but also to prevent other vaginal flora members which may reach the amniotic cavity. The possible emergence of resistance and fetal risks were our concern and further studies are needed to evaluate the benefit-hazard ratio. Although antibiotherapy seems to be beneficial in this case study, further investigations are needed to show their outcomes for prolonging latency durations of PPROM subjects. This possible limitation pertains to the generalizability of our study to the general population.

In conclusion, when PPROM is accompanied by bacterial vaginosis, expectant active management with corticosteroids and antibiotics may be suitable for carefully selected patients. In such a condition the final treatment option may be the patient's decision, which should be confirmed by a consent form. As we represent in our case, local clindamycin therapy may be a good choice for the prevention of ascending vaginal infections. Close and careful follow-up with appropriate antibiotics may prolong the latency period to bring a desperate fetus from the lower limits of viability to "life-zone".

Conflict of interest

No conflict of interest was declared by the authors.

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Conservative management of placenta previa percreta by leaving placental tissue in situ with arterial ligation and adjuvant methotrexate therapy

Plasenta previa perkreata'lı olgunun plasental dokunun çıkarılmadan, arter ligasyonu ve adjuvan metotreksat terapisi ile konservatif yönetimi

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Abstract

Placenta percreta is one of the life-threatening conditions in modern obstetrics. The rising caesarean section rate means rising placenta percreta rate. Treatment strategies range from a caesarean hysterectomy to leaving the placenta in situ with or without internal iliac artery ligation/uterine artery embolisation and/or methotrexate therapy. We describe a case of placenta previa percreta which we managed successfully with conservative modalities.

(J Turkish-German Gynecol Assoc 2011; 12: 127-9)

Key words: Placenta percreta/accreta, methotrexate, conservative management

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Özet

Plasenta perkreata modern obstetrideki hayatı tehdit eden patolojilerden biridir. Artan sezaryen doğum oranı artan plasenta perkreata oranı anlamına gelmektedir. Tedavi stratejileri sezaryen histerektomiden plasentanın içeride bırakılması veya internal iliak arter ligasyonu/uterin arter embolizasyonu ve/veya metotreksat tedavisine kadar uzanmaktadır. Bu yazıda konservatif yöntemler kullanarak başarılı şekilde yönettiğimiz bir plasenta previa perkreata tanılı olguyu takdim etmekteyiz. (J Turkish-German Gynecol Assoc 2011; 12: 127-9)

Anahtar kelimeler: Plasenta perkreata/akreata, metotreksat, konservatif yönetim

Geliş Tarihi: 15 Mayıs 2010 **Kabul Tarihi:** 03 Ağustos 2010

Introduction

Placenta previa is defined as a placenta implanted on the lower uterine segment and cervical ostium that prevents descent of the fetus. It is an important cause of second and third trimester vaginal bleeding. Placenta percreta is an adherence anomaly caused by the absence of both decidua basalis and Nitabuch's layer, which results in a direct attachment of chorionic villi to the myometrium. The risk of abnormal placental implantation increases with the number of previous cesarean sections (1): 4-fold after one, up to 11.3 fold after two. Furthermore, patients with a previous cesarean section and placenta previa are five times more likely to have placenta accreta or percreta. We report a case of placenta previa who had a previous cesarean section and anterior placenta percreta.

Case

A 34-year-old G2P1 woman with one previous cesarean section was diagnosed with anterior placenta previa totalis at the 38th week of gestation. Magnetic resonance imaging scan showed abnormal uterine bulging with dark intraplacental bands which were associated with myometrial invasion and also suspicious bladder invasion (Figure 1). She had undergone cesarean section electively. There were firm adhesions between the anterior part of the uterus and the bladder. The lower segment was totally adhered. An oblique incision was performed at the fundus of the uterus and a 3000 gr healthy baby was delivered. The placenta was left inside with the cord tied. The posterior branches of the internal iliac arteries were ligated bilaterally. After filling the bladder with 1000 cc physiologic saline, placental invasion was confirmed and the hysterectomy procedure were postponed. She was transfused with 1 unit of blood during the operation. In the follow up; she received methotrexate 100 mgr protocol by weekly intervals for five times (2). Her β-HCG level was 1241 mIU/ml on day 1 of operation and 16 mIU/ml on the 60th day. Doppler ultrasounds were performed in the postpartum 2 months period. There were no changes in the placental mass size, vascularisation, bladder invasion and the lost view of fatty planes between uterus and bladder. MRI scan showed heterogeneous signal intensity throughout the placenta. The patient underwent operation again to remove the remnant tissues. Bladder mucosa had over vascularisation but did not have any invasions. Bilateral ureteral double j catheters were introduced. The firm adhesions between the anterior part of the uterus and bladder were not affected by metho-

