ISSN 1309-0399

TURKISH-GERMAN GYNECOLOGICAL EDUCATION and RESEARCH FOUNDATION

Journal of the Turkish-German Gynecological Association

Volume 11
Issue 3
September
2010

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Cover Picture: HeLa cell proteomics, silver stain with permission of Associate Professor Murat Kasap, Kocaeli University, Clinical Investigation Laboratory

ISSN 1309-0399

A-I

Publisher | AVES
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Phone: +90 212 589 00 53 Fax: +90 212 589 00 94
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İmtiyaz Sahibi ve Sorumlu Yazı İşleri Müdürü: Cihat Ünlü
Basım Yeri: Görsel Dizayn Ofset Matbaacılık Tic. Ltd. Şti. - +90 212 671 91 00
Basım Tarihi: Eylül 2010
Yayın Türü: Yerel Süreli Yayın

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Aims and Scope

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Editorial

Dear Colleagues,

I am excited about the new issue of our journal with many interesting articles. Our journal is playing an important role in our field, indexed by many internationally accepted databases. JTGGA is distributed to more than 250 readers internationally and we expect to get more international readers in the near future.

As the date is approaching for our 9th congress-IX.Turkish-German Gynecology Congress, we are working harder to prepare a scientifically satisfactory and highly appreciated meeting for you. Many famous and pioneer speakers have already accepted our invitation to join our congress as members of the international faculty. Our scientific committee holds various organizing meetings and works hard to draft the scientific programme and pre-congress courses. Our web site-**www.tajev2011.org** has been created for the participants to keep track of the latest announcements and up-to-date information about the congress. The abstract submission for our congress has started and abstracts may be sent via the web site.

The International Federation of Fertility Societies (IFFS) is a significant international organization encompassing the national fertility societies of close to 70 nations. Founded over 50 years ago, its mission is to stimulate basic and clinical research, disseminate education and encourage superior clinical care of patients in infertility and reproductive medicine. "IFFS 20th World Congress on Fertility & Sterility" will be held in Münich, Germany on September 12th - 16th, 2010. The Congress Organizing Board has made the decision to host the societies from Austria, China, India, Egypt, Japan, Spain, Russia and Turkey in order to organize "Regional Meetings" at the Congress, encouraging an intra-cultural exchange of views and ideas.

The Congress Organizing Board has appointed the Turkish German Gynecological Education and Research Foundation to organize the "Turkish Regional Meeting" at the Congress. We are proud that our foundation has been selected to represent our country and Turkish science in an international organization.

Hope to stay in touch and meet in our next Issue, December 2010.

Kind regards,

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

Influence of cumulus cell coculture and cumulus-aided embryo transfer on embryonic development and pregnancy rates

Kumulus hücre kokültürünün ve kumulus destekli embryo transferinin embryonik gelişim ve gebelik oranlarına etkisi

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Abstract

Objective: In this study, we aimed to evaluate the influence of autologous cumulus oocyte complex (COC) coculture on embryonic development and quality, and investigate the implantation and pregnancy rates after cumulus-aided embryo transfer in the ICSI-ET cycles.

Material and Methods: Ninety five consecutive infertile women undergoing their first cycle of IVF teratment were included in the study. The cases were divided into two groups. Group 1 consisted of 48 women undergoing ICSI, along with autologous cumulus embryo coculture and cumulus-aided emryo transfer. Group 2 comprised 47 consecutive patients who consented to undergo ICSI and in whom autologous cumulus embryo coculture and cumulus-aided embryo transfer were not performed. Implantation and pregnancy rates were compared between the two groups.

Results: The demographic data and controlled ovarian hyperstimulation parameters were similar in the two groups. The fertilization and cleavage rates were found to be higher in group 1 when compared with group 2 (p=0.03 and 0.001, respectively). There were no statistical significant differences for the implantation and clinical pregnancy rates between the two groups.

Conclusion: Usage of autologous COCs as coculture may improve fertilization and cleavage rates. However, cumulus-aided embryo transfer does not produce an increase in implantation and pregnancy rates. (J Turkish-German Gynecol Assoc 2010; 11: 121-6)

Key words: Coculture, Cumulus cells, ICSI-embryo transfer, Pregnancy rate

Received: 9 July, 2010 **Accepted:** 12 August, 2010

Özet

Amaç: Bu çalışmada, otolog kumulus-oosit kompleks (COC) kokültürünün embryonik gelişim ve kalitesine etkisini değerlendirmek, ve ICSI-ET sikluslarında kumulus destekli embryo transferinin implantasyon ve gebelik oranlarına etkisini araştırmayı amaçladık.

Gereç ve Yöntemler: Yardımla üreme tedavisinin ilk siklusunda olan 95 infertil kadın hasta çalışmaya dahil edildi. Vakalar iki alt gruba ayrıldı. Grup1 otolog embryo kokültürü ve kumulus destekli embryo ttransferi uygulanan 48 hastayı kapsadı. Diğer grup ise rutin ICSI tedavisi uygulanan 47 hastayı içerdi. Bu grupta otolog kumulus embryo kokültürü ve kumulus destekli embryo transferi uygulanmadı. İmplantasyon ve gebelik oranları iki grup arasında karşılaştırıldı.

Bulgular: Demografik veriler ve kontrollü ovarian hiperstimulasyon parametreleri her iki grup arasında benzerdi. Fertilizasyon ve yarıklanma oranları grup1'de grup 2'den istatiksel olarak anlamlı derecede yüksek saptandı (sırasıyla p=0.03 ve 0.001). İmplantasyon ve gebelik oranları açısından her kik grup arasında istatiksel olarak anlamlı fark tespit edilmedi.

Sonuç: Otolog kumulus-oosit kompleksinin kokültür olarak kullanılması fertilizasyon ve yarıklanma oranlarını arttırabilir. Bununla birlikte, kumulus destekli embryo transferi implantasyon ve gebelik oranlarına katkı sağlamamaktadır.

(J Turkish-German Gynecol Assoc 2010; 11: 121-6)

Anahtar kelimeler: Kokültür, Kumulus hücreleri, ICSI-embryo transfer, Gebelik oranı

Geliş Tarihi: 09 Temmuz 2010 **Kabul Tarihi:** 12 Ağustos 2010

Introduction

The concept of improved preimplantation development and implantation ability by coculturing embryos in the presence of another cell type called feeder cells has led to the development of the coculture system. A number of cell types have been used for this purpose. These feeder cells have included human reproductive tissues, such as oviducts, endometrium, oviduct-endometrial sequential coculture, cumulus-granulosa cells and even cells from ovarian carcinoma (1-7). The suggested beneficial effects of cocultures were the secretion of embryotrophic factors such as nutrients and substrates, growth factors, and cytokines and the removal of potentially harmful substances including heavy metals, ammonium, and free radical formation, thus detoxifying the culture medium (8, 9). The main effect is to increase the metabolic chance of the human embryo to achieve the blastocyst stage and implant. However, the results concerning an actual increase of pregnancy rate on a large scale after the transfer of cocultured embryos in the uterus may be conflicting. There is also

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doi:10.5152/jtgga.2010.017

debate about usage of coculture or sequential culture media to obtain a large number of viable blastocysts.

Several studies have demonstrated faster cleavage rates, less fragmentation, and better implantation rates after using a coculture system (7, 10, 11). It has been reported that the coculture system increased blastulation rates to 55%-70% (12, 13). In most mammals, the oocyte in the Graafian follicle is surrounded by tightly packed layers of cumulus cells, forming the cumulus-oocyte complex (COC). During the preovulatory period, cumulus cells change from a compact cell mass into a dispersed structure of cells for the synthesis and deposition of a mucoid intercellular matrix, a process referred to as cumulus expansion. Cumulus expansion is thought to influence a variety of fundamental developmental changes during oocyte maturation. Volumetric expansion of COC correlates, with the outcome of oocyte maturation, fertilization, and embryo development. It may be hypothesized that coculture of embryos with cumulus cells that serve as a source of growth factors would lead to an increase in the adhesiveness of embryos. However, there is limited data about the effects of COC coculture on embryonic development and pregnancy rates in the literature.

In this study, we aimed to evaluate the usage of autologous COC coculture as a medium for embryonic development and quality and also establish implantation and pregnancy rates after cumulus-aided embryo transfer in the ICSI-ET cycles.

Material and Methods

Patient selection

The present study was a prospective, single-centre, randomized, controlled, group-comparative clinical trial assessing cumulus coculture and cumulus-aided embryo transfer in women undergoing IVF. A total of 113 consecutive women who underwent ICSI-ET cycles at the Assisted Reproductive Unit of Meram Medical Faculty from January 2008 to February 2009 were included in this study. The flowchart for the study population is shown in Fig. 1. Randomized assignment of the two procedures was performed by a computer-based program. The sequence of allocation to the two groups was not concealed and the study was not blind. Patients could participate in the study only once. The study was approved by the Ethics Committee of Meram Medical Faculty and informed consent was obtained from all patients before entry into the study.

Indications for ICSI-ET treatment included unexplained infertility, anovulation, male and tubal factors. Exclusion criteria were: presence of a clinically significant systemic disease; diabetes mellitus, polycystic ovaries or any other endocrine disorder and submucosal polyp, leio-myoma or uterine septum which were detected on hysteroscopy or hysterosalpingography.

The patients were divided into two groups - Group1 (study) consisting of 48 consecutive consenting patients who had undergone ICSI, along with autologous cumulus embryo coculture and cumulus-aided emryo transfer. Group 2 (control) comprised of 47 consecutive patients who consented to undergo ICSI without cumulus coculture and cumulus-aided embryo transfer.

Stimulation protocol and preparing procedure

All patients were started on 1 mg/day Leuprolide acetate (Lucrin, Abbot, France) on the 21st day prior to menstruation for pituitary desensitization. When exogenous gonadotropins were started in down-regulated women on day 2 of menstruation, the dose of Leuprolide acetate was decreased to 0.5 mg/day. It was administered daily until the day of HCG injection. Initial mean doses of 177.5 IU and 185 IU recombinant FSH (recFSH, Gonal F, Serono, Italy) or human menopausal gonadotropin (hMG, Menogon, Ferring, Germany) were started on the second day of menstruation for 6 days. FSH dose was adjusted individually according to the response of the ovaries and estradiol concentrations. When the leading follicle reached 18 mm in diameter or at least two follicles were >17 mm in diameter, a total of 10,000 units of HCG were administered intramuscularly. Oocyte retrieval was performed 35-37 h later by ultrasound-guided procedure.

After aspiration of COCs from the patient, whenever the collected COCs exceeded the 10 M II stage after hyaluronidase treatment; the excess COCs were spared for usage. We continued the study in these patients with excess numbers, and the women with the spare COC of $\geq$4 were included in the study group.

Group1 (study): After microinjection of 10 M II stage oocytes was completed, they were placed into their droplets (each 90 microliter Cleavage Medium, SAGE) as 2-3 oocytes per droplet, and for each of these droplets one COC was added and continued for embryo culture (5.0% CO2, 99.9% humidified). Whenever the embryos were changed to a new droplet, the COC was simultaneously carried. . During embryo transfer, the selected embryos were grouped in a medium pool (Blastocyst Medium supplemented with 30% HSA SAGE) and the larger and intact COC was selected to add to the pool (Figure 2). Embryo loading to the catheter (Wallace, Smiths, England) was done as selected embryos plus COC in a 50-70 microliter medium (Figure 3). The contents of the catheter were released into the uterus over a period of 8-10 seconds, and the catheter was held in position for about 15 seconds. If the excess COC number was not enough the patient was excluded from the study.

Group 2 (control): The same stimulation protocol was administered and oocyte retrieval was performed 35-37 h later. After microinjection of 10 M II stage oocytes was completed, they were placed into their droplets (each 75 microliter Cleavage Medium, SAGE) as 2-3 oocytes per droplet and continued for embryo culture without any COC addition. Culturing and embryo transfer technique was similar to group1 except for COC addition.

The luteal phase was supported with micronized vaginal progesterone, 600 mg/day, up to the tenth week of gestation in cases where pregnancy was achieved. Clinical pregnancy was confirmed 28-35 days after embryo transfer by a the presence of a gestational sac on ultrasound.

Sample size and statistical analysis

The sample size was estimated on the basis of the expected implantation rate. The reported implantation rate in patients undergoing cumulus-aided embryo transfer is 25.6% (14).

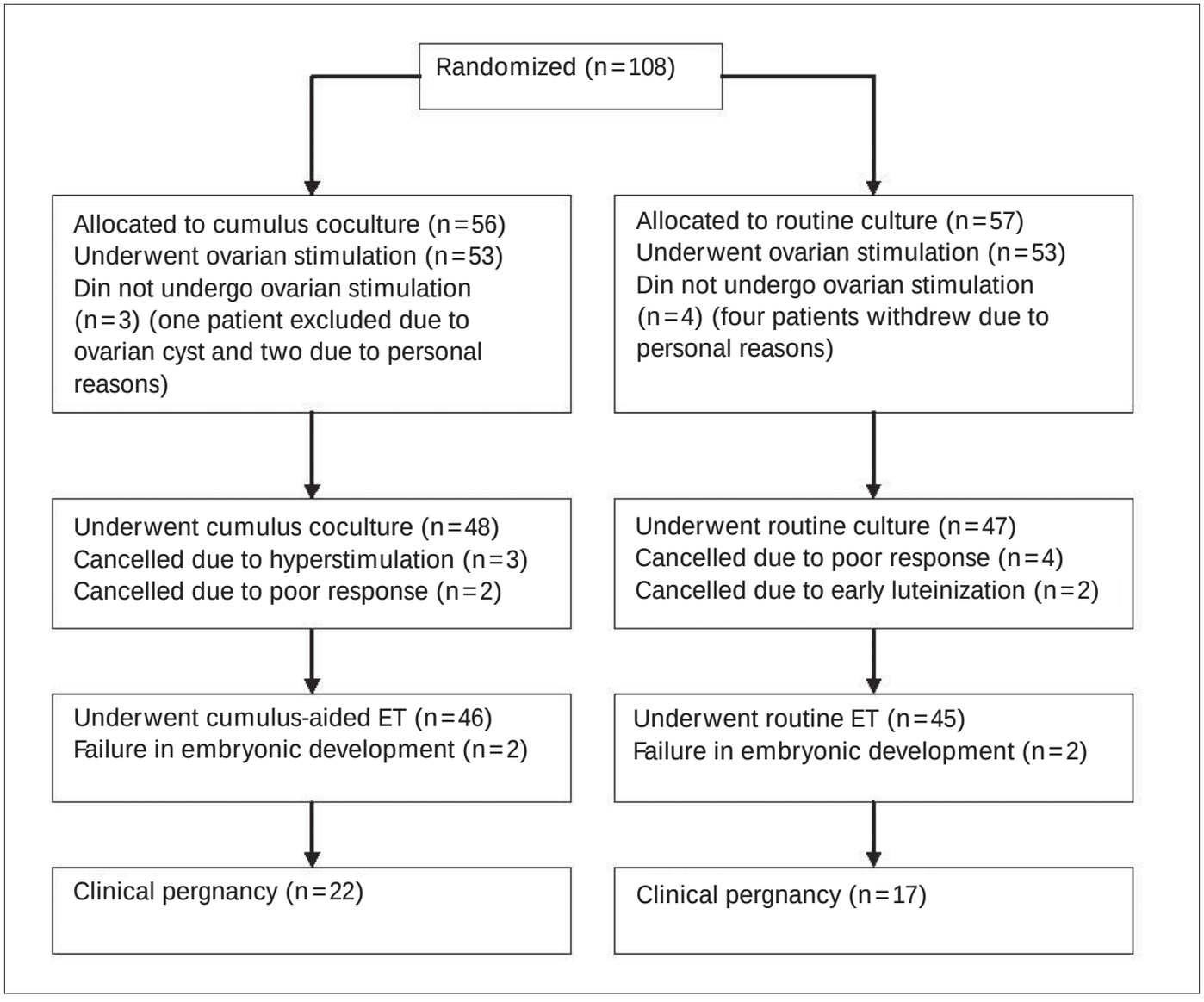

Figure 1. Flowchart of the study population

Assuming the implantation rate to be 5% different for women with cumulus-aided embryo transfer, 47 women in each group would be required to provide 80% power at the 5% significance level.
Values were expressed as mean±SD for continuous variables and the number and percentage for categorical variables. The χ^2 test and Student's t test were used to compare categorical and parametric data, respectively. The nonparametric Mann-Whitney U test was used to check for differences between numeric variables between the 2 groups. All P values were 2-tailed and $P<0.05$ was considered statistically significant.

Results

The demographic features of the study participantsare shown in Table 1. The mean ages, duration and causes of infertility (unexplained, anovulation, tubal and male factors) were similar in the two groups. The controlled ovarian hyperstimulation characteristics of both groups are listed in Table 2. The mean duration of stimulation, doses of daily administered recFSH, sum of recFSH doses, doses of daily administered HMG, sum of HMG doses, estradiol levels on hCG administration, endometrial thickness measured by transvaginal sonography on hCG administration day, mean number of oocytes at metaphase II stage (MII) per each cycle and total number of MII oocytes were similar in the two groups. There were no statistical significant differences between the study and control groups for cycle characteristics.
The parameters of the wo groups after ICSI procedure are displayed in Table 2. The fertilization and cleavage rates, total number of high quality embryos per transfer, numbers of embryos on day 2 and 3, mean number of embryos transferred, implantation rates and pregnancy rates were investigated after ICSI procedure. The fertilization rate was higher in Group 1 (study group) when compared with Group 2 (control group). The mean fertilization rates at 18-21 hours in Groups 1 and 2 were 56.63% and 44.37%,

respectively (Table 3). There was a statistically significant difference between the two groups (p= 0.03) for this parameter. The cleavage rate was found as 59.6% in the study group, and it was detected as 41.4% in control group. Group 1 had a significantly higher cleavage rate than Group 2 (p= 0.001).

The total number of high quality embryos per transfer, numbers of embryos on day 2 and 3, t mean number of embryos transferred, implantation and pregnancy rates were similar in the two groups. Statistically significant differences were not obtained between the study and control groups. Although the study group had higher clinical pregnancy rate than the control group, a statistically significant difference was not determined between the two groups.

Figure 2. Two ICSI performed embryos inside COC at 26. hour, one without any progression while the other developed to the two blastomer stage

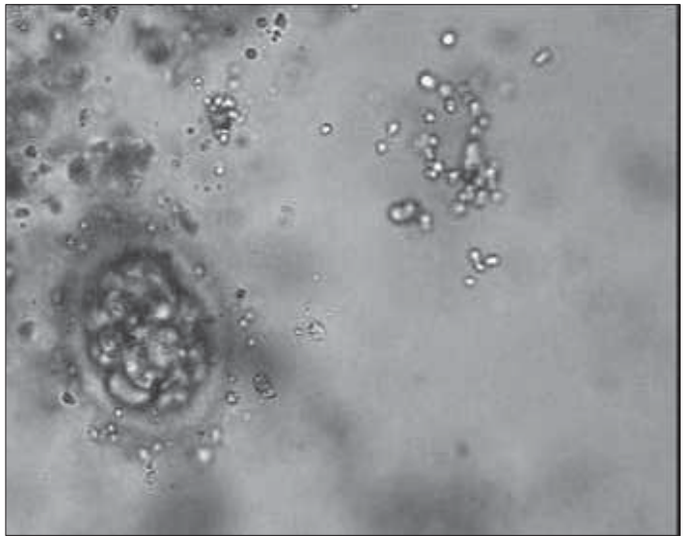

Figure 3. An early 4th. day embryo before embryo transfer, the embryo is embedded in COC. The embryo is suspended inside the matrix not touching the base of the culture dish which gives confluency to the image

Table 1. Demographic features of the groups

	Group 1 (n=48)	Group 2 (n=47)	p value
Mean age (year)	27.6±3.3	28.8±4.4	0.58
Infertility duration	6.7±2.1	5.8±2.8	0.27
Infertility causes			
Unexplained	17 (35%)	15 (32%)	0.74
Anovulation	13 (27%)	14 (30%)	0.24
Tubal factor	10 (21%)	11 (23%)	0.59
Male factor	8 (12%)	7 (15%)	0.35

Table 2. The ovarian hyperstimulation characteristics of both groups

Parameter	Group 1 (n=48)	Group 2 (n=47)	p value
Duration of stimulation/day	10.48±1	10.52±1.1	0.52
Daily FSH consumption (IU)	177.5±37.2	185±46.2	0.17
Total FSH consumption (IU)	1859.5±434.5	1884.5±502.3	0.74
Daily HMG consumption (IU)	84±24.6	78±14.8	0.42
Total HMG consumption (IU)	877.5±255.9	813±127.9	0.64
Total FSH+HMG consump. (IU)	2737±435.6	2697.5±539.3	0.22
Estradiol on hCG day (pg/ml)	2210±369.3	2166±425.5	0.82
End. thickness on hCG day (mm)	10.7±1.7	10.5±1.7	0.52
Total MII oocytes (n)	643	617	0.51
M II oocyte per cycle (n)	12.3±1.6	12.8±2.2	0.44

Table 3. The parameters of both groups after ICSI procedure

Parameter	Group 1 (n=50)	Group 2 (n=50)	p value
Fertilization rate (%)	56.6	44.3	0.03
Cleavage rate (%)	59.6	41.4	0.001
Total embryos (n)	217	206	0.18
Embryos on day2 (n)	31 (62%)	32 (64%)	0.53
Embryos on day3 (n)	19 (38%)	18 (36%)	0.61
Embryos / transfer (n)	2.8±0.5	2.9±0.5	0.27
Implantation rates (%)	34%	33%	0.46
Pregnancy rates (%)	22 (46%)	17 (36%)	0.09

Discussion

The use of another cell type for cocultures during IVF is currently finalized to obtain a high number of healthy and viable embryos with a high potential of implantation (14). Among the different cell lines that can be used as feeder cells for cocultures, COCs express some growth factors and cytokines, which are known to improve embryo morphology. There is little data about the effects of COCs coculture on human embryo development or pregnancy rates in IVF procedures. The present study investigated the efficacy of COCs coculture and also cumulus-aided embryo transfer on the results of ICSI procedures. We have demonstrated an increase in fertilization and cleavage rates after coculture of oocytes with COCs. Despite an increase in pregnancy rate, a significant difference was not obtained for this parameter.