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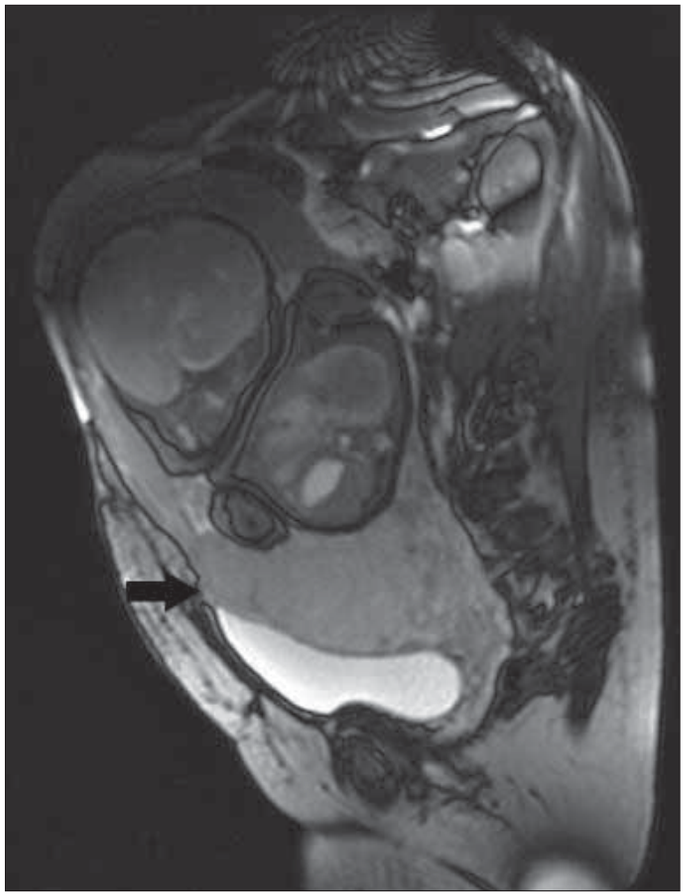

Figure 1. Abnormal uterine bulging. Sagittal T2W image of a uterus at 38 weeks gestation showing placenta previa and focal bulging of the lower uterine segment, altering the normal pear shape of the gravid uterus (arrow). The patient was proved to have placenta percreta at the time of C/S

trexate therapy, so the hysterectomy procedure could not be performed. The uterus was incised longitudinally at the fundal part and the placental mass were removed manually. 4 units of blood were also transfused. She was discharged 2 weeks later. Her regular mensturation returned 4 months after the operation. At the sixth month postparum, her β-HCG level was 0.1 mIU/ml, whereas abdominal ultrasound showed incomplete involution, with an echogenic focus in the myometrium suggestive of calcified placental remnants.

Discussion

The increasing rate of C/S is a growing world-wide problem in modern obstetrics. Especially women, who have had a prior C/S, have the risk of facing adherence anomalies of the placenta and their complications. Placenta accreta, placenta increta and placenta percreta represent a spectrum of abnormal implantation by the placenta. Placenta percreta is the most severe of the implantation anomalies, with invasion of the myometrium and uterine serosa, often with extension into neighboring organs (3). It has been reported that placenta accreta/percreta occurs in 5% of cases of placenta previa with unscarred uterus, rising to 25% with one previous cesarean section (4). The major risk factor for accreta is the presence of a placenta previa with a previous cesarean scar, a situation requiring hysterectomy in 66% of cases (5). Our case had had a prior C/S and placenta previa totalis which made us suspect adherent placental tissue.