A previous study, in which Vero epithelial cells derived from Gren monkey kidneys were used as coculture, reported an increased number of blastocytes in coculture groups (10). Another study showed an increase in the implantation rates per embryo in the pregnant patients when the embryos were cocultured with Vero cells (13). Ben-Cherit at al. examined the effect of human embryo coculture with an ovarian cancer cell line (7). They showed that coculture of early cleavage stage human embryos with epithelial cancer cells markedly improved in vitro human blastocyst formation compared with standard culture conditions. Freeman et al. performed a preliminary study to evaluate the effects of granulosa-lutein cell co-culture on human embryo development and pregnancy rates for IVF (11). They demonstrated that autologous granulosa-lutein cell co-culture improved embryo development, implantation and subsequent pregnancy rates for IVF.

Mansour et al. performed a prospective randomized research to study the value of co-culturing human pronucleate oocytes with their cumulus cells (16). They concluded that co-culture of human oocytes with their cumulus cells significantly decreased their fragmentation and increased the number of embryos that reached the 4-cell and 8-cell stages with regular blastomeres. Cumulus cells have been used as a feeder layer in a coculture system in another previous report (6). It was shown that the culture of human embryos with their cumulus cells in insemination drops of medium produced a significantly greater proportion of the fully expanded blastocysts. In a recent report, Kalthur et al. studied the influence of cumulus cell co-culture on sperm motility, and they showed that co-culture with cumulus cells enhanced sperm motility under in vitro conditions (17). As a different condition from our study, cumulus-aided embryo transfer was not performed in these studies. In the present study, the total and mean number of high quality embryos transferred were not improved when cumulus cells were used as a coculture.

A recent study from India was undertaken to evaluate the effect of the use of cumulus coculture and cumulus-aided embryo transfer on pregnancy rates (14). Women undergoing cumulus coculture and cumulus-aided embryo transfer were compared with those who underwent cumulus coculture but did not undergo cumulus-aided embryo transfer. This study demonstrated the efficacy of cumulus-aided embryo transfer, using autologous cumulus cells. However, there was no difference in fertilization rates and cleavage rates in this report between the study and control groups. The results of the study suggested that cumulus cells could play an important role in embryonic development and embryo-uterine adhesion. The present study was not in accord with the results of this report. We obtained significant improvements only in fertilization and cleavage rates in women with cumulus coculture. The number of embryos transferred, implantation and clinical pregnancy rates were similar in the two groups. However, despite the absence of a statistical difference, there was an increased trend towards cumulus-aided embryo transfer for pregnancy rates in our study. This might have resulted from the number of cases in our study.

Cumulus cells accompanying the ovulated oocytes are also capable of secreting many cytokines and growth factors, an ability that may be important in oocyte nourishment and may facilitate embryo cleavage following fertilization (18). Growth factors and cytokines secreted by the cumulus cells are utilized to improve the potential for implantation process. Huang and co-workers reported that the IL system is an important factor in embryo-maternal molecular communication during implantation process (19). Another study has shown that IL-6 secreted by cumulus cells plays a significant role at the time of implantation (20). Parikh et al postulated that the rationale of using the combined technique of cumulus coculture and cumulus-aided embryo transfer was to generate better-quality embryos and to increase the potential for implantation (17). They demonstrated a significant increase in the implantation rate in women undergoing cumulus-aided embryo transfer. In this report, the high-order multiple gestation rates were higher in the cumulus-aided embryo transfer group. Our study did not show any significant increase in implantation rate, but we obtained a trend toward an increase in pregnancy rate in women with cumulus-aided embryo transfer.

In conclusion, the use of cumulus cells as a coculture and the addition of cumulus cells into the uterus may contribute to the effort to improve implantation rates in the future. However, there is only limited data on cumulus coculture and cumulus-aided embryo transfer. Further studies with larger series are needed to show the effectiveness of cumulus coculture and cumulus-aided embryo transfer during IVF treatments.

Conflict of interest

No conflict of interest is declared by authors.

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Idiopathic granulomatous mastitis-utility of fine needle aspiration cytology (FNAC) in preventing unnecessary surgery

İdiyopatik granulomatöz mastitde gereksiz cerrahi girişimi önlemek için ince iğne aspirasyon sitolojisinin (İİAS) kullanılması

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Abstract

Objective: Granulomatous mastitis is a benign disorder which closely mimics malignancy clinico-radiologically. A simple and cost effective modality like fine needle aspiration cytology (FNAC) can help in prompt diagnosis and avoid unnecessary surgery.

Material and Methods: A retrospective study where data were collected for granulomatous lesions of the breast diagnosed by histopathology in a five year period and review of FNAC slides. Cases positive for Mycobacterium tuberculosis either on ZN stain or Polymerase chain reaction (PCR) and fungus were excluded. A total of 8 cases were included in our study for analysis.

Results: All the cases showed the presence of granuloma composed of epithelioid histiocytes against a background of giant cells, polymorphs with absence of necrosis.

Conclusion: Cytological diagnosis of granulomatous mastitis is difficult as it overlaps with other etiologies like tuberculosis which is prevalent in this part of the world. However, with the use of ancillary techniques like PCR and negative microbiological investigations, a definitive diagnosis can be made.

(J Turkish-German Gynecol Assoc 2010; 11: 127-30)

Key words: Granulomatous mastitis, fine needle aspiration cytology (FNAC)

Received: 14 May, 2010 **Accepted:** 12 August, 2010

Özet

Amaç: Granülomatöz mastit klinik ve radyolojik olarak kanseri taklit edebilen iyi huylu bir hastalıktır. Basit ve ucuz bir yöntem olan ince iğne aspirasyon sitolojisi (İİAS) gereksiz cerrahileri önlemede ve kesin tanı koymada etkin bir yoldur.

Gereç ve Yöntemler: Bu retrospektif çalışma dataları 5 yıllık süre ile histopatolojik olarak granulomatöz lezyon tanısı konan hastaların İİAS lamları ve kayıtları gözden geçirelerek toplanmıştır. ZN boyama veya PCR ile tanısı konmuş olan tüberküloz vakaları ve mantar enfeksiyonu olanlar çalışmaya alınmamıştır. toplam çalışmaya uygun 8 vaka incelenmiştir.

Bulgular: Tüm olgular epiteloid histiositler ve tabanda dev hücreler ve nekrozsuz polimorfmlar göstermiştir.

Sonuç: Granulomatoz mastitin sitolojik tanısı özellikle dünyanın bu bölgesinde olduğu gibi yaygın görülen tüberküloz varlığında oldukça zor konulur. Ancak PCR ve negatif mikrobiolojik araştırmalar sayesinde ayırıcı tanıya kolayca gidilir.

(J Turkish-German Gynecol Assoc 2010; 11: 127-30)

Anahtar kelimeler: Granulomatöz mastit, ince iğne aspirasyon sitolojisi (İİAS)

Geliş Tarihi: 14 Mayıs 2010 **Kabul Tarihi:** 12 Ağustos, 2010

Introduction

Idiopathic granulomatous mastitis is a rare benign breast disease, first described by Kessler and Wallooch (1) in 1972. In the literature, only a few large series have been reported, emphasizing the histological (2-5), imaging (6-8) and cytological (9-11) aspects, with series sizes ranging from 6-16 cases. The histological features of granulomatous mastitis have been well described as consisting of non-caseating granulomas within the breast parenchyma and lobulitis with or without neutrophilic micro abscesses (12, 13). However, cytological features have not been widely discussed.

The clinical and radiological presentation mimics carcinoma, often resulting in unnecessary radical surgery. Therefore, in our study we present eight cases of granulomatous mastitis with special emphasis on their cytological features.

With the increasing use of fine needle aspiration and cytology (FNAC) as the initial investigation for breast lesions, there is a need for an increased awareness of this disease entity so that prompt and correct line of management can be selected.

Material and Methods

The histopathology records of a tertiary care hospital were collected for cases of granulomatous lesion reported between July 2004 and July 2009. The histopathology slides and all the case histories of these patients were retrieved and re-analyzed. A total of 19 cases of granulomatous lesions of breast

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doi:10.5152/jtgga.2010.18

were diagnosed on histopathology during this period. Of these, FNAC slides of 14 patients could be retrieved. Among the latter, acid fast bacilli (AFB) were detected in Ziehl Neelsen (ZN) stained smear in one case only in which a straightforward diagnosis of tuberculosis of breast was made. In the remaining 13 cases, Polymerase chain reaction (PCR) was performed for the IS6110 sequence of mycobacterium tuberculosis either on material obtained on cytology or from paraffin-wax embedded material. PCR gave positive results in 5 cases and negative in the 8 cases. These PCR-negative cases gave negative results on Periodic Acid Schiff (PAS) and Grocott's Methanamine Silver staining and culture in Lowenstein-Jensen (LJ) media. These cases are included in our report of Idiopathic Granulomatous Mastitis.

(The FNAC were performed by trained and experienced pathologists using 10ml syringes fitted to a FNAC gun and using 22 to 23 gauge needles. The aspirated material was used to make direct smears for May Grunwald Giemsa (MGG) stain and alcohol-fixed smears for Haematoxylin and Eosin stain and/or Papanicolaou stains.

In all the cases, the following cytological parameters were evaluated-

a) Presence or absence of granulomas
b) Relative proportions of other cells like epithelioid histiocytes, lymphocytes, plasma cells and polymorphs.
c) Presence or absence of background necrosis
d) Multinucleated giant cells- proportion and morphology
e) Ductal cells- normal or atypical.

All the histopathological slides were reviewed and the diagnosis confirmed.

Results

All the patients were women and the mean age was 34.8 years. In 6 cases, the lesion was located on the left side and in the other 2 on the right (Table 1). At least 4 smears were prepared from each case and were stained with H& E and/or Papanicolaou stain, MGG, PAS and Grocott's Methanamine Silver stains.

All the 8 cases showed the presence of granulomas and giant cells in the smears (Fig. 1). The granulomas (Fig. 2) were composed of epithelioid histiocytes with abundant cytoplasm, oval to reniform nuclei, dispersed chromatin and conspicuous nucleoli. Background of all the smears showed epithelioid histiocytes with morphology similar to those seen constituting the granulomas. Polymorphs accounted for most of the inflammatory cells in all the cases. Three cases showed lymphocytes, while plasma cells were present in only one case. Caseous necrosis was absent in all the cases. The giant cells were both foreign body type and Langhan's type morphologically.

All the slides were negative for fungus on PAS stain and Grocott's Methanamine Silver stain. Culture did not yield any growth in any of the 8 cases. For all the cases, ZN stain staining for acid fast bacilli was non-contributory and Mycobacterium Tuberculosis DNA was not detected by PCR in the specimens (Fig. 3).

Histological review of all the cases confirmed the diagnosis of granulomatous mastitis (Fig. 4).

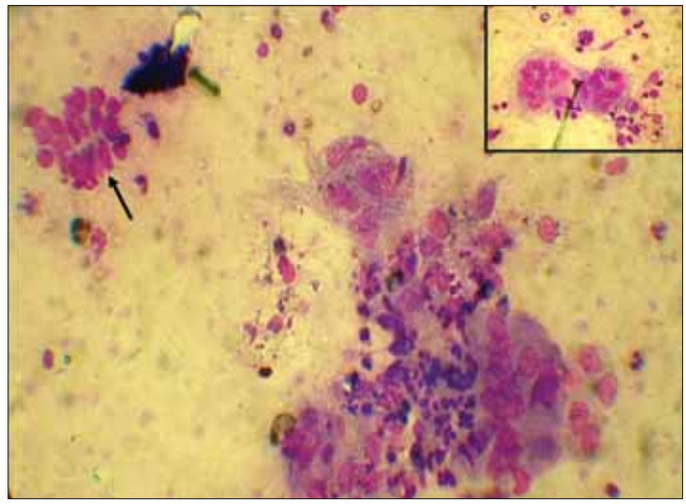

Figure 1. Collection of histiocytes, arrow (↑) showing benign ductal epithelial cells, inset showing giant cells. MGG, × 40

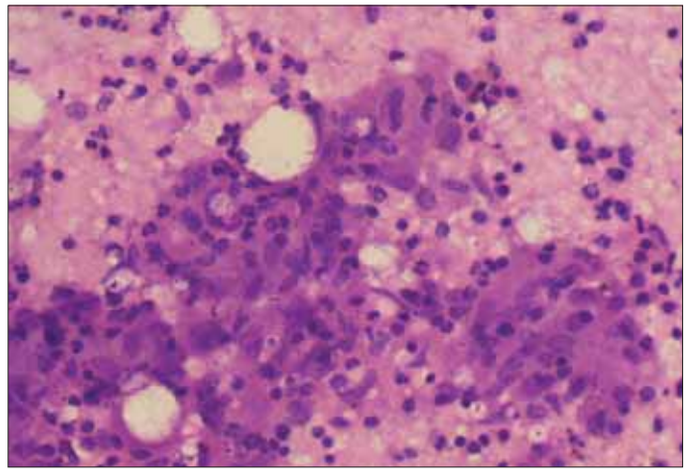

Figure 2. Granuloma comprising of epithelioid histiocytes with polymorphs in the background. H&E, × 40

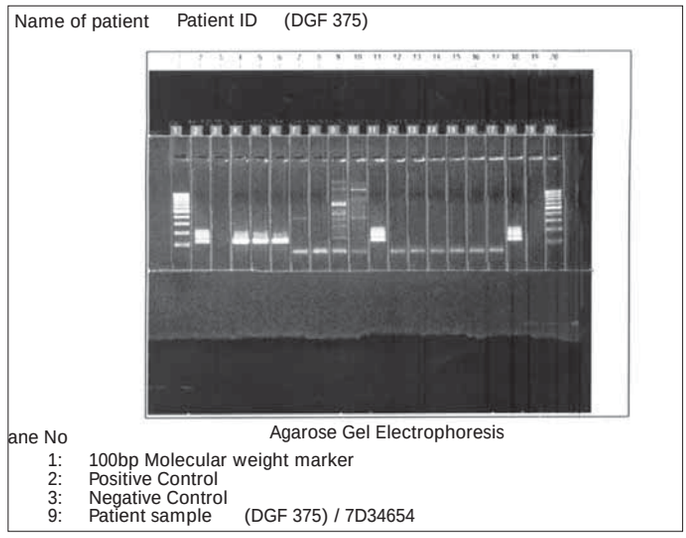

Figure 3. Photograph of agarose gel electrophoresis showing negative result for the sequence of Mycobacterium tuberculosis

Discussion

Granulomatous mastitis is an uncommon breast lesion that is well known for its worrisome clinical presentation, particularly in younger women (14). In our study, however, one patient was aged 61 years. Clinically and radiologically, granulomatous mastitis is difficult to distinguish from breast carcinoma (14). In our study, the radiological diagnoses of cases 1 and 7 were that of breast carcinoma and both patients had to undergo unnecessary mastectomy. This could have been avoided by performing core biopsy before mastectomy (15).

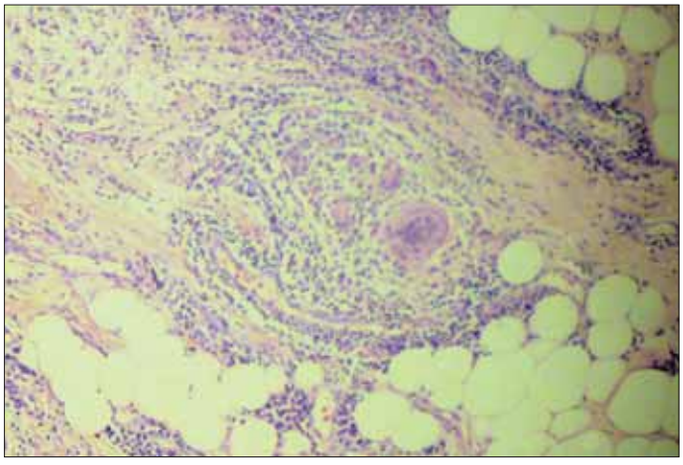

Figure 4. Histopathology section showing granuloma, giant cells & polymorphs involving breast lobule. H&E, × 40

The etiology of idiopathic granulomatous mastitis is still unknown. Kessler and Wallooch proposed an autoimmune pathogenesis (1). In a recently reported case, immunohistochemical staining showed that the lesion contained predominantly stromal T lymphocytes (16). However, there has been no evidence of systemic immune abnormalities such as the formation of autoantibodies or antigen-antibody complexes.

Among the only three large series describing the FNAC features of granulomatous mastitis in the literature, the usefulness of FNAC in granulomatous mastitis has been debated, with some authors confirming the useful role of FNAC (10, 11), whereas others have concluded that the various causes of granulomatous inflammation cannot be confidently differentiated by FNAC (9).

The presence of epithelioid histiocytes appears to be a common feature, having been reported in all cases of granuloma-

Table 1. Clinicopathological profile of patients included in the study

Case Number	Age	Sex	Clinical Features	Radiology	FNAC diagnosis	Histopathological diagnosis
1	30	F	Lump in left upper outer quadrant breast with axillary lymphadenopathy	Mass with angulated and spiculated margins surrounded by echogenic fibrous tissue ? Carcinoma	Granulomatous Mastitis	Idiopathic Granulomatous Mastitis
2	28	F	Lump in right upper inner quadrant breast	Not Done	Granulomatous Mastitis	Idiopathic Granulomatous Mastitis
3	25	F	Lump in left breast involving three-fourth of breast with sinus formation and few enlarged axillary lymph nodes	Increased soft tissue thickening and edema with multiple focal heterogenous area. ? Inflammatory	Granulomatous Mastitis	Idiopathic Granulomatous Mastitis
4	61	F	Right breast lump and nipple discharge	Not Done	Granulomatous Mastitis	Idiopathic Granulomatous Mastitis
5	35	F	Left breast lump	Not Done	Granulomatous Mastitis	Idiopathic Granulomatous Mastitis
6	36	F	Pain and ill defined lump in right breast	Benign breast disease	Granulomatous Mastitis	Idiopathic Granulomatous Mastitis
7	32	F	Lump in left breast with sinus formation	heterogeneous, with a thin rim of subcapsular radiolucent fat	Granulomatous Mastitis	Idiopathic Granulomatous Mastitis
8	32	F	Lump in left breast	spiculated margins with separate anterior focal asymmetry ? Carcinoma	Granulomatous Mastitis	Idiopathic Granulomatous Mastitis

tous mastitis in the literature (9-11, 14, 16, 17). In our study, all the cases showed epithelioid histiocytes whether single or in the form of granulomas.

The number of single epithelioid histiocytes was directly proportional to the number of granulomas in the smears. Our series showed the predominance of neutrophils over lymphocytes, similar to that of another study of nine cases, which demonstrated moderate to abundant numbers of neutrophils in the FNAC preparation. Most other reports have described a mixed inflammatory cell infiltrate. Another characteristic feature was the absence of caseous necrosis, which favors a diagnosis of granulomatous mastitis over an infective cause of granulomatous inflammation.

The single most important differential diagnosis of granulomatous mastitis in our part of the world is tuberculosis. The predominant presence of neutrophils in the background and the lack of caseous necrosis favor a diagnosis of granulomatous mastitis rather than tuberculosis (14). Granulomatous mastitis should also be distinguished from other chronic inflammatory breast diseases such as mammary duct ectasia, Wegener's granulomatosis, sarcoidosis and histoplasmosis (17). A diagnosis of granulomatous mastitis should also be considered when high numbers of epithelioid histiocytes are seen in smears, even in the absence of granulomas.

Conflict of interest

None declared.

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Effect of GnRH analogues and octreotide treatment on apoptosis and the cell proliferation of endometrium adenocarcinoma cell lines

Endometriyal adenokanser hücre serilerinde GnRH analogları ve oktreotidin apoptozis ve hücre proliferasyonu üzerindeki etkileri

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Abstract

Objective: The aim of this study was to compare apoptotic and antiproliferative effects of gonadotropin-releasing hormone analogues and their combination with octeotide on endometrioid endometrial cancer cell lines.

Material and Method: Women diagnosed with endometrioid adenocarcinoma at the department of Gynecology and Obstetric of Kocaeli University Medical School were included in this research. Endometrium cancer cell lines obtained from three patients were used for this study. After trypsinization in 0.5% in calcium magnesium, free phosphate buffer solution (CMFPBS) cells were seeded on glass slides in 24-well plates containing DMEM-F12 medium and 10% fetal calf serum as culture medium. Cells were incubated for 24 hours at 37ºC in 5% CO2. GnRH agonist leuprolide (Lucrin 1 μmol/L), GnRH antagonist ganirelix (Orgalutran 1 μmol/L), leuprolide with octreotide (Sandostatin 10-6 mol/L), ganirelix with octreotide and no drug were added to the wells. Apoptosis and cells proliferations were evaluated after 12, 24, 48 and 72th hours of incubation. The percentage of apoptotic cells was evaluated by TdT mediated biotin-dUTP nick-end labeling (TUNEL) method; cell proliferation was assessed by bromodeoxyuridine (BrdU) incorporation.

Results: Apoptotic index in grade I EEC cell line among ganirelix-octreotide treated cells and leuprolide-octreotide combination therapy were respectively higher than the untreated control ($p<0.001$, $p=0.001$). The number of apoptotic cells in grade II EEC cell line among leuprolide-octreotide and leuprolide were significantly ($p<0.001$, $p<0.001$) higher than in controls. In grade III EEC cell line, the number of TUNEL positive cells among leuprolide, ganirelix and ganirelix-octreotide therapiy groups were significantly higher than in untreated control. Time dependent antiproliferative effect was obtained with leuprolide and leuprolide-octreotide in grade I EEC ($p<0.001$, $p<0.001$). Grade II EEC cell line is not influenced by hormonotherapies. However, the antiproliferative effect was obtained with ganirelix, leuprolide and leuprolide-octreotide in grade III cell line.

Conclusion: GnRH analogues appears to have a direct effect, enhancing the apoptotic index and decreasing the cell proliferation in endometrial adenocancer cell lines.

(J Turkish-German Gynecol Assoc 2010; 11: 131-6)

Key words: Endometrial cancer, gonadotropin-releasing hormone analogues, octreotide, apoptosis, cell proliferation

Received: 1 July, 2010 **Accepted:** 12 August, 2010

Özet

Amaç: Bu çalışmanın amacı endometriyal adenokanser hücre serilerinde GnRH analogları ve oktreotidin apoptozis ve hücre proliferasyonu üzerindeki etkilerini karşılaştırmaktır.

Gereç ve Yöntemler: Kocaeli Üniversitesi Tıp Fakültesi Kadın hastalıkları ve Doğum bölümünde tanı almış olan endometriyal adenokanser vakaları çalışmaya alındı. Üç hastadan alınmış olan endometriyla kanser hücre serileri bu çalışmada kullanıldı. Tripinizasyon için %0.5 lik kalsiyum magnezyum, free phosphate buffer solusyonda (CMFPBS) bekletilen hücreler daha sonra DMEM-F12 mediyumu ve %10 dana fetus serumu içiren dishlere ekildi. Hücreler 37ºC in %5 CO2 de 24 saat inkübe edildiler. GnRH agonist leuprolide (Lucrin 1 μmol/L), GnRH antagonist ganirelix (Orgalutran 1 μmol/L), leuprolide ve octreotide (Sandostatin 10-6 mol/L), ganirelix ve octreotide ve ilaçsız gruplar oluşturuldu. Apoptozis ve hücre proliferasonu inkübasyondan sonraki 12, 24, 48 ve 72. saatlerde değerlendirildi. Apoptotik hücre oranı TUNEL yöntemi ile, hücre proliferasyonu ise bromodeoxyuridine (BrdU) ilavesiyle değerlendirildi.

Bulgular: Grade I endometriyal kanser hücre serisindeki apoptotik index ganirelixoctreotide ve leuprolide-octreotide combination tedavileri alan gruplarda tedavisiz gruba göre anlamlı olarak daha yüksek olarak saptandı ($p<0.001$, $p=0.001$). Grade II endometriyal kanser hücre serisindeki apoptotik index sayısı leuprolide-octreotide ve leuprolide gruplarında kontrol gruba göre anlamlı olarak daha yüksek olarak saptandı ($p<0.001$, $p<0.001$). Grade III endometriyal kanser hücre serisindeki TUNEL pozitif hücre oranı leuprolide, ganirelix and ganirelixoctreotide tedavisi alan gruplarda kontrol grubuna göre anlamlı olarak daha yüksek idi. Zamandan bağımsız antiproliferatif etkinlik leuprolide ve leuprolide-octreotide gruplarında grade 1 hücre serilerinde gözlendi ($p<0.001$, $p<0.001$). Grade 2 Grade I endometriyal kanser hücre serisinin hormon tedavisinden etkilenmediği görüldü. Buna karşın ganirelix, leuprolide ve leuprolide-octreotide gruplarında Grade III endometriyal kanser hücre serisinde antiproliferatif etkinlik saptandı.

Sonuç: Öyle görülmektedir ki, endometriyal adenokanser hücre serilerinde GnRH analoglarının apoptotik indeksi yükseltme ve hücre proliferasyonu azaltma mekanizmaları ile direkt etkisi vardır.

(J Turkish-German Gynecol Assoc 2010; 11: 131-6)

Anahtar kelimeler: Endometriyal kanser, gonadotropin-releasing hormone analogları, octreotide, apoptozis, hücre proliferasyonu

Geliş Tarihi: 01 Temmuz 2010 **Kabul Tarihi:** 12 Ağustos 2010

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doi:10.5152/jtgga.2010.19

Introduction

Endometrial carcinoma is the most common malignancy of the female genital tract (1). Despite the dominance of early stage disease, surgical treatment and/or irradiation are not curative for advanced endometrial cancer and the number of reported cancer deaths is increasing. Chronic elevated level of estrogen without the inhibitory effects of progesterone are considered stimuli for abnormal endometrial cell proliferation. Also, mutations in the tumour suppressor genes and microsatellite instability (MI) are common genetic abnormalities in endometrioid endometrial cancers (EEC), and distinguish these lesions from other histological subtypes of endometrial carcinomas (2).

Apoptosis and proliferation are the main factors in controlling both benign and malignant cell populations. Apoptosis is a physiological process leading to cell death characterized by cell shrinkage, membrane blebbing and DNA fragmentation and it is an important phenomenon existing in normal endometrium, regulated by sex steroids during the menstrual cycle (3, 4). In conditions where the concentrations of sex steroids are altered, the expression of apoptosis related proteins is susceptible to change. If the balance of the regulation of cell cycle is affected it thus induces a potential loss in the control of cell survival and may progress to cancer progression.

As endometrial cancer develops from generally hormone dependent cells, endocrine treatment has been the traditional palliative therapy of advanced or recurrent disease. Progestins have been used and currently GnRH analogues and their mechanism of effect have been investigated by many researchers. It has been demonstrated that about 50% to 80% of endometrial cancer express GnRH receptors, and recent researches have been focused on the possible use of GnRH agonists and antagonists as a potential target for the treatment of endometrial cancer (5-7).

Octreotide acetate is a synthetic octapeptide analogue of naturally occurring somatostatin with similar pharmalogical effects, but with a prolonged duration of action. It may act directly and specifically on neoplastic cells or indirectly via peptides and/or other substances that are crucial for neoplastic growth.

The aim of the study is to compare the apoptotic and proliferative effect of leuprolide, ganirelix, leuprolide combined with octreotide, ganirelix combined with octreotide and untreated control in human endometrioid adenocarcinoma cell culture to evaluate possible clinical use of these hormones for future treatment of advanced/ recurrent endometrial carsinoma.

Material and Methods

Tissue samples

Surgical specimens were obtained from three patients undergoing hysterectomy for endometrial cancer at the Department of Gynecology and Obstetrics of the Kocaeli University Medical School. All patients were post-menopausal, aged 56, 65 and 71 years. Tumor specimens were placed in ice cold phosphate buffer solution (PBS) immediately after surgical removal and representative portions were excised to prepare the materials for histological frozen section. Approximately 1 mm^3 tumor tissue were utilised for the cell culture and the residue was sent for histological examination. Tumor grading of the paraffin-embedded tissue blocks were compatible with the preoperative diagnosis. This investigation was approved by the Institutional Ethics Committee of the Kocaeli University, School of Medicine. Informed written consent was obtained from all subjects.

Cell culture

Samples of human endometrioid type adenocarcinoma were obtained under sterile conditions in the surgical pathology unit. The tissue was immediately placed into the culture medium and processed within 60 minutes of collection. Single cells obtained by mechanical disruption were separated from the clumps by sedimentation and then removed. The bigger clumps that sedimented were digested in a 37ºC shaking waterball for 1 hour with 1mg/ml collagenase B ve 0.1 mg/ml DNAase. The tissue was then washed in PBS.

After removal of the supernatant, pellets which had been diluted with one ml of the washing medium were filtrated through sieve number 46 (cell-dissociation sieve, Sigma-Aldrich) and the ultrafitrate was seeded on 25 cm^2 tissue culture flasks and embedded 10 ml DMEM-F12 medium with 10% fetal calf serum. The cells were cultured in a humidified atmosphere of 5% CO_2 and 95% air at 37ºC and passaged every 3 days after the 5th day of incubation. After trypsinization in 0.5% in calcium magnesium free phosphate buffer solution (CMF-PBS), the cells were seeded on glass slides in 24-well plates containing DMEM-F12 (Dulbecco's modified Eagle's minimal essential medium) medium and 10% fetal calf serum as culture medium.

Incubation

Cells were incubated for 24 hours at 37º C in 5% CO_2. Leuprolide acetate (Lucrin®, Abbott, Chicago MI, USA) as GnRH agonist and ganirelix (Orgalutran®, Organon) as GnRH antagonist in concentrations of 1 μmol/L; and a combination of leuprolide with octreotide 10^{-6} mol/L (Sandostatin®, Novartis, Quebec, Canada) and ganirelix with octeotide as somatostatin analogue were added to the wells. After 12 hours cells were removed for analysis of apoptosis and after 24, 48, and 72 hours groups, media were removed 1 hr before the end points of each interval and the cells were incubated in 1 ml of medium containing 20μM BrdU for the last hour.

Measurement of apoptosis

The percentage of apoptotic cells was assessed by the TUNEL technique following the manufacturer's instructions (In situ cell death detection kit, POD, Cat No.1 684 817, Roche Diagnostic) in the endometrial cancer cells cultures 12 hours after the addition of GnRH agonist Lucrin 1 μmol/L (Leuprolide®, Abbott, Chicago MI, USA), GnRH antagonist Orgalutran 1 μmol/L (Ganireliks®, Organon) and their combination with somatostatin analogue octreotide 10^{-6} mol/L (Sandostatin®, Novartis).

The cells were fixed for 30 min in 4% paraformaldehyde at room temperature. The cells were permeabilized with 0.1% TritonX-100 and 0.1% sodium citrate for 2 min at 4ºC and were then incubated at 37ºC for 60 min in the dark in 50 μL TUNEL reaction mixture. Thereafter they were incubated with 50 μL converter at room temperature for 20 min.

BrdU incorporation in vitro

Detections of BrdU-labeled cells was performed using standard avidin-biotin complex methods immunoperoxidase kits

(LabVision) with primary BrdU antibody (NeoMarkers). The cells were fixed in methanol at -18ºC for 1-2 min, allowed to air dry, then stored at -20ºC until all coverslips were ready for processing. Cells were rehydrated in the PBS for 5 min, followed by immersion in 2N HCl for 1 hr at room temperature. The cells were incubated in 0.1 M borate buffer (pH 8.5, 0.1 M boric acid, 25 mM $Na_2B_4O_7$ and 75 mM Na Cl) twice for 5 min each, followed by 3 washes in PBS.The cells were then incubated with BrdU Mouse Mab (Bu2a) at a dilution of 1:100 for 1 hr at 37ºC with biotinylated secondary antibody (LabVision Cat. TM-060-HL) for 20 min and with streptavidin/peroxidase (LabVision Cat. TA-060-HA) for 30 min at room temperature. Subsequenty, sections were subjected to color reaction with 0.002% 3.3- diaminobenzidine tetrahydrochloride containing 0.005% H_2O_2 in PBS (pH 7.4) and lightly counterstained with hematoxylin.

Cell counting

The number of TUNEL positive stained cells and BrdU labeled cells were quantified by two independent observers in a blind manner. Apoptotic cells were detected by their red colour (Figure 1). Each observer viewed randomly selected 1000 cells in a light microscope at a magnification of 40X. The number of apoptotic cells was determined by apoptotic index (i.e., number of apoptotic cells per 100 cells) and BrdU labeled cells were expressed as the percentage of positive cells (Figure 2). There was no significant difference between the results of two observers ($p=0.23$).

Statistical analysis

Statistical analysis were performed by Kruskal- Wallis nonparametric analysis using SPSS 11.5 (Statistical Programme For Social Sciences, IL, USA). The statistical significance of the difference between the control and hormonotherapy groups was determined by one-way Anova followed by Dunnett T3 test for multiple comparisons. A P value of less than 0.05 was considered significant.

Results

Apoptosis

Figure 3 demonstrates that the TUNEL positive cells count after the 12th hour of the treatment with leuprolide, ganirelix and their combination with octreotide was higher than the control group. In the grade I EEC cell line the number of apoptotic cell was higher (2.5 ± 0.52) than in the grade II (0.7 ± 0.48) and grade III (0.3 ± 0.48) adenocarcinoma cell line in the drug free group.
Apoptotic index in the grade I EEC cell line among ganirelix-octreotide treated cells (8.5 ± 0.671) and leuprolide-octreotide combination therapy (4.7 ± 0.3) were respectively higher than in the untreated control (2.5 ± 0.167), which was statiscally significant ($p<0.001$, $p=0.001$). The number of apoptotic cells in the grade II EEC cell line among leuprolide-octreotide (5.5 ± 0.167) and leuprolide (3.1 ± 0.18) were significantly ($p<0.001$, $p<0.001$) higher than in the untreated control (0.7 ± 0.153). In the grade III EEC cell line, the number of TUNEL positive cells among leuprolide (4.4 ± 0.819), ganirelix (4.2 ± 0.389) and ganirelix-octreotide (3.1 ± 0.277) therapies groups were significantly ($p<0.001$, $p<0.001$, $p=0.002$ respectively) higher than the untreated control (0.3 ± 0.153) (Table 1).
In the presence of 10^{-6} mol/L octreotide, the apoptotic index of the grade I ECC cell line was significantly increased when

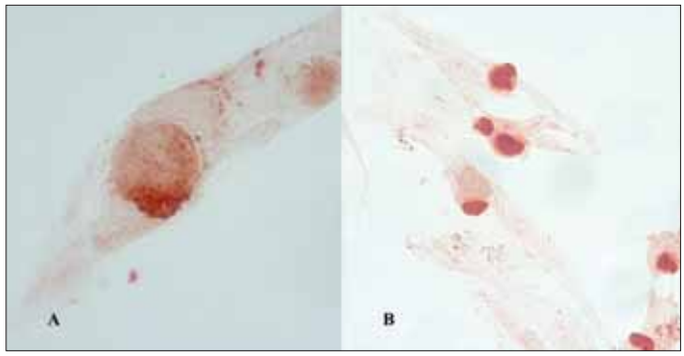

Figure 1 a-b. TUNEL positive cells

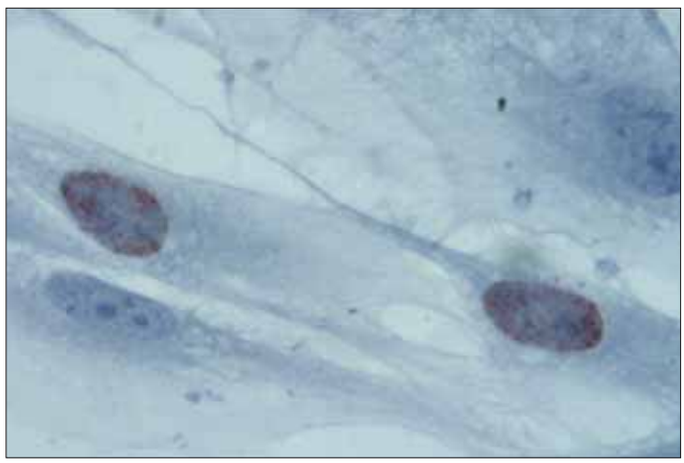

Figure 2. BrdU labeled endometrial cancer cells with light microscopic observation

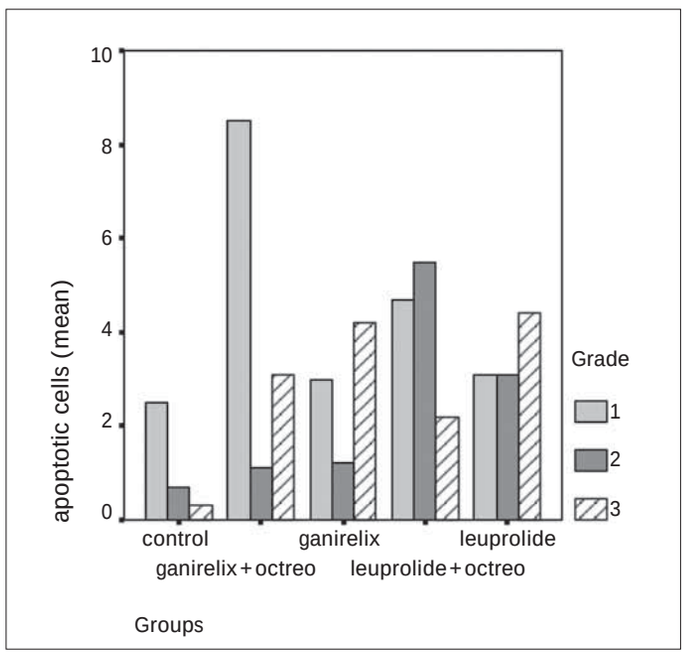

Figure 3. Mean of apoptotic cells in grade I, grade II and grade III endometrial adenocarcinoma, after treatment with GnRH analogues and analogues plus octreotide

compared with the ganirelix and leuprolide added groups ($p<0.001$, $p=0.025$ respectively). However, no additive effect was found with the combination of octreotide on the grade III ECC cell line.

Inhibition of cell proliferation

The inhibitory effect of ganirelix, leuprolide and their combination with octreotide on three different endometrial cancer cells lines (grade I-II-III) was confirmed using BrdU incorporation into untreated and treated EEC cells in vitro at the 24th, 48th and 72th hours. BrdU labeled cells among ganirelix (2.6±0.22), ganirelix and octreotide (3.7±0.26), leuprolide treated cells (3.9±0.1) were respectively lower than the number in untreated controls (7.8±0.36) which was statiscally significant ($p<0.001$) at the 24th hour of the study in grade I EEC. The number of BrdU positive cells at the 48th and 72th hours were statistically significant when compared with the controls ($p<0.001$) (Table 2). The antiproliferative response was also seen in the presence of untreated medium in grade II ECC (Table 3). The combination of ganirelix with octreotide did not produce any variation in cell proliferation compared with that obtained with ganirelix alone in grade I and grade II ECC cell lines, while ganirelix and leuprolide alone therapy groups were found more effective than the combination in the grade III EEC cell line (Table 4).

In grade I ECC, when the decreasing percentiles were compared between the groups, the leuprolide plus octreotide treatment group ($p=0.004$) and leuprolide treatment group ($p=0.04$) were found to have significantly higher decrease compared to the control group.

In grade II, no difference was found between the groups. On grade III ECC cell lines, ganirelix plus octreotide ($p<0.001$) and leurolide plus octreotide ($p<0.001$) had significantly smaller decrease when compared to the control group.

Discussion

GnRH is the primary hypothalamic regulator of reproductive function. Leuprolide acetate is a GnRH agonistic analogue used to treat a wide range of estrogen dependent disorders. It acts on the anterior pituitary, initially inducing a transient rise in gonadotropin release. With continued administration, GnRH causes pituitary desensitization leading to suppressed circulating levels of gonadotropins and estrogens and has been used in the therapy of some sex-hormone-dependent cancers, including breast, prostatic, endometrial and ovarian cancer (8-11). GnRH antagonists, unlike the agonistic analogues, do not induce an initial stimulation of gonadotropin release but cause immediate and rapid, reversible and dose dependant suppression of gonadotropin secretion by competitive receptor occupancy of GnRH receptors (12). The antiproliferative effects of GnRH-a seem to be not only through the suppression of gonadal steroids, but also a direct effect on cell growth, and a specific binding site for GnRH has been demonstrated in several tumors responsive to GnRH agonists (5, 13-15). A second type of GnRH receptor has been identified in endometrial and ovarian cancer cells, which transmits a significantly stronger antiproliferative effect than GnRH-I receptor. In mammals, GnRH-II is more widely identified than GnRH-I in periferal tissues, suggesting that GnRH-II may have additional functions (16). In a recent in vitro study, GnRHs treatment was found to cause an increase in integrin $\beta 3$ expression and evoked the activation of focal adhesion kinase (FAK), ERK1/2 and p38 MAPK compared to the control (17). GnRH-II treatment increases the expression of DNA damage-inducible gene 45 (GADD45α) in endometrial cancer cells (16). GnRH-II induces apoptosis through the binding of GnRH-I receptors, activation of ERK1/2 and p38 MAPK pathways, and induction of GADD45α signaling. These recent

Table 1. The mean of apoptotic cells in grade I, grade II and grade III endometrial adenocarcinoma, after treatment of GnRH analogues and analogues plus octreotide and their comparisons by groups

	Grade I (mean±SE)	Grade II (mean±SE)	Grade III (mean±SE)
Control	2.5±0.167	0.7±0.153	0.3±0.153
Ganirelix+Octreotide	8.5±0.671*	1.1±0.1	3.1±0.277***
Ganirelix	3±0.149	1.2±0.133	4.2±0.389*
Leuprolide+Octreotide	4.7±0.3**	5.5±0.167*	2.2±0.49
Leuprolide	3.1±0.18	3.1±0.18*	4.4±0.819*
p value	<0.001	<0.001	<0.001

* $p<0.001$
** $p=0.001$
*** $p=0.002$

Table 2. Mean of BrdU positive cells in grade I EEC cell line, after treatment with GnRH analogues and analogues plus octreotide, their comparisons by groups and the percentage of decreased proliferative cells

	24th hour (mean±SE)	48th hour (mean±SE)	72th hour (mean±SE)	Decreasing percentage %±SE (min-max)
Control	5.6±0.42	4.8±0.2	2.7±0.26	46.3±10 (-33-71)
Ganirelix+Octreotide	3.7±0.21	3.2±0.13	1±0	71.6±2.5 (50-75)
Ganirelix	2.6±0.22	2.4±0.16	1.1±0.1	51.6±8.7 (0-66.6)
Leuprolide+Octreotide	7.6±0.26	3.4±0.16	1±0.14	86.5±2.1 (71.4-100)
Leuprolide	3.9±0.1	3.5±0.16	0.4±0.16	90±4.08 (75-100)
p value	<0.001	<0.001	<0.001	<0.001

Table 3. Mean of BrdU positive cells in grade II EEC cell line, after treatment with GnRH analogues and analogues plus octreotide and their comparisons by groups and the percentage of decreased proliferative cells

	24th hour (mean ± SE)	48th hour (mean ± SE)	72th hour (mean ± SE)	Decreasing percentage % ± SE
Control	7.2 ± 0.46	1.8 ± 0.35	1.5 ± 0.22	77.8 ± 3.8 (57-90)
Ganirelix + Octreotide	3.7 ± 0.36	0.8 ± 0.13	0.6 ± 0.16	79.1 ± 5.9 (50-100)
Ganirelix	1.9 ± 0.37	0.9 ± 0.1	0.3 ± 0.15	83.3 ± 11.7 (0-100)
Leuprolide + Octreotide	3.1 ± 0.18	1.4 ± 0.22	0.5 ± 0.16	81.6 ± 6.3 (50-100)
Leuprolide	1.5 ± 0.22	3 ± 0	0.2 ± 0.13	85 ± 10.6 (0-100)
p value	< 0.001	< 0.001	< 0.001	0.175

Table 4. Mean of BrdU positive cells in grade III EEC cell line, after treatment with GnRH analogues and analogues plus octreotide and their comparisons by groups and the percentage of decreased proliferative cells

	24th hour (mean ± SE)	48th hour (mean ± SE)	72th hour (mean ± SE)	Decreasing percentage % ± SE (min-max)
Control	5.5 ± 0.16	5.5 ± 0.56	2.2 ± 0.29	59.6 ± 5.84 (20-80)
Ganirelix + Octreotide	3.8 ± 0.24	3.3 ± 0.21	3 ± 0.29	16 ± 11 (-50-75)
Ganirelix	5.2 ± 0.41	2.4 ± 0.16	2.1 ± 0.1	56.4 ± 5 (25-71.4)
Leuprolide + Octreotide	4.5 ± 0.26	2.3 ± 0.3	2.4 ± 0.22	44.1 ± 6.8 (0-80)
Leuprolide	7.5 ± 0.26	7.2 ± 0.24	1.4 ± 0.22	81.4 ± 2.5 (66.6-88.8)
p value	< 0.001	< 0.001	< 0.001	< 0.001

studies demonstrated that GnRH related inhibition of endometrial cancer cell proliferation is mediated by GnRH receptors and also related with mitogenic signal transduction molecules. Ganirelix is a synthetic third generation gonadotropin-releasing hormone antagonist that blocks GnRH receptors in the anterior pituitary gland, preventing endogenous GnRH from inducing LH and FSH release. GnRH antagonists have agonistic effects on this type II receptor (18). GnRH II has a strong antiproliferative effect without involving induction of apoptosis. It could be speculated that the additional receptors interact with pathways regulating the cell cycle (19).