Antenatal detection success of adherence by ultrasonography and/or MRI varies between 50 and 80%, but the distinction between the grades of placental invasion of the myometrium may be very difficult to discern (6). In a review by Comstock, several ultrasonographic features including the presence of placental lacunae, myometrial thickness, loss of the clear space between placenta and myometrium, and anomalies of the bladder-myometrium interface have been documented to be associated with a higher risk of placenta accreta (7). Lax et al. reported that MRI scans showing abnormal uterine bulging of the normal pear shape of the gravid uterus, heterogeneity of the signal intensity of the placenta on T2W images and the presence of T2 dark linear bands of intraplacental signal intensity have a statistically significant difference between patients with placental invasion and those with normal placentation (8). Similar findings were detected in the MRI scans of our case.

It is accepted that a patient with complete placenta previa needs to be delivered by C/S. There are two major management options for placenta percreta; cesarean hysterectomy and cesarean delivery with conservative management of the placenta. Hysterectomy has long been the primary treatment for placenta accreta by ACOG (9) but it is associated with a high morbidity and mortality, including the psychological consequences of loss of the uterus in a young woman. The novel approach of treating women with placenta left in situ with methotrexate, was first reported in 1986 (10). This significantly reduces morbidity from severe haemorrhage, particularly with bladder involvement (11). Lam et al. reported a case of successful management of placenta accreta without any hysterectomy or methotrexate therapy because of the patient's strong desire to retain the uterus and side effects of the drug (12). In another case report by Crespo et. al, the patient received 4 doses of methotrexate intramuscularly every other day, alternating with 4 doses of 15 mg folinic acid to reduce placental residual tissue which was retained in a cesarean delivery and seven months later, she passed a fleshy tissue mass vaginally then a subsequent ultrasonograhic examination documented an empty uterine cavity (13). In our case, we tried to avoid severe haemorrhage and bladder injury by leaving the placental tissue inside the uterus. We planned to perform hysterectomy but this procedure was postponed because of the placental invasions and firm adhesions. We ligated the internal iliac arteries and added methotrexate as adjuvant therapy.It decreased the level of β-hcg, but did not make any changes in placental mass or invasion so we had to perform another surgical intervention to remove placental tissue. We did not wish to perform bladder resection. We removed the placental residual mass manually with a uterine fundal incision. After the second complementary operation, she was not given any other therapy. Six months later, her regular mensturation returned and β-hcg level was 0.1 IU/ml. Ultrasonography showed a 6 week uterus and myometrial echogenic focus of calcified placental remnants.

Selective arterial embolisation is another therapy to avoid massive obstetric haemorrhage. The procedure, however, is usually available in only a small number of tertiary centres and requires appropriately trained interventional radiologists.

Conflict of interest

No conflict of interest was declared by the authors.

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Erratum

Dear Editor

We made some mistakes on Table 4 data of article entitled ' Comparison of ovulation induction and pregnacy outcomes in IVF patients with normal ovarian reserve who underwent long protocol with recombinant-FSH and highly purified-hMG' which was published in 2011 in your Journal. If it is possible to change the related paragraph as 'In our study, the clinical pregnancy rates were 18.2 in the rec-FSH and 13.9 in the HP-hMG groups and the ongoing pregnancy rates were 15.9 and 13.9, respectively', I will be pleasured. I would like to apologize for this error.

Best Regards,
Cem Çelik

Pelvic splenosis mimicking ovarian metastasis of breast carcinoma: a case report

Meme karsinomunda over metastazını taklit eden pelvik splenozis: Bir olgu sunumu

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Abstract

Splenosis is the heterotopic autotransplantation of splenic tissue that usually follows traumatic splenic rupture and splenectomy. Implanted splenic tissue may give rise to a mass or masses in the chest, abdomen, or pelvis which the clinician must distinguish from benign or malignant tumors. A 38-year-old multiparous woman presented for a routine gynecological examination during breast cancer treatment. She had undergone splenectomy following traumatic splenic rupture at the age of 13. Pelvic examination revealed a left adnexal mass. Transvaginal ultrasonography showed a 39x56x40 mm diameter hyperechoic, hypervascular solid tumor on the left ovary. Total abdominal hysterectomy and bilateral salpingo-oophorectomy was performed. The postoperative histological diagnosis was splenic tissue. Splenosis must be considered in the differential diagnosis of previosly splenectomized patients who present with unexplained masses.