Previous in vitro studies have shown that the number of Ishikawa endometrial cancer cells was reduced by the GnRH-I antagonist cetrorelix (SB-75) in a dose-dependent manner (13). This growth inhibitory effect of SB-75 was not found to be associated with a decrease in the number of cells in the S phase but was associated with an induction of apoptosis (13). The decrease in the rate of apoptosis in grade 3 adenocarcinoma in the drug free group may reflect loss of cell homeostasis control and decreased differentiation. It may stated that there are tissue-specific differences controlling the progression of cancer cells.

Heterogeneity of receptor density appears to be common in endometrial adenocarcinomas as the vast majority of tumors showed substantial receptor heterogeneity of both ER and PR within the tumors. Hormone receptor heterogeneity of endometrial carcinoma has been discussed also in the context of the primary tumors and the metastases having different hormone receptors status (20). In a recent study, Jeon et al demonstrated that GnRH receptor expression was not related to the histotype of endometrial cancer, disease stage, tumor differentiation, lymph node metastasis and myometrial invasion (7). In invitro conditions, GnRH analogues will show their effect via GnRH receptors. As previously described, GnRH agonists can induce Fas Ligand production in GnRH receptor bearing endometrial carcinoma. The Fas Ligand expression linked to the GnRH receptor activation may mediate the antiproliferative action of GnRH agonist by increasing apoptosis within the cancer cells, but the GnRH effect was abolished by the addition of the antagonist antide (21). In this presented study, apoptosis was found to be induced in both agonist and antagonist application. The cell culture studies demonstrated an increase in the programmed cell death in the grade I, II and III endometrial adenocancer cell lines at the 12th hour of the treatment groups. However, the proliferation index varied depending on the histological grade, which may contribute to the difference in tumor behavior. These apoptotic and antiproliferative effects occur at micromolar concentrations. In fact, when administrated subcutaneously, plasma concentrations of leuprolide at therapeutic doses are in the nanomolar range (22). Also, blood concentrations of antagonist analogues ranged between 30-60 ng/ml with an injected dose of 10 mg/day (18). Previous dose response experiments showed the antiprolifetive effect of the lower concentration of the GnRH analogues in the HEC-1a lines and Ishikawa cell lines (13, 23). Moreover, primary in vitro cultures are closer to in vivo biology when compared to cancer cell lines. Our results suggest that in endometrial cancer, GnRH is part of a negative autocrine system. This knowledge could help clinicians decide whether to use GnRH agonistic or antagonistic

analogue and a combination with octreotide to inhibit cell proliferation, in patients with recurrent and/or advanced disease. However, further studies are needed to evaluate the possible additive roles of somatostatin analogues in endometrial cancer. The insulin-like growth factor-1 (IGF-1) signalling pathway has important roles in regulating cellular proliferation and apoptosis. In vivo carcinogenesis models indicate that high levels of plasma IGF-1 are associated with increased risk of cancer (24). Therapeutic strategies that target the IGF-1 receptors or reduce serum levels of growth factor and IGF-1 may be important for the antineoplastic activity of cancer dependent IGF pathway (25). Many human tumors can express somatostatin receptors. In a series of 28 randomly selected endometrial carcinomas, sst_1 was present in 32%, sst_{2A} in 39%, sst_3 in 43%, sst_4 in 4% and sst_5 in 4% of cases. 36% expressed more than one sst receptor (26). Somatostatin analogue octreotide (SMS 201-995) bind with a high affinity for somatostatin receptors 2 and 5 but show a relatively low affinity for ssr 3 (27). Receptor positive endometrial carcinomas may be a potential target for somatostatin analogues.

There are few reports on the use of GnRH-a and somatostatin analogues in Gynecologic Oncology. The use of octreotide in gynecologic tumors is reported as case reports. Preclinical studies on tumor biology on somatostatin and its receptors are still under research. As in vitro studies do not parallel the in vivo milieu, these studies are not yet conclusive. Our study reports the effects of GnRH agonist, antagonist and octreotide in three different grade endometrium cancer cell lines. They were compared in terms of their effects on apoptosis and proliferation.

In summary, the current study suggests that GnRH agonistic and antagonistic analogues and their combination with octreotide induce apoptosis. This may be a part of a therapeutic mechanism. Since we did not evaluate the GnRH receptor expression and the molecular pathway of apoptosis in the presented study, the extent of growth inhibition may be affected by more precisely targeted application of GnRH analogues on appropriate GnRH receptor positive cells. Further studies would help provide a better understanding of the molecular mechanisms of apoptosis and cell cycle dynamics.

Conflict of interest

No conflict of interest is declared by authors.

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Experience of our surgery in iatrogenic vesicovaginal fistulas

İatrojenik vezikovajinal fistüllerde cerrahi deneyimlerimiz

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Abstract

Objective: In this study, transvesical and transvaginal approaches used in our clinic for the treatment of gynecologic iatrogenic vesicovaginal fistulas are discussed.

Material and Methods: 11 patients with vesicovaginal fistula admitted to the Department of Urogynecology, Zeynep Kamil Teaching-Research Hospital between 2005-2009 were enrolled in our study. Transvesical and transvaginal fistula repair were performed on all patients. All patients were treated by surgical repair, 4 cases by a classic transabdominal approach, 5 cases by an omental flap interposition and 2 cases by a martius flap interposition.

Results: The most common cause of iatrogenic vesicovaginal fistula in our patients was total abdominal hysterectomy for benign conditions (n=10/11). The mean patient age was 43 years (34-53) and the mean time from the causative surgery to the operation was 7.5 months (3-12). The surgical techniques were successful in all patients. There were no intraoperative complications and no postoperative recurrences.

Conclusion: The mouth of the fistula should be determined clearly on preoperative evaluation and surgery procedure should be planned according to the fistula aperture. The point to be careful of is excision of all diseased tissue in the bladder and vagina, complete separation of the bladder from the vagina with a margin of healthy tissue, and watertight closure of both bladder and vagina without tension. The aim of the vascularized tissue interposition between the closed bladder and the vagina is to provide the improvement of vascularity. We believe that in the treatment of supratrigonal and large fistulas, the transvesical approach with use of omental flap interposition is more effective, while, in the treatment of small and trigonal fistula,the transvaginal approach with use of martius flap interposition is an effective tecnique.

(J Turkish-German Gynecol Assoc 2010; 11: 137-40)

Key words: Iatrogenic vesicovaginal fistula, martius flap, omental flap

Received: 28 April, 2010 **Accepted:** 1 July, 2010

Özet

Amaç: Bu çalışmada kliniğimizde jinekolojik iatrojenik vezikovajinal fistül nedeniyle uyguladığımız transvezikal ve transvajinal tedavi yaklaşımımız tartışılmıştır.

Gereç ve Yöntemler: 2005-2009 yılları arasında Zeynep Kamil Eğitim Araştırma Hastanesi Ürojinekoloji Kliniğine başvuran 11 kadın hastaya vezikovajinal fistül tanısıyla transvajinal ve transvezikal fistül onarımı uyguladık. Bu hastalara yaklaşımda 4 hastaya klasik abdominal yaklaşım, 5 hastaya omentum interpozisyonu, 2 hastaya martius flep interpozisyonu yapıldı.

Bulgular: Hastalarımızda vezikovajinal fistülün en sık sebebi benign nedenlerle yapılan total abdominal histerektomi bulundu (n=10/11). Hastaların ortalama yaşı 43 yıl (34-53), ortalama semptomatik dönemi 7.5 ay (3-12) olarak hesaplandı. Cerrahi teknikler tüm hastalarda başarılı oldu. Hastaların hiçbirinde intraoperatif komplikasyon ve postoperatif dönemde nüks olmadı.

Sonuç: Vezikovajinal fistül hastalarında preoperatif değerlendirmede fistülün vajinal ve mesane ağızları net olarak tespit edilmelidir ve yapılacak cerrahi yaklaşıma buna göre karar verilmedir. Dikkat edilmesi gereken nokta mesane ve vajinadan hastalıklı dokuların çıkartılıp, tamamen ayrı ayrı tabakalı, sızıntı olmayacak şekilde, sağlam doku sınırından ve gerilim olmadan onarılmasıdır. Onarılan mesane ve vajina arasına vaskülerize doku interpozisyonu kanlanmayı artırmak için uygulanır. Fistülün küçük, trigonal yerleşimli olduğu olgularda martius flep interpozisyonunun kullanıldığı transvajinal yol etkili bir yöntem olmakla birlikte; fistülün büyük, supratrigonal yerleşimli olduğu olgularda abdominal yaklaşımın daha etkili olduğuna inanıyoruz.

(J Turkish-German Gynecol Assoc 2010; 11: 137-40)

Anahtar kelimeler: İatrojenik vezikovajinal fistül, martius flep, omental flep

Geliş Tarihi: 28 Nisan 2010 **Kabul Tarihi:** 01 Temmuz 2010

Introduction

Urogenital fistulae are among the most dreaded complications encountered in obstetrics and gynecology and constitute a major surgical challenge for the urogynecologist. A vesicovaginal fistula is rare but its impact lies in the social distress that results from persistent leakage of urine. The overall incidence varies between 0.5 and 1.5%, and bladder injuries are more common than ureteric ones (1, 2). The VVF is the most common and the vesicouterine fistula the least common variety among the genitourinary fistulae (3, 4). Early in the past century, the most common cause of the VVF was complicated childbirth with tissue injury. With modern obstetric services, these fistulas are rare, and the most common cause, 75-90% of all VVF, is total abdominal hysterectomy (5, 6). In addition, some rare etiologies have been men-

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doi:10.5152/jtgga.2010.20

tioned in the literature, such as pelvic trauma, radiation necrosis, illegal abortion, as well as radical pelvic surgery (7, 8). In recent years, the rise of laparoscopic approaches has brought about an increased rate of iatrogenic injury (9). In underdeveloped countries, the most common cause of VVF is still obstetric causes including complications of neglected, prolonged, or obstructed childbirth (10).

In the present study, we aimed to discuss the etiologies of 11 cases with gynecologic iatrogenic VVF which were evaluated and operated on in our clinic and the results of our experiences about applied surgery techniques in these cases.

Material and Methods

The 11 cases of vesicovaginal fistulae, which were operated on in our department of urogynecology from 2005 to 2009, were included in this study. The complaints of the patients were urinary leakage from the vagina or continuous urinary incontinence. Preoperative assessment of the patients included detailed history taking, physical examination, rectovaginal examination, appropriate laboratory investigations, pelvic-abdominal ultrasound if needed, and also intravenous urography. Vaginoscopy, cystoscopy, and retrograde catheterization were applied to determine the size, site, and extent of the fistula as well as to exclude or confirm the involvement of the ureter or neck of bladder. The methylene blue dye test was carried out with the help of a tampon inserted into vagina when the fistula was not obviously seen on cystoscopy. Before cystoscopy, an intravenous pyelograph (IVP) was carried out in order to observe the upper urinary tract. After observation of the localization of fistula in the bladder and vagina, the patients were prepared for operation. Local estrogen preparations for patients showing vaginal postmenopausal atrophy and antibiotherapy for patients having infection on the fistula tract were given. Mechanical bowel preparation, when necessary, was started 48 h before surgery. Ceftriaxone 1 g and metronidazole 500 mg were administered intravenously with induction of anesthesia. Before surgery, while patients were in the supine lithotomy position, vaginal and suprapubic areas were sterilized with a solution containing povidone-iodine. Transvaginal or transabdominal approaches were preferred as an operation technique. In the transvesical approach, layered closure with or without omental flap interposition (O'Conor technique) was used (11-13). Abdominal incision was made vertically, the bladder was mobilized widely from the underlying vagina and uterus to the level of the fistula while avoiding from ureter orifice, and the fistula tract was excised circumscribely from the living tissue margin. Closure of the bladder mucosa and muscle layers was carried out by separate suturing with interrupted 3-0 polyglactin sutures without tension in a nonoverlapping manner to achieve a watertight closure. The vaginal mucosa was then sutured separately with interrupted 3-0 polyglactin sutures. Separate closure of the bladder and vagina was achieved. The repair was reinforced with free omental flap between the bladder and vagina for the cases in which the O'Conor technique were performed. The abdomen was closed after fistula repair and the operation was completed. In the transvaginal approach, a martius flap interposition was made to reinforce repair after the fistulous tract was excised (11, 14). The fistula tract was excised following wide mobilization of the vagina for about 2 cm from the edge of the fistula. Bladder mucosa and muscular layers were repaired separately and rotated to the inner edge of the fistula with interrupted 3-0 polyglactin sutures. A vertical incision was made on the right labium major and a martius labial fat pad (Bulbocavernosus fat tissue) interposition was made from under the vaginal mucosa and labium minor and was sutured on the bladder fascia. Mobilized vaginal mucosa was repaired vertically with 2-0 polyglactin sutures without tension in a manner that covered the fat flap. The vertical incision on the labium majus was repaired separately with 3-0 silk sutures. The operation was completed with insertion of a tampon into the vagina.

Results

The mean age of patients was 43 years (range 34-53). In ten patients, the etiology of the fistula was hysterectomy performed for benign conditions, while in one patient it was a history of repeated cesarean section (Table 1). While in ten patients VVF was primary, only one patient had secondary VVF. The mean symptomatic period beginning from their operation date until referral to our hospital was 7.5 months (range 3-12). Fistula size varied between 5 mm and 20 mm (average 15) and the diameter of fistular orifices varied between 2 mm and 15 mm (average 5) (Table 2). The locations of fistulas were supratrigonal in eight patients and trigonal in three patients. Nine patients were repaired transabdominally and two patients were repaired transvaginally. Two patients with small fistula (≤5mm) and trigonal were repaired vaginally and a martius flap interposition was applied. One patient with fistula, which was 10 mm and trigonal, was repaired abdominally by the O'Connor technique with an omental flap interposition. A double J catheter was placed near the mouth of the fistula in trigonal fistulas. Four patients with a fistula size greater than 10 mm and supratrigonal

Table 1. The causes of vesicovaginal fistula

Etiology	n
Hysterectomy for benign conditions*	10
Repeated cesarean	1
*Total abdominal hysterectomy was performed for these patients	

Table 2. The mean age, parity, symptomatic period, diameter of fistulas, size of the fistulas

Mean age (years)	43 (34-53)
Mean parity	2.3 (2-4)
Mean symptomatic period (month)	7.5 (3-12)
Mean diameter of fistulas (mm)	5 (2-15)
Mean size of the fistulas (mm)	15 (5-20)

were repaired abdominally and an omental flap interposition was made. The other four patients with a fistula size less than 10 mm and supratrigonal were repaired abdominally and a layered closure was made without omental flap interposition (Table 3). The average operative time was 35 min (25-45) for layered closure, 75 min. (50-80) for omental flap (O'Conor technique) and 120 min (110-130) for martius flap. There were no intraoperative complications. Urinary tract infection was seen in only one patient and the other patients had no postoperative complications. Blood transfusion was not required in any of the patients. The bladder was drained by foley catheter for three weeks in trigonal fistulas and one week in supratrigonal fistulas after operation and the catheter was extracted after patients could successfully urinate. Prophylactic antibiotic was continued parenterally for the first 48 h postoperatively and orally until the catheter was removed. All patients were examined postoperatively after an average of 4 month (range 2-6) and recurrence of fistula was not observed.

Discussion

The VVF is the most common among the genitourinary fistulae and in developed countries, 90% of vesicovaginal fistulae are caused by gynecological procedures (3, 15). Hysterectomy, both by transabdominal and transvaginal approaches, is the most common procedure, comprising 75% of fistulae (16). The overall incidence varies between 0.5 and 1.5%, and bladder injuries are more common than ureteric ones (1, 2). In pelvic surgery the development risk of VVF is 0.22% in laparoscopic hysterectomy, 0.1% in abdominal hysterectomy and 0.02% in vaginal hysterectomy (9). They emerge as a complication of gynecological surgery in developed countries, or obstetric trauma in third world countries. Obstetric fistulas are usually larger than post-hysterectomy fistulas and located more distally (5, 17). The main symptom of VVF is leakage of urine from the vagina, apparent only when the bladder is full, or constantly in the presence of a large fistula. After gynecological surgery, leakage usually appears after removal of the urinary catheter (18). Cystoscopy with vaginal examination is the most important clinical examination in the evaluation of VVF. The instillation of methylene-blue solution into the bladder is helpful in the diagnosis of small fistulas. Intravenous urography with cystography in the lateral position is necessary to discover the position of the fistulous channel and to exclude ureterovaginal fistula. VVF can be treated with surgery or conservatively and the timing of repair remains controversial. According to the literature, it is apparent that there is no consensus as to the definition of late (2-4 months) and early (1-3 months) repair (19, 20). Conservative approaches such as catheter drainage, occlusion with fibrin, peeling of the tract epithelium with metal screw and steroid use have been reported in the literature for closure of small fistulas (21). We preferred late repair in all operated patients, since we believe that we relatively decreased the number of postoperative surgery failures caused by necrosis and/or inflammation of the fistula edge, through saving time for creating a fibrous tissue. In our surgeries, the preferred approaches were transvaginal and transabdominal. Important technical details include excision of all diseased tissue in the bladder and vagina, complete separation of the bladder from the vagina with a margin of healthy tissue, and watertight closure of both bladder and vagina without tension. Interposition of vascularized tissue (peritoneum, omentum, labial fat pad, gracilis muscle, myocutaneous muscle flaps) between the closed bladder and the vagina is recommended to improve vascularity (22, 23). Fistulas greater than 3 cm, associated with radiotherapy or malignancy,and which involve the trigone, bladder neck and urethra are complicated fistulas and interposition of vascularized tissue can be used successfully in these cases (24-26). The first operation applied to patients with VVF is the most important operation which determines the rate of success in treatment. We consider that interposition of vascularized tissue is a reasonable approach for patients eligible at the first attempt. The applied surgical approaches are dependent on many factors, together with the experience and training of the surgeon (11). In the literature, the overall success rate for simple fistulae repaired by the transabdominal layered closure technique is 87.5% and by the transvaginal route 87.09% (27-29). We have achieved success in all 11 patients (n=11/11) on whom we operated.

Table 3. The locations and diameter of fistulas and surgical techniques

Diameter	n	Location	Surgical techniques
≤5mm	2	Trigonal	Martius flap interposition
10mm	1	Trigonal	Omental flap interposition
≥10mm	4	Supratrigonal	Omental flap interposition
<10mm	4	Supratrigonal	Only layered closure

In conclusion, VVF is an iatrogenic and treatable condition which is hygienically, socially and psychologically devastating. We consider that, in the treatment of supratrigonal and large fistulas, the transabdominal approach using omental flap interposition is more effective,while in the treatment of small and trigonal fistula, the transvaginal approach using martius flap interposition is an effective technique. In our cases, both transvaginal and transabdominal approaches have been used considering the size and location of the fistula, and no recurrence has been observed.

As in many other developed countries, vesicovaginal fistula is also seen after simple type 1 hysterectomy in our country. Most of these fistulas are supratrigonal. Operations should not be performed unless the intravesical and intravaginal location and tracing of fistulas are known.

Conflict of interest

No conflict of interest is declared by authors.