(J Turkish-German Gynecol Assoc 2011; 12: 130-2)

Key words: Splenosis, splenectomy, adnexal mass

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Özet

Splenozis, travmatik dalak rüptürü ve splenektomiyi takiben dalak dokusunun heterotopik ototransplantasyonudur. İmplante olan dalak dokusu klinisyenin karşısına göğüs boşluğu, karın boşluğu veya pelviste, iyi yada kötü huylu tümörlerden ayırmasını gerektiren kitle veya kitleler olarak çıkabilir. Otuzsekiz yaşında multipar kadın hasta meme kanseri tedavisi sırasında rutin jinekolojik muayene için başvurdu. Onüç yaşında, travmatik dalak rüptürünü takiben splenektomi geçirmişti. Pelvik muayenede sol adneksiyal kitlesi vardı. Transvaginal ultrasonografide sol over üzerinde 39x56x40 mm çaplarında hiperekoik, hipervasküler solid tümör saptandı. Total abdominal histerektomi ve salpingoooferektomi yapıldı. Operasyon sonrası patolojik tanı dalak dokusu olarak geldi. Splenozis daha önce splenektomi geçiren hastalarda açıklanamayan bir kitle saptanıldığında ayırıcı tanıda düşünülmesi gereken bir durumdur.

(J Turkish-German Gynecol Assoc 2011; 12: 130-2)

Anahtar kelimeler: Splenozis, splenektomi, adneksiyal kitle

Geliş Tarihi: 20 Haziran 2010 **Kabul Tarihi:** 20 Ağustos 2010

Introduction

Splenosis is the heterotopic autotransplantation of splenic tissue that usually follows traumatic splenic rupture and splenectomy (1). Implanted splenic tissue may give rise to a mass or masses in the chest, abdomen, or pelvis which the clinician must distinguish from benign or malignant tumors (2). Splenosis is usually asymptomatic, but there are reported complications directly related to splenosis. Occasionally, patients present with nonspecific abdominal pain due to infarction, an enlarging abdominal mass with associated infection, intestinal obstruction due to adhesive bands of implants, gastrointestinal hemorrhage or hydronephrosis (3). Management of splenosis depends on the patient's symptoms. In general, it is accepted that asymptomatic implants should not be removed because splenic tissue may be immunologically functional and thus useful for the patient. Since this benign condition may mimic metastases, it should be kept in mind in managing cancer patients with a history of post-traumatic splenectomy, in order to avoid unnecessary surgery or chemotherapy (4).

We report a case presenting with a pelvic mass, which was preoperatively mistaken for metastasis of breast carcinoma.

Case Report

A 38-year-old woman presented at the gynecology clinic for routine gynecological examination during breast cancer treatment. She had a locally advanced breast cancer (T4bN2M0). She had no family history of breast carcinoma. She had been treated with preoperative chemotherapy, (consisting of 5-fluorouracil 600 mg/m^2, doxorubicin 60 mg/m^2, and cyclophosphamide 600 mg/m^2 for four cycles with 21-day interval) and modified radical mastectomy. There was no evidence of residual tumor in the breast, but 14 of the resected 25 lymph nodes were metastatic. Histopathological examination had revealed a histological grade 3 invasive ductal carcinoma of the left breast. Estrogen receptor (ER) of the tumor was

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positive but progesterone receptor (PgR) was negative by immunohistochemistry. Negative c-erbB-2 overexpression by immunohistochemical staining was also observed in this tumor. She had a history of splenectomy due to traumatic rupture after a motor vehicle accident at the age of 13 years. On pelvic examination, the uterus was found of normal size. The left ovary was enlarged to 5 cm. Transvaginal ultrasound (TVUSG) scanning showed a 60x30mm hyperechoic, hypervascular solid tumor which seemed to be of left ovarian origin. Carbohydrate antigen-125 (Ca-125) value was within normal limits. Magnetic resonance imaging (MRI) showed a 39x56x40 mm diameter mass hypointense on T1-weighted and hyperintense on T2-weighted image on the left adnexal side. Further exploration of the pelvic mass was required, because exact diagnosis and final treatment could not be determined. Before the operation, the patient was advised about the various surgical options and it was agreed that if the diagnosis was not definitive during surgery or if there was the slightest doubt, then total abdominal hysterectomy and bilateral salpingo-ooperectomy would be performed. On laparotomy, a normal sized uterus with a bluish-red mass of 4x6 cm in diameter with a soft consistency was observed on its posterior left side. Bilateral ovaries, and peritoneal surfaces were viewed to be normal. There were no pelvic adhesions. Since the exact nature of the macroscopic lesion could not be determined, as previosly agreed with the patient, total abdominal hysterectomy and bilateral salpingo-oophorectomy was performed. Microscopy of the left ovary showed focal microscopic metastases (Figure 1) of breast carcinoma. The bluish-red mass of 6x3.5x2.5 cm in diameter with soft consistency which was observed on the posterior left side of uterus had a white pulp composed of lymphoid aggregates. It was embedded in a highly vascular red pulp composed of broad anastomosing venous sinuses (Figure 2).

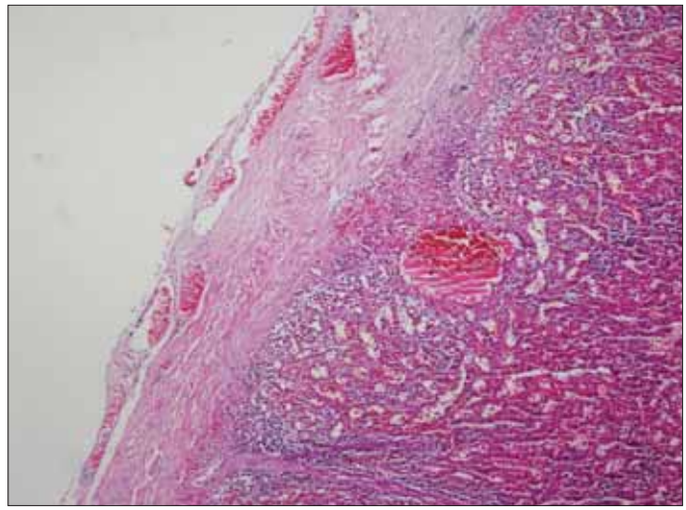

Figure 1. **Ovarian metastases were pathologically consistent with primary invasive ductal carcinoma (Hematoxylin&Eosin x200)**

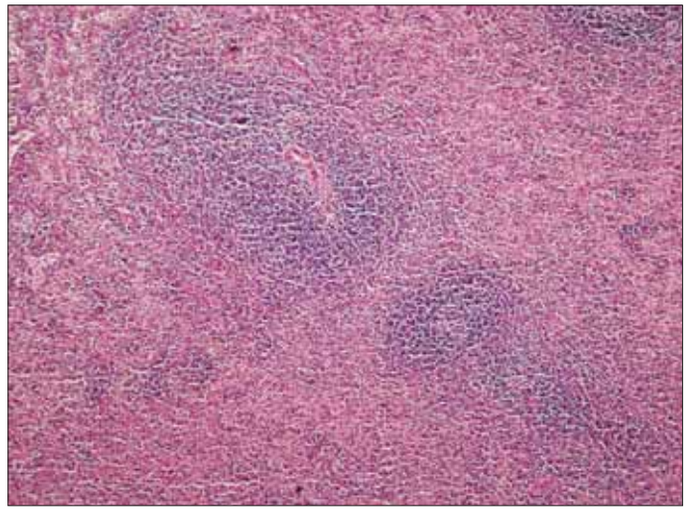

Figure 2. **Bluish-red mass of 6x3.5x2.5 cm in diameter on the posterior serosal side of uterus had white and red pulp of splenic tissue (Hematoxylin&Eosin x100)**