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Etonogestrel contraceptive implant (Implanon): analysis of patient compliance and adverse effects in the breastfeeding period

Etonogestrel gebelik önleyici implant (Implanon): Emzirme döneminde hasta uyumu ve yan etkilerin analizi

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Abstract

Objective: To analyse the compliance of patients and side effects of Implanon® during breast feeding.

Material and Methods: Prospective study of 61 postpartum women who chose Implanon® for long term contraception between April 2007 and December 2009. Compliance, side effects and removals were recorded.

Results: Amenorrhoea, prolonged bleeding, frequent bleeding and infrequent bleeding were reported in 20 (32%), 13 (21%), 4 (6.5%) and 2 (3.2%) patients, respectively. Non-menstrual side effects experienced by participants included; weight gain reported by 10 patients (16%), anxiety by 6 (9.8%), breast tenderness by 4 (6.5%), headache by 4 (6.5%), pain at the insertion site by two (3.2%), hirstutism by two (3.2%), acne by 1 (1.6%), loss of libido by 1 (1.6%), weight gain and headache by two (3.2%), weight gain and anxiety by two (1.6%). The mean breastfeeding period was 16±7.4 /months. During the follow up, Implanon® was removed from 24 patients (39%).

Conclusion: If patients are well informed about its expected side effects before placement, Implanon® is well tolerated and i an acceptable choice for women who have recently experienced labor and are looking for long term reversible contraception.

(J Turkish-German Gynecol Assoc 2010; 11: 141-4)

Key words: Implanon, etonogestrel, postpartum contraception, breastfeeding, compliance, side effect, removal

Received: 5 April, 2010 **Accepted:** 5 August, 2010

Özet

Amaç: Emzirme döneminde Implanon' un hasta uyumu ve yan etkilerini analiz etmek.

Gereç ve Yöntemler: Nisan 2007 ile Aralık 2009 arasında uzun dönem korunma için Implanon' u seçen doğum yapmış 61 kadının prospektif çalışması. Uyum, yan etkiler ve implantın çıkarılması kaydedildi.

Bulgular: Amenore, uzayan, sık veya nadir kanamalar 20 (%32), 13 (%21), 4 (%6.5) and 2 (%3.2) şeklinde tespit edildi. Deneklerde görülen non menstrual yan etkiler sırasıyla; kilo alma 10 hasta (%16), anksiyete 6 (%9.8), meme gerginliği 4 (%6.5), baş ağrısı 4 (%6.5), girişim yapılan yerde ağrı 2 (%3.2), hirsutizm 2 (%3.2), akne 1 (%1.6), libido kaybı 1 (%1.6), kilo alma ve baş ağrısı2 (%3.2), kilo alma ve anksiyete 1 (%1.6) idi. Hastaların ortalama emzirme süresi 16±7.4 ay idi. Takip süresince 24 hastadan (%39) Implanon® çıkarıldı.

Sonuç: Yerleştirilmeden önce, hastalar beklenen yan etkileri hakkında iyi bilgilendirilirse Implanon® yeni doğum yapmış uzun dönemli, geri dönüşü olan bir korunma yöntemi arayan kadınlar için tolere ve kabul edilebilir bir yöntem olabilir.

(J Turkish-German Gynecol Assoc 2010; 11: 141-4)

Anahtar kelimeler: İmplanon, etonogestrel, postpartum korunma, emzirme, uyum, yan etki, çıkarma

Geliş Tarihi: 05 Nisan 2010 **Kabul Tarihi:** 05 Ağustos 2010

Introduction

One of the most sensitive and intimate decisions made by an individual or by a couple is that of fertility control. These decisions may be more complex for the postpartum period, which is characterized by changes in women's priorities, attitudes and lifestyles. In this period, both making a choice regarding a contraceptive method and deciding the best time for initiation of contraceptive method is unclear. Breastfeeding influences the need and timing of postpartum contraception. Lactation can only serve as a contraceptive method for up to six months, but the failure of this method has been addressed in various studies (1, 2). Reluctance to use other methods of contraception, contraceptive failure and non-use of contraceptive services are leading causes of unintended pregnancy during breastfeeding. It is well reported that pregnancy which continues to childbirth is often associated with delay of initiation of prenatal care and shorter interpregnancy intervals and may negatively influence the health of the child and mother (3-6). When choosing a method of post-partum contraception, it is important that it does not interfere with lactation or have negative effects on the infant. For contraceptive purposes, non-hormonal methods should be the first choice for breastfeeding women. However,depending on medical or personal reasons, women may desire to use a hormonal contraceptive method. In this case, the progestagen-only method

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doi:10.5152/jtgga.2010.21

is advised after 6 weeks postpartum. Implanon® (NV Organon, Oss, The Netherlends) is a single-rod, progestogen-only contraceptive containing 68 mg of the active compound.

In terms of hormonal methods, combined oestrogen-progestin oral contraceptives have been shown to impair milk secretion (7), whereas contraception with progestin alone, whether delivered by oral, subdermal or intrauterine routes, appears to have no deleterious effects on milk production or infant growth when used by breastfeeding women (8).

The objective of the present study was to determine compliance and the efficacy of the patients with the single-rod contraceptive implant inserted during the immediate postpartum period. To the best of our knowledge, this is the first report investigating experiences of Turkish women with Implanon® in the postpartum period.

Material and Methods

This cohort study was conducted between April 2005 and December 2007 at the Obstetrics and Gynaecology Department of Fatih University, Medical School, Ankara, Turkey. The study was approved by the Hospital's Ethical Committee and all participants gave verbal informed consent. All underwent physical and pelvic examinations, as well as cervical cytological screening. Sixty-one women who had recently given birth and were breastfeeding and intended to use Implanon® for long-term contraception, were included in this study.

Exclusion criteria were being a smoker, a body mass index (BMI kg/m^2) $\geq$30, a history of thromboembolic events, pelvic infection, cervical cytological abnormality or a systemic disease (diabetes mellitus, cardiovascular disease, liver disease, thyroid disease, autoimmune diseases).

Patient compliance, side effects and removals were recorded at the end of the two years. The implant was inserted in the inner aspect of the non-dominant upper arm under local anaesthesia at least 6 weeks after delivery, by the same physician. Follow up of the patients was made by scheduled appointments or contact via a telephone consultation regarding side-effects, including bleeding pattern and whether or not they continued with the Implanon®. Women were informed about side effects of Implanon® before its placement. Of the 80 potential participants, 19 refused to participate. The remaining 61 women were mentally and physically healthy, and had no contraindication for Implanon® use.

All patients were followed up at 3 month intervals, for at least 1 year, with regard to menstrual and non-menstrual side effects of Implanon® and compliance with Implanon®. They kept a diary in which all genital bleeding and spotting episodes were mentioned. Amenorrhoea was defined as the absence of menstruation for 3 months. Recurring bleeding at 28±7 days intervals was defined as regular bleeding. Irregular bleeding included frequent, infrequent and prolonged bleeding episodes. Frequent bleeding was defined as having more than five bleeding or spotting episodes within 3 months. Infrequent bleeding was defined as having fewer than three bleeding or spotting episodes within 3 months. Prolonged bleeding was defined as having one or more bleeding or spotting episodes within 3 months and lasting more than 14 days each.

The treatment duration was defined as the number of days elapsed between implant insertion and its removal. The extent of exposure to the implant was expressed in woman-years. A woman-year was defined as a period of 365.25 days, which corresponds to approximately 13 cycles (364 d). The pregnancy rate was expressed as the Pearl Index (PI), which indicates the number of pregnancies per 100 woman-years of exposure. Numbers and percentages were calculated, using SPSS 13.0 for Windows.

Results

The 61 breastfeeding women were evaluated at the end of the 24th month. The side effects and continuation were assessed during this period. The mean age of the participants was 29.95±5.04 years (22-41). The mean BMI of the patients was 26.27±3.23. Twenty-three patients (23/61, 37%) were primiparous, 28 (28/61, 45%) had delivered twice, 5 (5/61, 8%) three times, 4 (4/61, 6.5%) four times, 1 (1/61, 1.6%) five times. At the time Implanon® was placed, all of the patients were in the postpartum period and breastfeeding. The majority of women (n=54, 88.5%) received counseling prior to the day of the fitting. Only 7 (11.4%) women were fitted with Implanon® on the day of the visit. The mean days of postpartum placement of Implanon was 48.12±13.192. Former methods of contraception were RIA (n=21, 34%), condom (20/61, 32%), coitus interruptus (8/61, 13%), pills (6/61, 11%), injectables (1/61, 1%). Five primigravid (5/61, 8.1%) women were not using any contraceptive method.

Age, parity, previous use of contraceptive methods and time of the placement of Implanon® are summarized in Table 1.

When patients were evaluated for side effects over 2 years, amenorrhoea was reported by 20 patients (32%), prolonged bleeding by 13, (21%), frequent bleeding by 4, (6.5%), and infrequent bleeding by 2 (3.2%) as seen in Table 2.

Non-menstrual side effects experienced by participants included; weight gain reported by 10 patients (16%), anxiety by 6 (9.8%), breast tenderness by 4 (6.5%), headache by 4 (6.5%), pain at the insertion site by two (3.2%), hirstutism by two (3.2%), acne by 1 (1.6%), loss of libido by 1 (1.6%), weight gain and headache by two (1.6%) and weight gain and anxiety by two (1.6%) Table 3.

Contrary to the expectation from progestogen-only implant, we did not detect any weight gain at the end of the study (24 month). Furthermore, BMI demonstrated a decrease from 26.27±3.23 to 24.54±1.06 ($p<0.05$). This reduction of weight may be an attribution to the breastfeeding effect on weight. Although, the change in BMI was not statistically significant in 10 (16%) patients who reported weight gain at the end of the study, only 3 (4.9%) patients requested removal of Implanon® (BMI= 27.01±2.17, $P>0.05$).

In our study, regarding continuation rates of Implanont at 6 months after insertion, (n=56) 91% of women continued, (n=47) 77% continued for 1 year, (n=40) 65% continued for 18 month and (n=37) 60 % continued for 2 years. During the follow up, Implanon® was removed from 24 patients (39%). The indications for removals were frequent/prolonged bleeding in eleven patients (18%), anxiety in two (3.2%), weight gain in three (4.9%), pain at the insertion site in two (3.2%), breast tenderness in two (3.2%), hirsutism in one (1.6%), and weight gain and anxiety in three (4.9%). Reasons for removal of Implanon® are listed in Table 3. No problems were encountered during the placement or removal of the implant. The mean breastfeeding period of patients was 16±7.4 /months.

Table 1. Baseline characteristic of patients and time of placement of Implanon

Characteristics	no. (%)
Age	
20-30 years	37
31-40 years	23
>41 year	1
Parity	
1	23
2	28
3	5
4	4
5	1
BMI (kg/m^2)	26.27±3.23
Postpartum placement (day)	48.19±13.19
Breastfeeding period of patients (month)	16±7.4

Table 2. Bleeding patterns of subjects during treatment with Implanon

	n	%
Amenorrhoea	20	32
Prolonged bleeding	13	21
Frequent bleeding	4	6.5
Infrequent bleeding	2	3.2

Table 3. Non-menstrual side effects during Implanon use, reasons for removal of Implanon

Side effects	
Weight gain	10 (16%)
Nervousness	6 (9.8%)
Headache	4 (6.5%)
Breast tendernass	4 (6.5%)
Acne	1 (1.6%)
Loss of libido	1 (1.6%)
Pain at the insertion site	2 (3.2%)
Hirsutism	2 (3.2%)
Reasons for removal	
Frequent and prolonged bleeding	11 (18%)
Weight gain	3 (4.9%)
Anxiety	2 (3.2%)
Weight gain and nervousness	3 (4.9%)
Breast tendernass	2 (3.2%)
Pain at the insertion site	2 (3.2%)
Hirsutism	1 (1.6%)

During the study period, which comprised 730.5 woman-years of observation, no pregnancies occurred (Pearl index score=0).

Discussion

The postpartum period presents an increased risk of pregnancy in the life of women. It is of great importance to the new mother that the next pregnancy is postponed. Women are more likely to report pregnancies as unplanned/untimed when they occur within an interval of 24 months or less. The World Health Organization (WHO) recommends a minimum of 2 years interval between pregnancies to reduce the incidence of maternal and fetal risks in each pregnancy (9). According to our study, Implanon® is a useful choice in women who desired a long-term and reliable contraception after postpartum period.

Since Implanon® is a progestogen-only contraceptive, it is not surprising that menstrual anomalies are more frequent than during spontaneous menstrual cycles. To our knowledge, this is the first report investigating compliance and side effects of Implanon® inserted during the postpartum period in Turkish women. The present study indicated that Implanon® provided an excellent contraceptive cover for the full period of 2 years to women of a wide range of ages and weights. No pregnancies occurred in the two years of use.

Normally the most frequent side effects of Implanon® were related to the disturbed bleeding pattern. Our report showed that, like spontaneous menstrual cycles, Implanon® is associated with disturbed bleeding pattern in breastfeeding women. In our study, when patients were evaluated for menstrual irregularity over 2 years, amenorrhoea (32%) was the most common problem reported for these women in the first 12 months after weaning. Prolonged or frequent bleedings were infrequent in the first 12 months after treatment initiation but the proportion of bleedings lasting more than 10 days was increased after 12 months. Irregular bleeding occured in 30% of our patients after 12 months. Although the incidence of amenorrhoea was just below 20% during most of the time, this rate was increased to 32% in our patients in whom Implanon® was inserted in the early postpartum period. This can be related with the effect of breastfeeding on the menstrual cycle. It is well known that elevated levels of prolactin that occur with breastfeeding inhibit the pulsatile secretion of gonadotropin-releasing hormone from the hypothalamus. This in turn interferes with the hypothalamic- pituitary- ovarian axis, preventing estrogen secretion and ovulation (10).

No unexplained adverse maternal effects have been reported during use of Implanon® in the postpartum period (11).

In clinical trials, the most common non-menstrual side effects experienced when wearing an implant are reported to range from 15-25% (12-15). In our study, the most frequent non-menstrual side effects experienced when wearing an implant, are; weight increases, anxiety, breast tenderness and headache (6.5 to 16%). Pain at the insertion site, hirstutism, acne and loss of libido are less frequently reported (1.6-3.2%).

The mean time of implanon removal after insertion was 15.14±7.21 month. A study of 329 users of Implanon® in Scotland (16) used 2 years 9 months as an end point and reported a comparable continuation rate of 47% of women with implant at that point. A study by Harvey et al. from family planning clinics in Queensland, Australia reported their continuation rates of Implanon at 6 months after insertion as;

94% of women continued, 74% continued for 1 year and 50% continued for 2 years (17). Our continuation rates in this Turkish study of 77% for 1 year and 60 % for 2 years are consistent with findings from a review of evidence from UK and Europe, which concluded that 20-25% and up to 44% of women will discontinue within 1 year and 2 years, respectively (18). Another study from Spain reported similar continuation rates (91.0%) in a follow-up of 372 women at 1 year after insertion of implanon (19). From Turkey, a study by Gezginç et al. (13) also reported that 75% of patients used Implanon® for at least 1 year of follow-up and 25% patients requested removal of the implant. Contrary to our high continuation rates at 6 months, Yıldızbaş et al. reported the high discontinuation rates (19.5%) among women in eastern Turkey after 6 months of use of this contraceptive modality. In terms of mood changes and weight gain, they also reported a significantly increased frequency of headache, nausea and dizziness at the end of the study. Probably these differencescan be related to a different patient profile of healthy sexually active women and the short period of their study (12).

The main reason for removal during the breastfeeding period was the bleeding problem (frequent/prolonged bleeding). The most common non-menstrual side effects leading to removal were weight gain and anxiety together (20-22). Similary, in our study, the most common indications for removal were frequent/prolonged bleeding (18%). Given the frequency of side effects such as mood changes and weight gain in the patients with Implanon® during breastfeeding period these symptoms should be specifically discussed with women, as different women may give a different level of priority when choosing a method of contraception, especially the postpartum period because, in the postpartum period, women may be more receptive to changing their contraceptive after delivery. Cwiak et al.'s study showed that (23) over 40% of peripartum women indicated a desire to change their methods.

Although prolonged breastfeeding is encouraged in Turkey, breastfeeding patterns and bottle supplementation are different among individuals. Hence, the postpartum period presents an increased risk of unplanned pregnancies. Introducing of new reliable contraceptive method such as Implanon® in the postpartum period may prevent unintended pregnancy. In our study, most of the patients (65%) were not aware of Implanon® as a contraceptive choice. As far as we know, there is no survey of the knowledge, attitudes and practices of postpartum contraception in Turkey. Our study is based on a small number of patients experience with Implanon® which was inserted during the postpartum period, but to the best of our knowledge no data exists about postpartum compliance and side effects of Implanon® in Turkish women. Our study also has no control group, hence the performance of Implanon® cannot be compared with the performance of other contraceptive methods during the breastfeeding period.

In conclusion, despite the frequency of side effects, a high proportion of women still use Implanon® as a method of contraception at the end of 2 years. This demonstrates that Implanon® is well tolerated and is an acceptable choice for women who have had a recent birth and are searching for a long term reversible contraception.

Conflict of interest

No conflict of interest is declared by authors.

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Ultrasound findings in aneuploidy fetusus: Evaluation of 332 cases

Anöploidisi olan fetüslerin ultrasonografi bulguları: 332 vakanın değerlendirilmesi

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Abstract

Objective: To evaluate the ultrasound findings found on ultrasound examination among cases that had aneuploidy at amniocentesis.

Material and Methods: This prospective study was performed at Dicle University, School of Medicine, Department of Obstetrics and Gynecology. 332 cases applied to our department for prenatal diagnosis and amniocentesis (AC) was performed. Of these cases, twenty were found to have aneuploidy evaluated. The factors recorded were; mean age, gestational weeks, AC indications, ultrasound findings (by Toshiba 140A and GE Voluson 730 Pro 4D ultrasound device) and fetal anomalies.

Results: 332 cases have had AC by an experienced specialist, in a two year period. The mean age of the cases was 32.20±6.03 years (22-44), and gestational weeks 16.45±1.46 (13-19). AC indications were; high double and/or triple test with ultrasound findings and abnormal ultrasound findings. In 8 (2.40%) cases there was no reproduction on cell culture. In 14 (4.21%) cases, different types of chromosomal anomalies were detected. In these cases, peripheral blood was taken from the parents and if, at least in one of them this situation was present, this would be accepted as normal. In 20 (6.02%) cases aneuploidy (numerical chromosomal anomalies) were detected and 11 of them (55.00%) were trisomy 21. In all of these aneuploidy cases, different types of ultrasound findings were detected; most of them had multiple ultrasound findings, and some of them had one anomaly. Of all 20 aneuploidy cases; termination of pregnancy was decided in 17 (85%) of them. 3 (15%) of these cases decided to carry on their pregnancy. Of the 3 cases; one baby was delivered spontaneously and live, one had died in utero and labor was induced and the third pregnancy is ongoing.

Conclusion: The importance of ultrasound in fetal anomaly screening is incontrovertible and positive ultrasound findings are the most important indications of amniocentesis. For this reason, before amniocentesis, we advise a detailed ultrasound examination by an experienced specialist. (J Turkish-German Gynecol Assoc 2010; 11: 145-8)

Key words: Amniocentesis, aneuploidy, ultrasound findings

Received: 1 March, 2010 **Accepted:** 3 August, 2010

Özet

Amaç: Amniyosentez ile anöploidi tanısını almış olan vakaların ultrasonografi bulgularının gözden geçirilmesi.

Gereç ve Yöntemler: Bu prospektif çalışma Dicle Üniversitesi Tıp fakültesi Kadın hastalıkalrı ve Doğum bölümünde gerçekleştirildi. Prenatal tanı ünitemize gelen ve amniyosentez yapılmış 332 vaka ele alındı. Bunlardan 20 vakada anöploidi saptandı. Yaş, gebelik haftası, amniyosentez endikasyonları, ultrason bulguları (Toshiba 140A and GE Voluson 730 Pro 4D ultrason cihazı ile) ve fetal anomaliler kaydedildi.

Bulgular: Bu 332 amniyosentez 2 yıl süresince deneyimli bir hekim tarafından yapıldı. vakalrın ortalama yaşı 32.20±6.03 yıl (22-44) ve gebelik haftaları 16.45±1.46 (13-19) idi. Amniyosentez endikasyonları; yüksek ikili ve/veya üçlü test ile pozitif ultrasonografi bulguları idi. Sekiz (%2.4) vakada kültürde hücre üretilemedi. Ondört vakada (%4.2), değişik tip kromozom anomalileri saptandı. Bu vakalarda ebeveyynlerden periferik kan külütrü yapıldı ve en az birinde bu varsa bu durum normal olarak değerlendirilid. Yirmi (%6.0) vakada anöploidi saptandı. Bunların 11 tanesi ise (%55.0) trizomi 21 idi. Tüm bu anöploidi vakalarında değişik ultrason bulguları tespit edilmişti. bu vakaların 17 tanesi /%85) terminasyonu kabul etti, 3 (%15) tanesi ise gebeliğin devamına karar verdi. Bu gebeliklerin ikisi doğum ile sonlandı, bir bebek yaşıyor diğeri ise kaybedildi.

Sonuç: Fetal anomali taramasında ultrasonografinin rolu tartışılmazdır ve pozitif ultrason bulgusu amniyosentezin en önemli endikasyonlarını oluşturur. Bu nedenle amniyosentez öncesi deneyimli bir uzman tarafından detaylı ultrason incelemesini öneriyoruz.