Discussion

Splenosis is the autotransplantation of splenic tissue that usually follows traumatic rupture of the spleen. Buchbinder and Lipkoff first reported splenosis in 1939 (5). Since then, fewer than 100 cases have been reported in the literature. Gynaecological cases (4, 6, 7) are only a minority of these because splenic tissue is capable of implanting on peritoneal surfaces, abdominal wall and omentum. Based on the location of splenic nodules, differential diagnoses to be considered may be endometriosis in the presence of pelvic implants (7), peritoneal mesothelioma in the case of peritoneal seeding (8), renal cancer (9) in the case of renal implants, abdominal lymphomas in the case of retroperitoneal locations mimicking lymph nodes (10), hepatic adenomas in the case of intra-hepatic implants (11) and peritoneal metastases. Ectopic splenic tissue most frequently occurs in the abdominal cavity, especially on the serosal surfaces of the small and large bowel, in the parietal peritoneum, the mesentery, and the diaphragm. Uncommon locations of splenosis have been reported on the female genital organs (7), the thoracic cavity (2), or as in our case, as an adnexial mass.

Muller followed patients who underwent posttraumatic splenectomy with ultrasonography and reported that presumed splenosis occurred in one third of the patients, but this was not histologically confirmed (12). The incidence of splenosis is unknown since it is usually an incidental finding at surgery or autopsy (6).

Splenectomy for non-traumatic reasons may also lead to splenosis if splenic tissue is spilled onto the peritoneal surface at the time of surgery or if morcellation extraction of the spleen was used. Investigation in mice showed that growth of ectopic splenic tissue can be affected if splenectomy is incomplete and the circulating mediators released by the residual spleen are active (6). The pathogenesis of splenosis commences at the time of splenic rupture or splenectomy, when the splenic pulp disperses into the peritoneal cavity (9, 11). It is supposed that the number of nodules of ectopic splenic tissue that develop in the peritoneal cavity correlates with the severity of splenic injury. Another mechanism of splenic tissue transplantation is splenic vein emboli or hematogenous spread of splenic pulp, which is suggested by cases of intrahepatic and intracranial

splenosis (13, 14). One theory suggests that splenic erythrocytic progenitor cells enter the liver via the portal vein, and then grow in response to tissue hypoxia (15).

Splenosis is usually asymptomatic. It is rarely of clinical significance. Occasionally, patients present with nonspecific abdominal pain due to infarction, an enlarging abdominal mass with associated infection, intestinal obstruction due to adhesive bands of implants, gastrointestinal hemorrhage or hydronephrosis. Pleurisy and hemoptysis may be the symptoms when thoracic splenosis occurs (16). Recurrence of Felty's syndrome also has been reported as a complication of splenosis, because splenic implants resume splenic function in 1-3 months (17).

The average reported interval between the spleen trauma and the diagnosis of splenosis is 19 years. Our patient had a 25 year interval between splenectomy and final diagnosis. The presumed diagnosis of splenosis can be made by the absence of Howell-Jolly bodies, siderocytes and other postcellular abnormalities on a peripheral blood smear.

Because most patients with splenosis are asymptomatic, ectopic splenic tissue is found incidentally during US, Computed Tomography (CT), or MRI examinations. MRI may be considered as an alternative modality for the identification of splenosis, in case of uncertainty of diagnosis with other examinations. Splenic implants have been described as hypointense on T1-weighted images and hyperintense on T2-weighted images, therefore similar to normal splenic tissue (18). In our case, magnetic resonance imaging showed a 56x40 mm diameter mass hypointense on T1-weighted and hyperintense on T2-weighted image.

The development of high frequency transvaginal scanning has facilitated the diagnosis of relatively small pelvic masses, and the advancement of laparoscopic surgical techniques may increase the frequency of diagnosis splenosis. Although the usual imaging modalities (US, CT, MRI) are helpful to localize and determine the size, structure and relations with adjacent organs, they are not specific. More specific and diagnostic studies using agents that are sequestered by reticulendothelial tissue, like 99m technetium sulphur colloid, 99m technetium labelled heat-denatured autologous red blood cells or 111 In-labelled platelet scans (19, 20) and recently ferumoxide-enhaced MRI (21) have been used. Another specific method for the diagnosis of splenosis is MRI examination with intravenous administration of superparamagnetic iron oxide (SPIO), which is used for delineation of hepatic and splenic disease. Ectopic splenic tissue demonstrates the same decrease in signal intensity as the normal spleen after administration of SPIO particles (22).

Management of splenosis depends on the patient's symptoms. In general, it is accepted that asymptomatic implants should not be removed because splenic tissue may be immunologically functional and thus useful for the patient. Furthermore, unnecessary excisions of the implants may lead to serious bleeding and damage to the surrounding organs. Since this benign condition may mimic metastases, it should be kept in mind in managing cancer patients with a history of post-traumatic splenectomy, in order to avoid unnecessary surgery or chemotherapy.

In conclusion, splenosis must be considered in the differential diagnosis of previosly splenectomized patients who present with unexplained masses.

Conflict of interest

No conflict of interest was declared by the authors.

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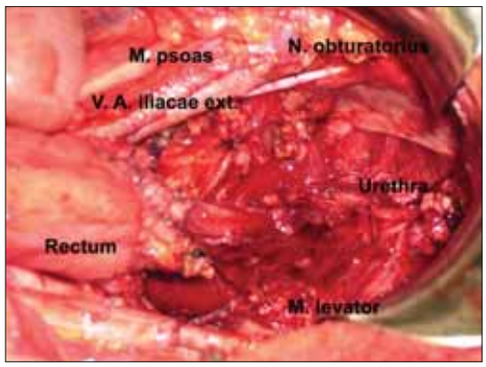
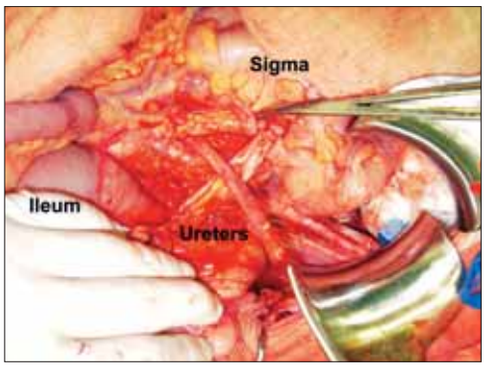
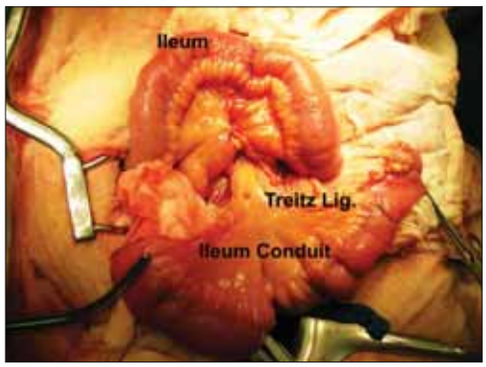
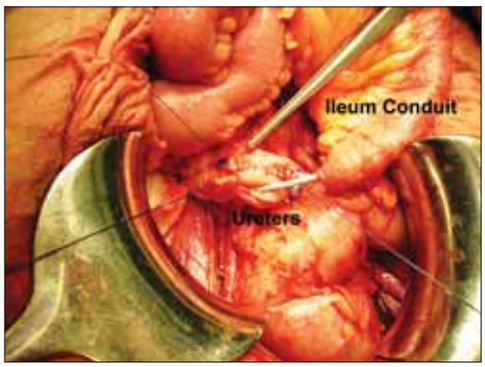
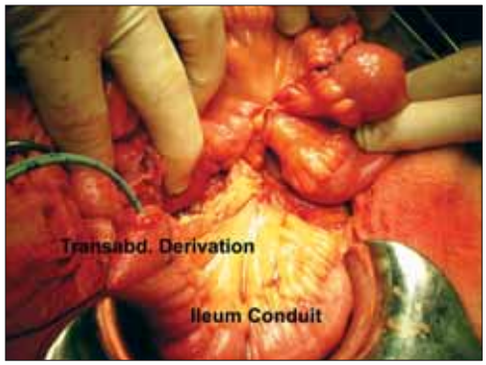
Which operation is described?