(J Turkish-German Gynecol Assoc 2010; 11: 145-8)

Anahtar kelimeler: Amniyosentez, anöploidi, ultrason bulguları

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

Introduction

Aneuploidies are the most frequent chromosomal anomalies; the incidence was reported as 9 in 1000 live births (1). The most frequent methods used for diagnosing chromosomal abnormalities is karyotyping of fetal cells by invasive procedures such as chorionic villus sampling and amniocentesis (2). Down syndrome, among all aneuploidies, is the most commonly encountered chromosomal abnormality in newborns, with an incidence of 1 in 800 live births. Because of decreased mentality and congenital heart disease, Down syndrome, therefore, has a substantial socioeconomic impact (3, 4). With a detailed ultrasound examination by an experienced specialist before invasive prenatal testing, many fetal anomalies may be detected. Therefore, this analysis is called genetic sonography (5).

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doi:10.5152/jtgga.2010.22

The aim of this study was to evaluate the findings detected on ultrasound examination for prenatal diagnosis in the cases where amniocentesis was performed.

Material and Methods

This prospective study was performed on 332 cases who applied to our department for prenatal diagnosis and amniocentesis (AC), the findings of 20 cases having aneuploidy evaluated, from January 2007 to December 2008, at Dicle University, School of Medicine, Department of Obstetrics and Gynecology. This is a reference hospital in the South Eastern region of Turkey. Most of the patients applying to our department have a lower socioeconomic status and the ratio of interrelative marriages is higher than the other regions of Turkey.

The factors recorded were; mean age, gestational weeks, gravidy and parity of the cases. The ultrasound examination and invasive testing were made by a single experienced specialist. The ultrasound findings were recorded on the amniocentesis form.

Indication for amniocentesis, ultrasound findings, fetal anomalies, karyotype results and treatments were also evaluated. The genetic sonogram was made by our Genetic Department after the AC.

Results

During the study period, 332 cases that had AC for prenatal diagnosis by an experienced specialist were included in the present study. The mean age of the cases was 32.20±6.03 years (22-44) and gestational weeks 16.45±1.46 (13-19). AC indications were; high double (n=5, 25%) or triple test (n=4, 20%) with ultrasound findings and abnormal ultrasound findings (n=8, 40) (Table 1). In 8 (2.40%) cases, there was no reproduction on cell culture. In 14 (4.21%) cases, different types of chromosomal anomalies were detected. In these cases, peripheral blood was taken from the parents and if, at least in one of them this situation was present, this would be accepted as normal. In 20 (6.02%) cases aneuploidy (numerical chromosomal anomalies) was detected and 11 of them (55.00%) were trisomy 21 (Figure 1).

In all of the aneuploidy cases, different types of ultrasound findings were detected; most of them had multiple ultrasound findings, and some had one anomaly. Of all 20 aneuploidy cases; ,termination of pregnancy were decided in 17 (85%) of them, 3 (15%) of these cases decided to carry on with their pregnancy. Of the 3 cases; one baby was delivered spontaneously and live, one died in utero and labor was induced and one pregnancy is still ongoing (Table 2).

Discussion

Prenatal diagnosis for fetal aneuploidy began in the 1960's only considering the mother age (5). Later, the American College of Obstetricians and Gynecologists (ACOG) advised prenatal screening for cases 35 years and over (6). With only an advanced maternal age indication only 30% of the Down

Table 1. Demographic characteristics and amniocentesis indications of the aneuploidy cases

Age	Karyotype	G	P	A	Y	GW	Amniocentesis Indication
29	Klinefelter	3	2	0	2	17	Ultrasound findings
28	Triploidy	1	0	0	0	15	Ultrasound findings, high triple test
41	Trisomy 13	14	11	2	10	17	Ultrasound findings, maternal age
34	Trisomy 13	4	2	1	2	17	Ultrasound findings, high double test
37	Trisomy 18	8	7	0	7	16	Ultrasound findings
25	Trisomy 18	3	2	0	1	17	Ultrasound findings
27	Trisomy 21	2	1	0	1	16	Ultrasound findings, high triple test
32	Trisomy 21	2	1	0	1	17	Ultrasound findings, high double test
35	Trisomy 21	2	1	0	1	16	Ultrasound findings, high double test
38	Trisomy 21	4	3	0	3	16	Ultrasound findings, hightriple test
37	Trisomy 21	5	3	1	3	13	Ultrasound findings
35	Trisomy 21	5	3	1	3	17	Ultrasound findings
39	Trisomy 21	4	3	0	3	18	Ultrasound findings, maternal age
31	Trisomy 21	1	0	0	0	17	Ultrasound findings, high double test
32	Trisomy 21	2	1	0	1	17	Ultrasound findings, high double test
44	Trisomy 21	4	2	1	2	17	Ultrasound findings, maternal age
26	Trisomy 21	2	0	1	0	18	Ultrasound findings, high triple test
22	Turner	2	1	0	0	16	Ultrasound findings
25	Turner	1	0	0	0	19	Ultrasound findings
27	Turner	1	0	0	0	13	Ultrasound findings

Table 2. Ultrasound findings and prognosis of 20 aneuploidy cases

Karyotype	Ultrasound Findings	Prognosis
Klinefelter	Oligohydramnios, hyperecogenic bowel	Termination
Triploidy	CCA , ventriculomegaly, symmetric IUGR	Termination
Trizomy 13	Echogenic focus in the heart, VSD, hydrocephaly, CCA, talipes, omphalocele, spina bifida, dilated right heart	Termination
Trisomy 13	VSD, pistol shot on hand, left CPC, cryptorchidism	Termination
Trisomy 18	Cystic hygroma, omphalocele	Termination
Trisomy 18	Calvarium offication defect, hypodensity in all bones, dysplasia of skeleton, achondroplasia	Termination
Trisomy 21	Thick NT, aplasic NB, short extremities	Living
Trisomy 21	Hypoplasic NB, Echogenic focus in the heart	Termination
Trisomy 21	Bilateral CPC, short extremites	Termination
Trisomy 21	Cystic hygroma, hyperecogenic bowel	Termination
Trisomy 21	Anasarca edema, hyperecogenic bowel, cystic hygroma	Termination
Trisomy 21	Cystic hygroma, Anasarca edema, bilateral pyelectasia, aplasic NB	Termination
Trisomy 21	Short extremites, VSD	IUMF
Trisomy 21	Thick NT, aplasic NB, short extremites	Termination
Trisomy 21	Hyperecogenic bowel, Echogenic focus in the heart, Thick NF, hypoplasic NB	Termination
Trisomy 21	Hyperecogenic bowel, left CPC, bilateral pylectasia, thick NF hypoplasic NB, Face angel : 95°	Termination
Trisomy 21	Hypoplasic NB, Thick NF, Face angel 101°	Pregnancy going on
Turner	Cystic hygroma	Termination
Turner	Cystic hygroma, short extremities	Termination
Turner	Cystic hygroma	Termination

IUGR: Intrauterine growth restriction, CCA: Corpus callosum agenesis, VSD: Ventricular septal defect,NT: nuchal translucency, NB: Nasal bone, IUMF: Intra uterine death of fetus, NF: nuchal fold

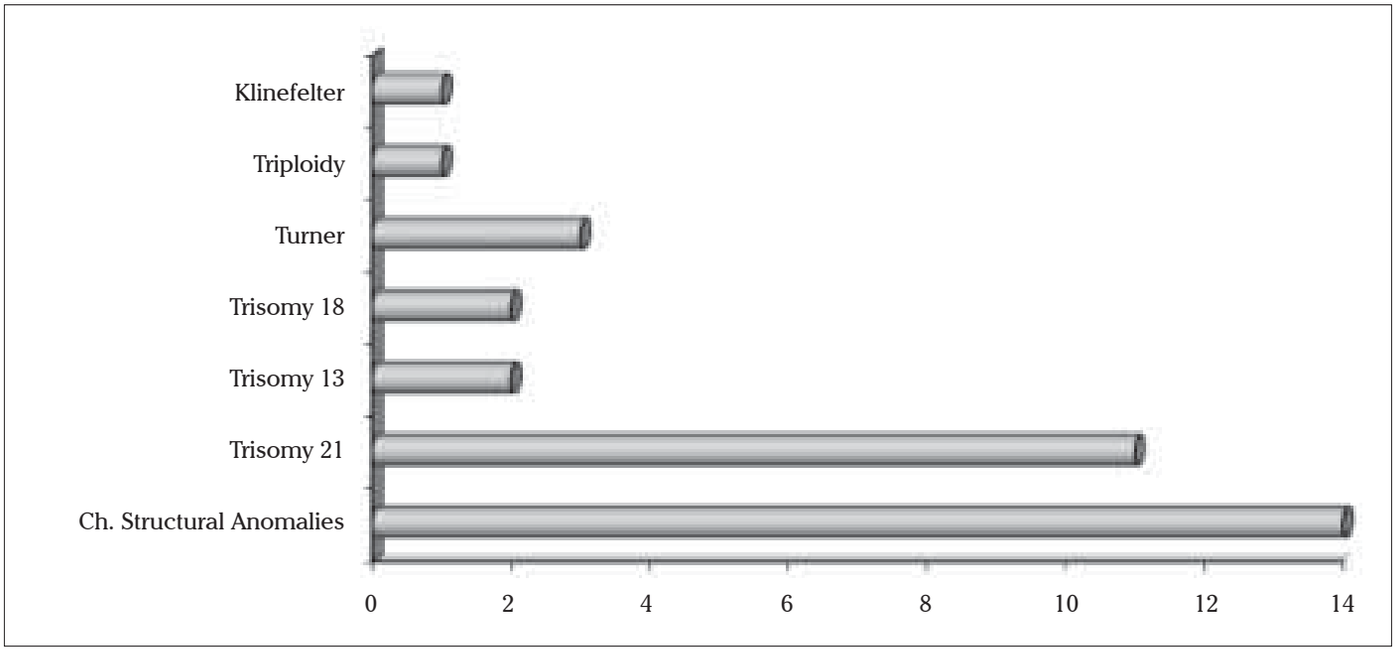

Figure 1. The cases that structural and numerical chromosomal anomailes detected on amniocentesis

syndromes can be detected (7). Hence, this indication lost its importance.

Nowadays, the main indications are advanced maternal age, biochemical tests and positive ultrasound findings (6). The importance of ultrasound examination also named genetic sonography, has been reported in many studies. The main indication of genetic sonographic examination is the detection of fetal anomalies (8, 9). The risk of fetal aneuploidy increases with the detection of soft markers. The risk increases more with the number of the soft markers (10, 11).

Genetic sonogram has some difficulties such as maternal obesity. Tsai et al. reported in their study that the detection rates of abnormalities on ultrasound screening related inversely with maternal obesity (12)

Nyberg et al. reported that most soft markers in aneuploidy cases were hyperecogenic bowel, ventriculomegaly and cardiovascular system malformations (13). In most of our cases the anomalies were similar to Nyberg's study (Table 2). Of our 20 aneuploidy cases, 7 (14%) were over 35 years age, and advanced maternal age was an amniocentesis indication in only 3 (6%) of them. Chelli et al found that first trimester ultrasound screening may allow early detection of a large number of aneuploidies and fetal malformations (13). In 9 (18%) of the cases, the indication was a high double or triple test. However, in all of the 20 cases, there were ultrasound findings. We consider that this higher ratio of our study is related to higher frequency of the intermarriages and the experience of the specialist who performed the prenatal diagnosis.

In conclusion, nowadays, the importance of ultrasound in fetal anomaly screening is incontrovertible and positive ultrasound findings are the most important indications of amniocentesis. For this reason, before amniocentesis we advise detailed ultrasound examination by an experienced specialist.

Conflict of interest

No conflict of interest is declared by authors.

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Comparison of serum androgens and endometrial thickness in obese and non-obese postmenopausal women

Obez ve obez olmayan postmenopozal hastalarda serum androjen seviyeleri ve endometrial kalınlığın karşılaştırılması

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Abstract

Objective: In this study, we investigated whether serum androgen levels and endometrial thickness differed in obese and non-obese women.

Material and Methods: Thirtytwo non-obese (BMI <30) and 48 obese (BMI ≥ 30) women were enrolled. Blood samples were analyzed for testosterone, free testosterone, androstenedione, DHEAS, and SHBG, and transvaginal ultrasonography was performed.

Results: Obese women had significantly higher free testosterone and endometrial thickness and significantly lower SHBG. Eight of 17 women with endometrial thickness >5 mm had significant pathology.

Conclusion: These results suggest that obesity may be a risk factor for endometrial carcinoma and other pathologies in post-menopausal women through an action on androgen concentrations.

(J Turkish-German Gynecol Assoc 2010; 11: 149-51)

Key words: Serum androgene, endometrial thickness, obesity, post-menopause

Received: 13 November, 2010 **Accepted:** 3 August, 2010

Özet

Amaç: Bu çalışmamızda, endometrial kalınlık ve serum androjen seviyelerinin obez ve obez olmayan hastalardaki farklılığı araştırılmıştır.

Gereç ve Yöntemler: 32 obez olmayan (BMI<30) ve 48 obez (BMI≥30) hasta çalışmaya dahil edildi. Hastaların serum testosteron, serbest testosteron, androstenedion, DHEAS, SHBG seviyeleri ölçüldü ve transvajinal ultrasonoğrafi yapıldı.

Bulgular: Obez hastaların serbest testosteron seviyeleri ve endometrial kalınlığı anlamlı olarak yüksek tespit edilirken; SHBG seviyeleri anlamlı olarak düşük bulundu. Endometrial kalınlığı 5mm'den büyük olan 17 hastadan 8 tanesinde endometrial patoloji tespit edildi.

Sonuç: Bu sonuçlarla; obezitenin, post-menopozal kadınlarda androjen hormonları üzerinden endometrial karsinoma ve diğer patolojiler için bir risk faktörü oluşturabileceği akılda bulundurulmalıdır.

(J Turkish-German Gynecol Assoc 2010; 11: 149-51)

Anahtar kelimeler: Serum androjen, endometrial kalınlık, obezite, post-menopoz

Geliş Tarihi: 13 Kasım 2010 **Kabul Tarihi:** 03 Ağustos 2010

Introduction

The major hormonal changes associated with the occurrence of the final menstrual period (FMP) in normal subjects include a profound fall in circulating estradiol (E_2), primarily of ovarian origin, beginning up to about one year earlier, and an accompanying large increase in the circulating gonadotropins, FSH and LH (1, 2). Changes in circulating androgens are more complex. In cross-sectional studies, levels of testosterone [T] have been reported to be lower in postmenopausal than premenopausal women (3, 4), whereas in the few longitudinal studies, either no change (5) or a small fall, of the order of 15% (2), in total T has been reported. The major adrenal androgen, dehydroepiandrosterone sulfate (DHEAS), reaches its peak levels in young women in their twenties and declines progressively thereafter, with no obvious relationship to the menopause (6). Serum sex hormone binding globulin (SHBG) levels fall to a small degree postmenopausally (1). SHBG is responsible for the transport of sex hormones in the plasma, especially testosterone, dehydrotestosterone and estradiol (higher affinity for testosterone and lower for estradiol). It has a negative relationship with free testosterone and is used as an indirect indicator of androgenity (7).

Transvaginal sonography (TVS) is a non-invasive and reliable method for evaluating the uterus and endometrium (8).

In this study we investigated whether serum androgen levels and endometrial thickness differed in obese and non-obese postmenopausal women.

Material and Methods

Eighty postmenopausal women who applied to the menopause clinic of İstanbul Education and Research Hospital, Department of Obstetrics and Gynecology, between July 2004, were prospectively included in the study. These women had experienced natural menopause, had an FSH

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doi:10.5152/jtgga.2010.23

level greater than 40 mIU/ml, were non-smokers; and at least one year had passed since their last menstrual period. Seven women who had previously used hormone treatment or a medication that could affect body mass index, and three women who had genital or systemic pathology, were excluded from the study. PAP smears and physical examinations were performed after obtaining patient histories. Complete blood count, liver and renal functional tests, hormone profile (FSH, LH, E2, progesterone, prolactin, testosterone, free testosterone, androstenedione, DHEAS, SHBG, and fasting insulin levels) were carried out for each woman.

After excluding any systemic disease, age, height and weight of each woman were noted at the first visit. Blood samples were taken after 12 hours fasting for the evaluation of sex steroids. Samples were centrifuged at 3000 rpm and serums were stored under -20ºC until evaluation of SHBG. A chemiluminescent enzyme immunometric assay technique was used for the determination of hormone concentrations, except SHBG, for which an enzyme linked immunosorbant assay (ELISA) was used.

Transvaginal ultrasonography was performed with a Toshiba 2700 SA ultrasonography machine, and the criteria given by the American College of Obstetricians and Gynecology in 1995 was used for the measurement of endometrial thickness. The evaluation was performed in the three planes (anterior-posterior, long axis, semi-axial). Fluid collection in the cavity was not added to the thickness when making the measurement. We performed fractional curettage when we detected an endometrial thickness value of 5 mm or more.

The women were allocated into two groups according to Body Mass Index (BMI), which was calculated as weight (kg) / height² (m^2), and a cut-off value for obesity of 30 kg/m^2 used. Those with BMI > 30 kg/m^2 were considered as the study group.

Statistical Package for Social Sciences (SPSS v. 11.0) was used for statistical analysis. Two independent sampled Student's t-test were used for the comparison of data between the two groups. Results were recorded as mean ± SD. $p < 0.05$ was considered significant. This study was approved by the local Ethics Committee.

Results

The mean ages and post-menopause intervals of the women in the study group were 52.54 ± 6.87 years and 5.43 ± 5.62 years respectively. The mean height was 156.17 ± 6.26 cm, weight 89.02 ± 19.73 kg, BMI 32.85 ± 6.79 kg/m^2, and endometrial thickness 5.56 ± 4.06 mm.

The demographic characteristics of the women according to BMI are listed in Table 1. The hormonal concentrations and endometrial thicknesses of the two groups are compared in Table 2.

SHBG levels were significantly lower in the group in which BMI was greater than 30 kg/m^2, whereas free testosterone and endometrial thicknesses were significantly higher (Table 2).

Only one of the 17 women whose endometrial thickness was higher than 5 mm had a BMI less than 30 kg/m^2. Fractional curettage was performed in these 17 women, and endometrial carcinoma was found in two, complex atypical hyperplasia in one, simple endometrial hyperplasia in one, and endometrial polyp in four. No pathology was detected in nine of these women.

Discussion

The relation between obesity and the increased endometrial cancer risk is well established in a review by Kaaks et al. and explained by the increasing amount of bioavailable estrogens in the circulation and the endometrial tissue (9). After the menopause, the major source of estrogen is via peripheral aromatization of androgens, especially in the adipose tissue (9). Furthermore, hyperinsulinemia in obese women inhibits the synthesis of SHBG (9). As a result, obesity is associated with increasing levels of estrodial unbound to SHBG, which can freely diffuse to target tissues.

Many articles have reported a decrease in SHBG as the BMI increases (10-12), and we also found lower SHBG concentrations in the group which had the higher BMI.

Douchi et al. reported a prospective study in which a significant relationship was demonstrated between endometrial thickness and obesity (13). Warming et al. demonstrated a positive correlation between BMI and endometrial thickness in a study that included healthy post-menopausal women (14). Serin et al. reported that increased endometrial thickness was associated with obesity but not with hypertension (15). We also observed increased endometrial thickness among women with high BMI and furthermore, independently of these surveys, we evaluated the role of androgens for this relation and detected high levels of free testosterone in obese women with increased endometrial thickness.

Decreased SHBG concentrations as a result of increased BMI in post-menopausal women increase the free androgen and estrogen levels in the circulation, with a resultant increased risk of endometrial carcinoma. An increased endometrial thickness with increased BMI could be an indicator of this probable risk. The question then arises whether this increased

Table 1. Demographic characteristics of the two groups according to BMI

	BMI < 30 (n=32)		BMI ≥ 30 (n=48)		
	Mean	± SD	Mean	± SD	p
Age (years)	53.06	6.58	52.54	6.87	0.813
Parity (n)	3.31	1.66	3.08	1.50	0.653
Years post-menopause	6.56	8.35	5.43	5.62	0.613
Height (cm)	155.25	5.30	156.17	6.26	0.633
Weight (kg)	66.56	7.22	89.02	19.73	0.0001

Table 2. Comparison of hormone concentrations and endometrial thickness according to BMI

	BMI < 30 (n=32)		BMI ≥ 30 (n=48)		
	Mean	**± SD**	**Mean**	**± SD**	**p**
FSH (mIU/ml)	64.13	19.54	65.05	22.65	0.895
LH (mIU/ml)	28.59	16.30	23.31	15.26	0.321
Prolactin (ng/ml)	6.14	4.34	8.51	3.75	0.085
Estradiol (pg/ml)	26.15	9.64	21.78	4.06	0.061
Progesterone (ng/ml)	0.28	0.08	0.27	0.09	0.675
Testosterone (ng/ml)	0.24	0.15	0.20	0.16	0.458
Androstenedione (ng/ml)	13.66	10.42	13.28	6.63	0.889
Insulin (μIU/ml)	12.39	9.97	15.01	12.20	0.480
Free testosterone (pg/ml)	1.21	0.70	1.81	0.71	0.013
SHBG (nmol/l)	95.01	64.77	53.96	33.07	0.012
Endometrial thickness (mm)	3.77	3.96	5.56	4.06	0.008

endometrial carcinoma risk is solely due to the increased estrogen concentration or not.

Allen et al. have evaluated the endogenous sex hormones and endometrial cancer risk in women in a prospective case control study (16). They concluded that high levels of free testosterone and estrogens are associated with an increased endometrial cancer risk in postmenopausal women (16). Furthermore, they suggested that the association of endometrial cancer risk with free testosterone levels is a result of peripheral conversion of androgens into estradiol. Also, SHBG was significantly inversely and BMI positively associated with risk.

In another prospective case control study by Lukanova et al,. it was concluded that increased endometrial cancer risk in postmenopausal women with high blood concentrations of androgens seems to be primarily due to their role as precursor hormones for estrogen synthesis (17).

Interestingly, we have detected endometrial adenocarcinoma in two subjects, complex atypical hyperplasia in one, and simple endometrial hyperplasia in three. Free testosterone levels of these cases were high and SHBG levels were low.

Although our study did not definitively demonstrate a relationship between elevation of androgens and endometrial pathologies, it indicates that the probability of such a relationship should be kept in mind. Considering free testosterone and SHBG concentrations together with the endometrial thickness may help to detect endometrial pathologies.

Future studies are needed to demonstrate conclusively that elevated androgen levels, as well as increased estrogen, can give rise to endometrial pathologies in postmenopausal women.

Conflicts of interest

No conflict of interest is declared by authors.

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Comparison of standard mammography with digital mammography and digital infrared thermal imaging for breast cancer screening

Meme kanseri taramasında standart mamografi ile dijital mamografi ve dijital infrared termal görüntülemenin karşılaştırılması

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Abstract

Breast cancer is the most common malignancy in women. Screen-film mammography (SFM) has been considered the gold standard for breast cancer screening and detection. Despite its recognized value in detecting and characterizing breast disease, mammography has important limitations and its false-negative rate ranges from 4% to 34%. Given these limitations, development of imaging modalities that would enhance, complement, or replace mammography has been a priority. Digital mammography (FFDM) and digital infrared thermal imaging (DITI) are some of these alternative modalities.

(J Turkish-German Gynecol Assoc 2010; 11: 152-7)

Key words: Mammography, digital mammography, digital infrared thermal imaging, breast cancer

Received: 9 June, 2010 **Accepted:** 6 August, 2010

Özet

Meme kanseri kadınlarda görülen en yaygın malignitedir. Mamografi hem tarama hem de saptama bakımından altın standarttır. meme kanserindeki bu değerine rağmen mamografide önemli kısıtlamalar mevcuttur ve yanlış negatiflik oranı %4-34 arasında değişmektedir. Bu kısıtlamalar nedeniyle görüntüleme yönteminin geliştirmek elzem olmuştur. Dijital mamografi ve dijital infrared termal görüntüleme diğer bazı alternatif yöntemlerdir.

(J Turkish-German Gynecol Assoc 2010; 11: 152-7)

Anahtar kelimeler: Mamografi, dijital mamografi, dijital infrared termal görüntüleme, meme kanseri

Geliş Tarihi: 09 Haziran 2010 **Kabul Tarihi:** 06 Ağustos 2010

Introduction

Breast cancer is the most common malignancy and the second most common cause of cancer death in women in the US (1). According to the American Cancer Society report on cancer facts, it accounts for about 30% of all cancers in women. Approximately one in every eight women is diagnosed with breast cancer by the age of 90, with an absolute lifetime risk of 14.4% (2).

In Turkey, the ratio of breast cancer is 24.1%, ranking first of all the cancers in women (3). The incidence of breast cancer has increased, and the estimated number of breast cancer cases was 44,253 in 2007 (4, 5). There is a geographical heterogeneity regarding breast cancer incidence and survival rates in Turkey. The incidence in Western Turkey (50/100,000 in 2000) is more than twice that of the Eastern part (20/100,000) (5-7). Five year survival rates for breast cancer are 85% in Western and 60% in Eastern Turkey (7).

Breast cancer survival depends upon its earliest possible detection because survival rate increases with earlier detection with a possibility of complete cure. Breast cancer has a ten year survival rate for Stage 0of 95%; Stage I, 88%; Stage II, 66%; Stage III, 36%; and stage IV, 7% (8). Larger tumor size at diagnosis is also associated with decreased survival (9).

Screening might produce greater benefits for early detection if it were more sensitive and specific. A wide variety of new technologies, including alternative imaging modalities, and improvements in x-ray mammography, are being investigated with the aim of improving early-detection rates.

Many imaging modalities can be used for breast screening, such as X-ray; ultrasonography, magnetic resonance imaging (MRI); computed tomography (CT); ultrasound; positron emission tomography (PET) scans, digital mammography (FFDM) and lastly digital infrared thermal imaging (DITI) which is currently increasing in popularity.

This review of the scientific literature aims to assess the safety and efficacy of screen-film mammography (SFM) and ultrasonography compared to FFDM and DITI for the detection of breast cancer.

Mammography

SFM has been considered the gold standard for breast cancer screening and detection (1). It has been used currently as the most effective method of detecting asymptomatic breast

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doi:10.5152/jtgga.2010.24

cancer. Its use for screening has been widely promoted by the National Cancer Institute and other organizations (1, 3). Over 70% of women in the US over the age of 40 have had a mammogram within the past 2 years (8).

A prior research has investigated the efficacy of SFM at reducing mortality from breast cancer (9). Meta-analyses of randomized-control trials of mammography screening show a 25-30% reduction in breast cancer mortality for women over 50, and a smaller, more equivocal effect in women aged 40-49. Most experts agree that mammography screening is beneficial for women between 50-69 years old (10).

Despite its recognized value in detecting and characterizing breast disease, mammography has important limitations: first, its false-negative rate ranges from 4% to 34%, depending on the definition of a false negative and on the length of follow-up after a "normal" mammogram. Second, screening mammography is less sensitive in women with radiographically dense breast tissue. This is of particular concern because the amount of fibroglandular tissue may represent an independent risk factor for developing breast cancer. Thirdly, screening mammography also suffers from a high false-positive rate: on average, 75% of breast biopsies prompted by a "suspicious" mammographic abnormality have proved to be benign (3). The other drawbacks include discomfort due to breast compression, variability in radiological interpretation, and a slight risk of inducing cancer due to the ionizing radiation exposure (11). Another important point is over treatment which was explained in a new study by Gotzsche reporting that screening actually leads to more aggressive treatment, increasing the number of mastectomies by about 20% and the number of mastectomies and tumorectomies by about 30% (12-14).

Given these deficits, development of new imaging modalities or techniques that would enhance, complement, or replace mammography has been a priority (15). Current alternatives to screen-film mammography have included full-field digital mammography and digital infrared thermal imaging.

Digital mammography

High-quality full-field digital mammography (FFDM) addresses some of the limitations seen with SFM and is increasingly used for both diagnostic and screening mammography (16).

In women with an nonhomogeneous or extremely dense parenchymal breast pattern, the detection of breast cancer is difficult due to the similar X-ray absorption of carcinoma to the surrounding normal dense breast tissue (17). Digital mammography is superior to screen-film mammography in younger women with dense breasts due to its ability to selectively optimize contrast in areas of dense parenchyma (18). This advantage is especially important in women with a genetic predisposition for breast cancer, where intensified early detection programs may need to start from 25 to 30 years of age.

As screening programs involve large populations of healthy patients, digital mammography should offer considerable benefits in terms of radiation dose, image quality, data transfer and data archiving (16, 18). Two different technologies have been developed in digital mammography (17). Off-line systems with a cassette-based removable detector use an external reading device to generate the digital image; whereas in on-line systems, the detector is integrated into the digital mammography unit and the digital image data are directly read by the system in quasi real time.

Full-field digital mammography (Figure 1) allows direct image analysis from high-resolution monitors. High-resolution digital monitors allow the reader to adjust image contrast, magnification, and use other image-processing algorithms that might improve the accuracy of interpretation. As the technology matures and radiologists gain experience, the accuracy of digital mammography for diagnostic imaging is likely to improve.

The full-field digital mammography system used delivers an average of 27% dose less than screen film mammography (19). Such dose-gain changes with breast thickness, from about 15% for thin (30-40 mm) and thick (<70 mm) breasts up to 30-40% for more typical intermediate thickness values (19). Dose equivalence for very thin breasts below 30 mm was found.

The interpretation of mammograms, regardless of the technology used, remains a difficult art. A large percentage of breast cancers are missed, and false-positive mammograms are also a substantial problem. Furthermore, the long-term benefits from mammography screening, especially in women under the age of 50, are relatively small and continue to be debated, partly because of the lower incidence of breast cancer among younger women and the lower sensitivity of mammography in this group due to the density of their breast tissue (17).

The American College of Radiology Imaging Network (ACRIN) collected screening mammography studies performed by using both digital and screen-film mammography in 49,528 women

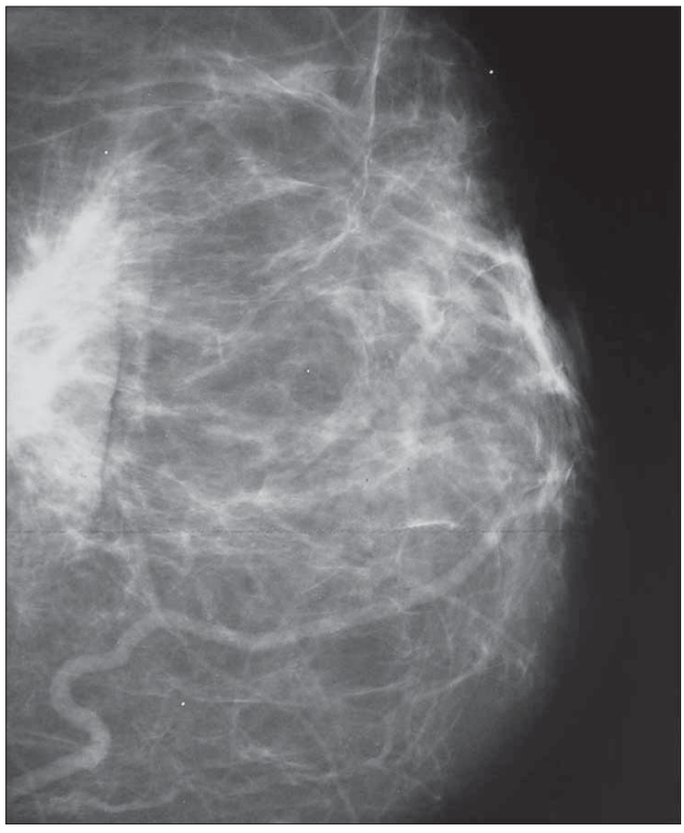

Figure 1. **Image of digital mammography**

(mean age, 54.6 years; range, 19-92 years) for a huge Digital Mammographic Imaging Screening Trial (DMIST). The study has confirmed that diagnostic accuracy with digital mammography is significantly better than that with screen/film for subgroups of patients, such as young women, women with dense breasts, and pre-menopausal women (20). The improved sensitivity of FFDM in this group is expected to lead to improvements in long-term outcomes. However, randomized clinical trials of women between the ages of 40 and 49 years have suggested that only equivocal mortality benefits are obtained with screening mammography. The delicate balance of risks and benefits can be affected by small differences in the accuracy of both the mammographic technology and image interpretation. Thus, it will be increasingly important to carefully evaluate the characteristics of any technology that may replace SFM.

Digital full-field mammography could become the method of choice in the detection and characterization of breast cancer. The limitation of digital mammography is the initial cost of the system and the discrepancy between this cost and the current rate of reimbursement. Other aspects like limited detector size or the lack of an automatic exposure control of some systems should be solved in time.

Overall, the evidence to date does not support the use of FFDM for screening or detection of breast cancer in all women. Although the DMIST demonstrated equivalence of the two technologies, FFDM is significantly more expensive. FFDM does have the potential to achieve better sensitivity and specificity than SFM in some subgroups of women (19). If FFDM would improve sensitivity slightly while sacrificing specificity, the overall harm from increased false positive results and increased biopsies would likely outweigh the increased detection rate, as the vast majority of women undergoing screening mammography do not have breast cancer.

Breast ultrasonography

Breast ultrasonography (Figure 2) is a relatively inexpensive and effective method of differentiating breast masses. Cysts and solid lesions are difficult to differentiate on mammography alone, and ultrasound is especially important in this situation since it can differentiate cystic or solid tumor. USG is also superior to mammography in the evaluation of breast abscesses (21-23). On the other hand, small calcifications are not easily seen on screening with ultrasound only, so mammography combined with breast ultrasonography are the standard imaging techniques for detection and evaluation of breast disease (24) in individuals over 30 years with a small palpable lump.

USG does not expose a patient to ionizing radiation, which is particularly important for pregnant patients and young patients. With the help of Doppler ultrasonography, detection of the presence and degree of vascularity of breast masses become easier and enables correct identification and treatment of masses. US is also useful in the guidance of biopsies and therapeutic procedures.

The choice of primary breast imaging in examining women with symptoms is partly based on age. It was shown by many studies that there is a progressive improvement in sensitivity of mammography in women 60 years or older relative to younger women (25). However, in women 45 years or younger, ultrasound has a significantly greater sensitivity than mammography.

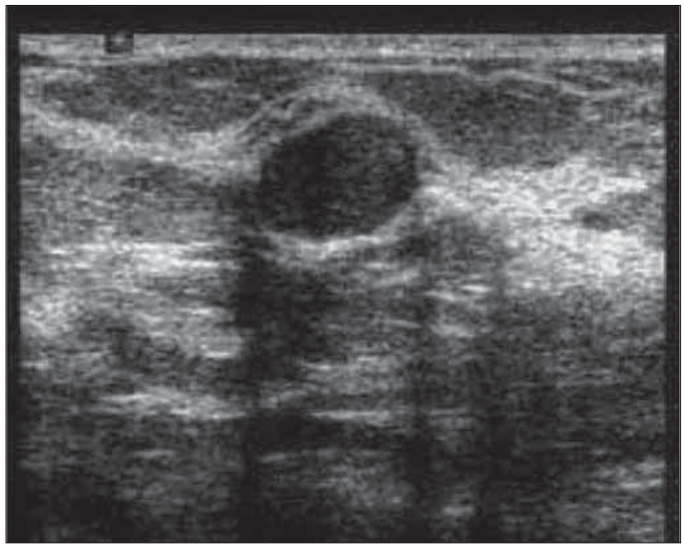

Figure 2. Image of breast ultrasonography

US is useful in the evaluation of palpable masses that are mammographically occult, in the evaluation of clinically suspected breast lesions in women younger than 30 years of age, and in the evaluation of many abnormalities seen on mammograms. Dense fibroglandular tissue is the most important inherent limitation of mammography in the diagnosis of breast cancer. USG is more sensitive than mammography in detecting lesions in women with dense breast tissue (24, 25).

In the study of Berg et al. 2809 women, with at least heterogeneously dense breast tissue in at least 1 quadrant, were recruited to undergo mammographic and physician-performed ultrasonographic examinations. Forty participants (41 breasts) were diagnosed with cancer: 8 suspicious on both ultrasound and mammography, 12 on ultrasound alone, 12 on mammography alone, and 8 participants (9 breasts) on neither. The diagnostic yield for mammography was 7.6 per 1000 women screened (20 of 2637) and increased to 11.8 per 1000 (31 of 2637) with combined mammography plus ultrasound. The diagnostic accuracy for mammography was 0.78 and increased to 0.91 for mammography plus ultrasound (ACRIN).

Although some researchers have reported reasonable results from US breast screening, a number of serious issues such as interobserver and intraobserver variability, need to be solved before the practice is recommended for general application. Thus experts suggest that women younger than 35 years be examined with ultrasound, and women 35 years and older be examined with mammography, as the primary breast imaging modality (21).

Digital infrared thermal imaging (Diti)

Thermography is a technique for measuring the body surface temperature and is used in medical applications. Infrared imaging is a physiological test that measures the subtle physiological changes that might be caused by many conditions, such as contusions, fractures, burns, carcinomas, dermatological diseases, rheumatoid arthritis, diabetes mellitus and associated pathology, deep venous thrombosis, liver disease, bacterial infections, and others (25, 26).

These conditions are commonly associated with regional vasodilation, hyperthermia, hyperperfusion, hypermetabolism, and hypervascularization (27-29), which generate a higher-temperature heat source. Unlike imaging techniques such as X-ray, ultrasonography, MRI, and other structural imaging tools that primarily provide information on the anatomical structures, infrared imaging provides functional information that is not easily measured by other methods. Thus, correct use of DITI (Figure 3) images requires in-depth physiological knowledge for effective interpretation. It has been developed to assist physicians in differentiating benign tissue from malignant tissue by characterizing different patterns in the infrared signal emitted by the tissue.

All types of cancer cells deelop angiogenesis which is necessary to sustain the growth of a tumor and an unbalanced metabolic activity that leads to the utilization of a large amount of blood glucose and release of large amounts of lactate into the blood (30). The use of Digital Infrared Imaging is based on the principle that metabolic activity and vascular circulation in both pre-cancerous tissue and the area surrounding a developing cancer is almost always higher than in normal tissue. Because of its extreme sensitivity, these factors have enabled DITI to be a viable technique for visualizing the abnormality. Infrared imaging may find thermal signs suggesting a pre-cancerous state of the breast or the presence an early tumor that is not yet large enough to be detected by physical examination, mammography, or other types of structural imaging. It also provides more dynamic information of the tumor since the tumor can be small in size but be fast growing, making it appear as a high-temperature spot in the DITI. It is also reported that the results of thermography can be correct 8-10 years before mammography can detect a mass in the patient's body (31).

Computerized thermal imaging (CTI) is a new, non-invasive imaging method that is being developed using the principles of traditional thermography but with the addition of digital image reconstruction. Computerized thermal imaging (CTI) is a heat sensing and processing system that uses a thermal sensitive camera to capture a digital image based on heat radiating from the body. After the breast images have been taken, they are analyzed by a computer algorithm and displayed for interpretation by the physician. Breast images are displayed in different colors (red, orange, and yellow) on a computer monitor. Any suspicious area (abnormal heat area) is marked on the digital breast image.

Previous studies reported (15) that a significant number of cancers (30-65%) can be visualized on prior mammograms on retrospective review. Double reading of mammograms by two radiologists can improve the detection rate of cancer but is expensive and time consuming. The goal of computer aid detection is to improve detection rates in a more efficient and cost-effective manner, as human examination of images is often influenced by various factors such as fatigue, carelessness, andothers. The detection accuracy is also confined by the limitations of the human visual system. In addition to all these factors, a shortage of qualified radiologists also causes an urgent demand for the development of computer technologies. The computerized system collects a series of infrared breast images while the cooled air surrounds the breast. Then the system analyzes and interprets the infrared data using algorithms

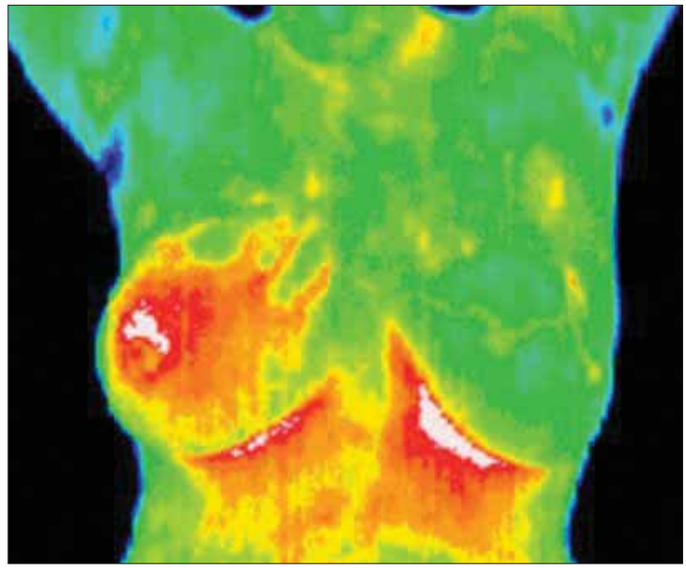

Figure 3. **Image of digital infrared thermal imaging (Inflammatory carcinoma of right breast)**

that correlate infrared data about the breast being examined to infrared patterns that are associated with either benign or malignant breast tissue. The end result is a numeric score for a given suspicious lesion after the region-of-interest placement. The CTI technology is designed to electronically store the digital breast images and provide the patient with an electronic copy of the images, which may be helpful if she visits another imaging facility. By maintaining close monitoring with infrared imaging, self breast examinations, clinical examinations, mammography, and other tests, a woman has a much better chance of detecting cancer at its earliest stage and preventing invasive tumor growth. In addition, infrared imaging is noninvasive, risk free, patient friendly, and the cost is considerably low. These features, together with its early detection capability, have enabled infrared imaging to be a strong candidate as a complementary diagnostic tool to traditional mammography.

DITI does not use ionizing radiation so this makes it very valuable in the diagnostic procedure of pregnant and younger women. It requires no physical contact, there are no liquids to drink. Difficulties in reading mammograms can occur in women who are on hormone replacement, nursing or have fibrocystic, large, dense, or enhanced breasts. These types of breast differences do not cause difficulties in reading digital infrared scans. It does not require painful levels of breast compression, is not likely to be limited by radiographically dense breast composition, and provides quantitative data that can reduce the interpretive variability associated with mammography.

Infrared imaging data for each subject were acquired during a single imaging session. The subject lay prone on the imaging bed during the procedure with both breasts suspended through openings in the top of the bed. Each breast was imaged individually while the contralateral breast was shielded from the cooled air by a protective gown. Infrared imaging began with a brief period of temperature stasis, after which a stream of cool air was circulated within the refrigeration chamber around the uncovered suspended breast. Multiple infrared images were

obtained in rapid sequence by the infrared camera both before and during the cooling phase. After the first breast was imaged, the process was repeated for the contralateral breast. The entire session required approximately 15 min, with actual imaging time lasting approximately 3 min per breast (32).

Thermography was approved by the U.S. Food and Drugs Administration (FDA) in 1982 as a supplement to mammography in helping to detect breast cancer. To date, CTI is only available for eligible women who participate in CTI clinical trials. The technology is currently being reviewed but has not been approved by the FDA.

The infrared imaging system has a high negative predictive value which is essential for its clinical use, whereas the positive predictive value does not have as great a clinical utility. Therefore, it is not designed to be a screening tool for identifying or localizing malignancies or to delay biopsy of highly suspicious lesions. It was suggested that the infrared imaging assessment would have adjunctive value to standard clinical practice when both mammography and sonography are commonly used in the decision to recommend biopsy.