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Bricker conduit during Type I supralevatoric anterior evisceration for relapsed cervical carcinoma”

Local recurrence of cervical cancer after radiation therapy is probably one of the most complications. Up to 70% of patients with cervical cancer receive radiation at some point in their treatment. It is well known that one out of three of these patients will suffer recurrent or persistent disease. In more than 80% of these cases, the disease will recur within the first 2 years after treatment (1).

Recurrent local disease after radiation therapy cannot be easily treated. Chemotherapy has been used in this situation with very poor results. Typically, the effectiveness of chemotherapy is very low after radiation failure, and chemotherapy is used only with palliative intent (2).

Re-irradiation may be proposed in very highly selected cases after a long interval free of disease. However, re-irradiation dramatically increases the rate of severe complications, especially fistulas, if used shortly after the first treatment Only a pelvic exenteration or evisceration can achieve tumor-free margins in these cases. During the last 60 years, this surgery has been proven successful in selected cases of recurrent pelvic cervical cancer after radiation, obtaining 5-year overall survival rates higher than 30% (3, 4).

In 1948, Brunschwig was the first surgeon to publish his preliminary experiencewith pelvic exenteration. Soon, his technique started to be used in other American institutions, becoming the gold standard of treatment in recurrent cervical cancer after radiation (5-7).

However, urinery diversion can be needed after bladder resection. The goal of any form of urinary diversion is to deliver the urine to outside with a minimum interference of life style, with a maximum protection of the urinary tract. Since Bricker first described his procedure in 1950, the ileal conduit has been the gold standard for urinary diversions after cystectomy for bladder cancer or after exenteration for a gynecological relapse in irradiated patients (Fig. 1, 2). A 15-to 20-cm-long distal ileal segment is isolated (Fig. 3), and the ureters are implanted in the proximal end (Fig. 4 and 5) or in the antimesenteric edge.The stoma is usually below and to the right of the umbilicus (8).

Most gynecologic oncologists who perform exenteration use this maneuver. With Magrina’s classification, exenteration is divided into supralevator (type I), infralevator (type II) and infralevator with vulvectomy (type III), and, an additional category, extended (7). This division can help to facilitate communication when referring to these patients. It can also facilitate a more detailed analysis of operative risk factors, complications, and results and can increase our knowledge of the indications and limitations of the different exenterative procedures.

In the gynecologic oncology setting, a colostomy is also widely used, but there are a number of reasons to choose an ileostomy as the preferred temporary stoma in these patients: 1. Ileostomy not only protects the colorectal anastomosis but also may protect the small bowel anastomosis that closes the donor area for the urinary conduit. 2. Commencement of small bowel function is almost immediate, allowing patients to eat sooner. 3. The rate of complications in the small bowel is significantly lower upon closing the stoma. Long-term complications of ileal conduit diversion are frequent; the most common are stomal or peristomal problems, parastomal hernia, conduit stenosis, and upper tract deterioration.

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CONGRESS CALENDAR

3-6 July 2011	**27th Annual Meeting of ESHRE** *Stockholm, Sweden* http://www.eshre.eu/home
11-17 September 2011	**ESGO-Oncology (17th)** *Milan, Italyp* www2.kenes.com/esgo17
11-17 September 2011	**ACOG (21th)** *Chicago, USA* www.agosonline.org
18-22 September 2011	**ISUOG (21th)** *Los Angeles, USA* http://www.isuog.org
5-9 October 2011	**UTD (3rd)** *Antalya, Turkey* http://www.utd.org.tr
15-19 September 2011	**ASRM (67th)** *Orlando, Florida, USA* http://www.asrm.org
1-2 December 2011	**3rd ANNUAL SEMINAR LAPAROSCOPIC & ROBOTIC Hysterectomy, and Intensive Hands-on Laparoscopic Suturing&Knot Tying** *New York, USA* www.nywomenshealth.com
1-4 July 2012	**28th Annual Meeting of ESHRE** *Istanbul, Turkey* http://www.eshre.eu/home