Infrared imaging is an economic and safe modality that provides physiologic data about a lesion. The physiologic view provided by infrared imaging complements the anatomic view provided by mammography, with a very high sensitivity and negative predictive value in masses. Infrared imaging holds great promise in the management of breast lesions. It can be used as an adjunct for further evaluating a mammographically apparent breast abnormality when the radiologist has a low-to-moderate suspicion that a malignancy is present. Thus, this dynamic computerized infrared imaging system could be a valuable addition to the physicians' armamentarium of diagnostic tools.

Conclusion

We reviewed the literature on the accuracy of new technologies proposed for breast cancer screening. Two potential tests were identified Full-field digital mammography (FFDM) and Dijital infrared termal imaging (DITI), for which primary studies met quality and applicability criteria and provided adequate data on test accuracy. These technologies have been assessed in cross-sectional studies of test accuracy where the new test is compared to standard film mammography. As a result, much attention has been devoted to developing improved radiographic techniques for breast cancer screening and evaluation. X-ray mammography is inexpensive and reliable, but the patient is exposed to ionizing radiation and the test is uncomfortable to the patient due to breast compression. If the breasts are dense or have implants, it is very difficult to obtain adequate images.

Direct comparision of digital to film mammography revealed that the recall rate, biopsy rate, and specificity of FFDM were almost identical to those of SFM. However, FFDM had higher sensitivity for breast cancer than SFM, particularly among younger women with denser breasts. The area under the curve was significantly greater for FFDM compared with SFM for women under the age of 50 years, women who were not postmenopausal, and women with heterogeneously or extremely dense breasts. On the other hand, SFM may be more suitable for older women with less dense breasts.

Breast ultrasonography is a relatively inexpensive, and effective method with non-ionizing radiation. It can be used in young and/or pregnant women. It is superior to mammography in differentiation of solid/cystic masses and in evaluation of dense fibroglandular breast tissue, which is the most important inherent limitation of mammography in the diagnosis of breast cancer. Although some researchers have reported reasonable results from US breast screening, experts recommend mammography as a primary screening method combined with breast ultrasonography in older women.

DITI, can be used as a tool for breast cancer detection. It is a comfortable, simple and safe method. It can be used also for young women with dense breasts for whom mammography is not very effective and for pregnant women. The evidence is currently insufficient to support the use of any of these new technologies in population screening, but would support further evaluation.

Conflict of interest

No conflict of interest is declared by authors.

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Is uterine prolapse a cause of primary infertility?

Uterin prolapsus infertilite nedeni olabilir mi?

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Abstract

Presented in this report is apparently the first case of its kind in the medical literature where a woman with 11 years of primary infertility not only conceived following conservative surgery for uterine prolapse but also had a successful obstetrical outcome.
(J Turkish-German Gynecol Assoc 2010; 11: 158-9)
Key words: Uterine prolapse, primary infertility, cervicopexy
Received: 15 December, 2009 **Accepted:** 24 January, 2010

Özet

Bu vaka tıbbi literaturde bildirilen ve 11 yıllık primer infertilitesi olup tedavi ile hem bu sorunu hem de obstetrik problemleri düzelmiş olan ilk vakadır. (J Turkish-German Gynecol Assoc 2010; 11: 158-9)
Anahtar kelimeler: Uterin prolapsus, primer infertilite, servikopeksi
Geliş Tarihi: 15 Aralık 2009 **Kabul Tarihi:** 24 Ocak 2010

Introduction

Uterovaginal prolapse is mostly attributed to childbirth injury, but paradoxically, hardly any data is available as to prolapse being a cause of infertility. Preservation of fertility is one of the criteria deciding the choice of management, and the surgical procedure offered depends upon the age of the patient and her desire for preservation of menstrual and reproductive capabilities. However, in our case the question was not just of preservation but rather the initiation of fertility.

Case Report

Written consent was obtained from the patient and the Departmental Ethical Committee approved this report.

A case is presented of a patient aged 32 years, who reported with complaints of primary infertility for 11 years and a mass protruding at the vaginal introitus since 8 years.

The wife of a non-smoker, non-alcholic office clerk, she had lived with her husband since 11 years and there was no history of any coital difficulties or any menstrual abnormalities. She had no chronic cough and no urinary or bowel problems. Her past history revealed a history of management of infertility;the husband's semen analysis was normal, she had had a dilatation and curettage about 9 years previously and the histo-pathology reported the secretory phase of the endometrium. Her fallopian tubes were patent on hysterosalpingography. Other infertility investigations were also normal. Four years previously, she had had Intra-Uterine Insemination in two cycles with the husband's semen but that exercise also proved futile. On examination, she was of average build and height with normal pulse and blood pressure. Her respiratory, cardio-vascular systems and per-abdomen examination did not reveal any abnormality.

Pelvic examination findings were: cervix lprotruding out of the vaginal introitus with normal sized uterus in the vaginal axis (cervical length-2.5 cm and total utero-cervical length-7 cm).

Her routine investigations and the couple's infertility profile were normal and the only positive clinical finding was third degree uterine prolapse. Based on the above review, repair of the prolapse was planned by adopting a conservative approach. The trans abdominal route was followed and bilateral slings of rectus sheath strips were carved out with free medial ends and lateral ends kept intact and then tied on the anterior surface of the exposed cervix, traversing retro-peritoneally almost parallel to the round ligaments (Purandre's Cervicopexy under spinal anaesthesia). The patient was discharged on the fourth day and the subcuticular sutures were removed on her revisit on the seventh day. The post-operative period was uneventful. Regular follow-up confirmed symptomatic & clinical relief from the prolapse.

About five months later, the patient missed her periods and tested positive for hCG. Her routine ante-natal visits were uneventful. At term, Lower Segment Caesarean Section was performed with no difficulty encountered during surgery and she was delivered of a male child. of 2500 grams. Regular follow-up after caesarean, to date (9 months), she has no complaints at allrelating to genital prolapse.

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doi:10.5152/jtgga.2010.25

Discussion

Genital prolapse is a disorder of pelvic support and is one of the most frequent problems encountered in day to day gynaecological practice, as our social and cultural background predisposes this condition to occur at an age earlier than in any other part of the world (1). Many approaches have been performed for normal anatomic restoration of the prolapsed organ to preserve the uterine function, allowing future child bearing. The age old Manchester-Fothergill procedure may lead to cervical incompetence culminating in miscarriages & premature deliveries (2). Moreover, the importance of conserving the cervix for conception has also been realised1, though of an optimal length (3). The first report of a few successful pregnancies (5 out of 19) was reported was of a sling surgery :sacro-spinous fixation (4). Tio date, review of the literature does not show even a single case where uterine prolapse could be ascertained as a cause for infertility.

Conclusion

In those cases of unexplained infertility with concurrent uterine prolapse, conservative surgery of the latter, by means of sling supports not only relieves the symptoms of the descensus, but may also help in achieving conception with a successful outcome, thereby curing the primary problem of long standing nulliparity. This option needs further evaluation, so that it can be offered to suitable candidates.

Conflict of interest

No conflict of interest is declared by authors.

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Pulmonary embolus arising from sloughed off myoma in late puerperium

Geç lohusalıkta myoma bağlı pulmoner embolizm

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Abstract

Pulmonary embolus is a rare and serious complication of myoma uteri in the puerperium that resulted in late postpartum hysterectomy

A 38-year-old, multiparous woman with a large myoma located on the left lateral wall of the uterus underwent emergency cesarean section due to fetal distres at 28 weeks. During the operation, a 15 cm sized intramural myoma was left without any intervention. On the 40th day postpartum the patient returned to the clinic with sepsis and pulmonary embolus because of obstruction of lochia drainage by the sloughed off myoma. The patient underwent hysterectomy and medical therapy for pulmonary embolus.

We presented an unusual complication of uterine leiomyoma in the late postpartum period after cesarean section. Whatever the mode of sloughing off ofthe myoma, the results of the obstruction of lochia drainage may be devastating as in our case. To avoid these complications, clinicians must be aware of these symptoms and prompt intervention is essential.

(J Turkish-German Gynecol Assoc 2010; 11: 160-2)

Key words: Myoma uteri, pregnancy, puerperium, pulmonary embolus, sepsis

Received: 22 December, 2009 **Accepted:** 11 February, 2010

Özet

Lohusalıkta myoma uteriye bağlı oluşan pulmoner emboli seyrek ve ciddi bir komplikasyondur. Otuzsekiz yaşında, lateral uterus duvarında 15 cm myomu olan multipar hasta, 28 hafta gebe iken fetal distress nedeniyle acil sezaryene alındı. Operasyonda myoma dokunulmadı. Hasta lohusalığının 40. gününde myomun drenajı bozması nedeniyle loşiye bağlı pulmoner emboli ve sepsis yakınması ile müracaat etti. Hastaya acil histerektomi yapıldı, pulmoner emboli için de medikal tedavi planlandı. Burada geç postpartum görülen bir pulmoner emboli vakası bildirilmiştir. Burada asıl sorun myomun loşi akışını bloke etmesi idi. Bu nedenle klinisyen uyanık olmalı ve gerekli girişimi yapmalıdır.

(J Turkish-German Gynecol Assoc 2010; 11: 160-2)

Anahtar kelimeler: Myoma uteri, gebelik, lohusalık, pulmoner emboli, sepsis

Geliş Tarihi: 22 Aralık 2009 **Kabul Tarihi:** 11 Şubat 2010

Uterine leiomyomas (fibroids, myomas) are the most common benign tumors of the uterus with an incidence of 20-25% in reproductive years (1, 2). In pregnancy, leiomyomas occur approximately 1% of the time, with a range 0.3% to 2.6% (3). The reported incidence of myomas complicating pregnancy is less than 1% and these complications are spontaneous abortions, fetal growth restriction, preterm labor, dystocia, fetal malpresentation, abruptio placenta, retained placenta and postpartum hemorrhage (4-7). Many of these complications are closely related with the size and the location of the myoma. In addition, some authors suggest that leiomyomas cause an increased incidence of cesarean delivery and cesarean hysterectomies (8).

The purpose of this case report is to remind clinicians that leiomyoma in pregnancy may cause rare but serious complications like sepsis and pulmonary embolism in the late postpartum period and this may result in loss of fertility.

Case

A 38-year-old woman gravida 7, para 4 was admitted to the clinic of the obtetrics and gynaecology of Ege University with high blood pressure. Although she did not know her exact last menstural period, ultrasonographic examination revealed a 28 week fetus and a myoma which was 110*190 mm in dimension. It was localized in the left lateral wall of the uterus. Blood pressure was 150/90 mm Hg. Routine biochemical, hematological and urine parameters were studied. These tests were all remarkable with excess protein in urine. In view of the foreging, the patient was diagnosed as preeclamsia. Basal cardiotocography revealed repetitious late deceletations that shows fetal distress so a cesarean section was performed. We delivered a 1220 gr male fetus with thick meconium in the amniotic fluid. He was transferred to the neonatal intensive care unit. During the operation a 15 cm

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doi:10.5152/jtgga.2010.26

intramural myoma was observed on the left lateral wall of the uterus. The myoma was left in place and bilateral tubal ligation of Pomeroy was executed with the patient's consent. The patient was discharged on the 7th postoperative day

On the 40th postpartum day the patient was admitted to our clinic with fever, abdominal pain, malodorous vaginal discharge and dyspnea. Physical examination revealed that the patient has abdominal and uterine tenderness and a purulant malodorous discharge from the cervix was observed on speculum examination. Uterus was in subinvolution and the fundus was palpated 3 cm over the umblicus. Her vital signs, blood pressure, fever, heart rate, respiratory rate were 120/80 mm Hg, 38˚C,130 pulse/min. and 36/min respectively. Emergency arterial blood gas analyses revealed respiratory alkalosis. In complete blood count study hemoglobin, wbc and platelets were 9.9g/dl, 16000/mm³ and 110000/mm³ respectively. D-dimer was over 4355micg/l and C-reactive protein was 35.49 mg/dl. In this presentation we suspected sepsis and pulmonary embolism so thoracic angio tomographic(CT), abdominal CT and echographic investigations were performed.

In the cardiac echo examination a 22*14 mm sized thrombus was diagnosed in the bifurcation of the pulmonary artery (Figure 1) and on thoracic angio CT, multiple thrombi were diagnosed bilaterally in the pulmonary arteries especially on the right side (Figure 2). Also on the right lower segment an acute infarct was observed. Abdominal CT reported 9 cm sized mass and pyometria in the cavity of the uterus.

With the diagnoses of postpartum endometritis, sepsis and secondary pulmonary embolism, the patient was given intravenous broad spectrum antibiotherapy and low molecular weighted heparin. After stabilisation, the patient underwent hysterectomy. Abdominal inspection during surgery revealed that the uterus was in subinvolution without free fluid in the abdomen. Hysterectomy was performed without complication. On macroscopic inspection, the uterus was incised longitudinally from fundus to cervix. We observed that the uterine cavity was full of pyometria and a 12 cm sized myoma was protruding from the submucous layer to the internal cervical os and was obstructing the drainage of lochia (Figure 3).

During the postoperative period, fever was decreased and uneventful. Microscopic examination of operation material was reported as necrotic myoma, acute endometritis and pyometria.The patient was discharged with anticoagulant therapy on the 12th postoperative day.

Discussion

Pregnancy and postpartum complications seem to be more common with larger and submucosal myomas that located below the placental attacthment (5). There is a variable progression of myoma in pregnancy reported by several studies (5, 9). Enlargement of the myoma in pregnancy was seen only 32% of the pregnants and the greatest enlargement was observed before 10th week of gestation. Conversly reduction of the size of myoma occurs in puerperium period. In our case we did not see significant reduction in size of the myoma (operation day size 15 cm, postpartum 42th day size 12 cm) but change of location

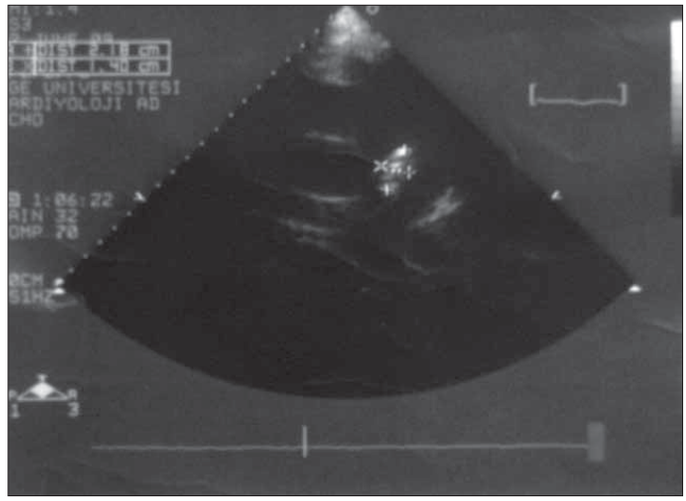

Figure 1. Thrombus in echocardiography was seen as echogenic focus

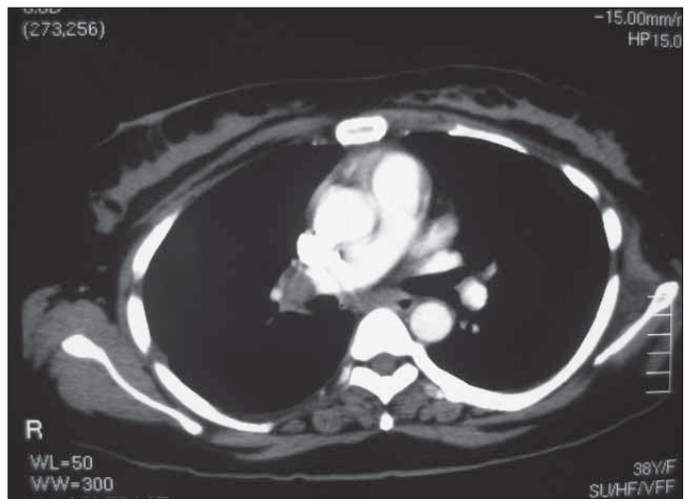

Figure 2. Multiple thrombi was diagnosed in the bifucation of pulmonary artery on thoracic angio CT

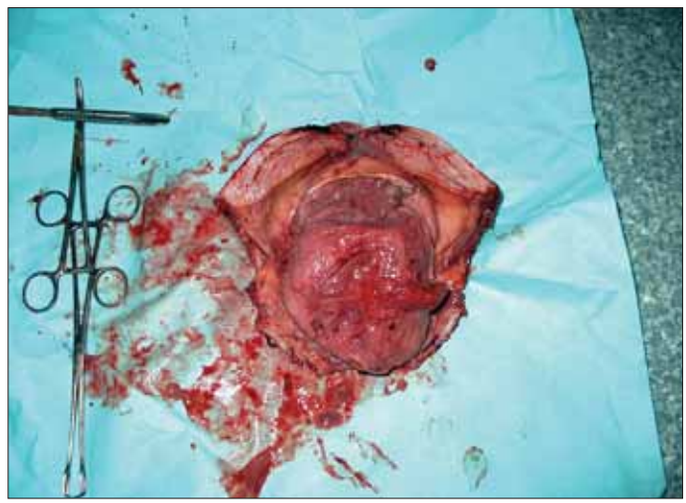

Figure 3. Uterine cavity and myoma protruding from submucous layer was seen after hysterectomy

from intramural to submucosal in uterus. Changing location of myomas in puerperial period was defined by many authors in literature (10-12). Route of myoma in portpartum period either subserosal or submucosal may be seen. Some authors suggest that this changing location especially to subserosal route makes operations of myomectomy easier than achieved in delivery period (11). Although subserosal transformation is good candidate for minimal invasive surgery, submucosal transformation accompany with some complications especially by obtructing the drainage of lochia such as postpartum haemorrhage, infectious complications, disseminated intravascular coagulation and pulmonary embolus.

Murakami et al. (10) discussed about mechanism of sloughing off the myomas during the puerperium. In summary they suggest that there may be three way of expulsion of the myomas to the endometrial cavity. First one is reduction of the blood supply by the delivery during the puerperium. This causes relative ischemia and than degeneration. Normal uterine tissue may provoke expulsion of the degenerative myoma. Similar actions and pathways are seen after uterine artery embolization (13-15), bipolar coagulation of uterine artery (16), and gonadotropin releasing hormone (GnRH) agonist therapy (17-19). Second way is rapid involusion of uterus and on the other side myomas may be the out of step with this involusion. As a result, prompt involusion may expulses myomas to the either submucosal or subserosal localization. Same mechanism may be seen in GnRH agonist therapy and uterine artery embolization which cause rapid atrophy of uterus. Third way is the endometrial changes over the myomas makes it easier to be sloughed off. However this may be seen in puerperial 3. or 4. weeks, it may be seen in other hormonal therapies like GnRH agonist therapy (18). In our case we also observed same mechanism but different results. In postpartum period intramural myoma transformed to submucosal myoma and this myoma sloughed off to the uterine cavity. This myoma obstructed the passage; lochia and necrotic myoma became infected. This infection and necrosis progresses to sepsis and maybe pulmonary embolism.

Akrivis et al. (12) described a case of primary postpartum haemorrhage due to a large submucosal leiomyoma. It was the same way of sloughing off the myoma but different complication. Complication was postpartum haemorrhage due to atonia which was resulted deep anemia and emergency postpartum hysterectomy. Authors suggest that obstruction of passage by myoma was the cause of atony bleeding.

The maternal hypercoagulable state is a physiological preparation for delivery however, this hypercoagulability is associated with an increased risk of venous thromboembolism. The elements of Virchow's triad (venous stasis, vascular damage, and hypercoagulability) are all present during pregnancy and the postpartum period. Additionally obesitiy, immobilisation, thrombophilia, smoking, advanced age, intrapartum haemorrhage, parity, infection and cesarean section are the risk factors for pulmonary embolism either intrapartum or postpartum period (20). In our case although most of the risk factor were already present, infection caused by incomplete drainage and mass effect of uterus with giant myoma might be additional reason for pulmonary embolus.

In conclusion we presented an unusual complication of uterine leiomyoma in late postpartum period after cesarean section. Whatever the way of sloughing off the myoma is, results of the obstruction of lochia drainage may be devastating as we see in our case. To avoid these complications, clinicians must be aware of these symptoms and promptly intervention is needed.

Conflict of interest

No conflict of interest is declared by authors.

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Transient osteoporosis of pregnancy: case report

Gebelikte geçici osteoporoz: Vaka sunumu

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Abstract

Transient osteoporosis of pregnancy is a rarely observed skeletal pathology developing in the last months of pregnancy. Meticulous evaluation is important for the differential diagnosis of severe and progressive hip and/or groin pain in pregnant patients. MRI is a valuable and safe technique for demonstrating bone marrow edema and skeletal abnormalities during pregnancy. Avoidance of vaginal delivery and non-weight bearing measures are essential in order to prevent complications such as hip fractures related to transient osteoporosis of pregnancy. We present the diagnostic evaluation and treatment of an uncommon case of transient osteoporosis of pregnancy with resolution of symptoms and postpartum.

(J Turkish-German Gynecol Assoc 2010; 11: 163-4)

Key words: Osteoporosis, pregnancy, hip pain

Received: 12 November, 2009 **Accepted:** 24 January, 2010

Özet

Gebelikte geçici osteoporoz seyrek olarak gebeliğin son haftasında iskelet gelişim patolojilerine neden olur. Özenli tanı ve yaklaşım özellikle kalça ve omurga ağrısı çeken hastalarda önem kazanır. Vaginal doğumdan kaçınmak geçici osteoporoza bağlı kalça kırıklarının önlenmesinde önem arzeder. Yazımızda böyle bir vaka bildirilmiştir.

(J Turkish-German Gynecol Assoc 2010; 11: 163-4)

Anahtar kelimeler: Osteoporoz, gebelik, kalça ağrısı

Geliş Tarihi: 12 Kasım 2009 **Kabul Tarihi:** 24 Ocak 2010

Introduction

Musculoskeletal symptoms such as hip, pelvis and groin pain are common complaints of pregnancy. They are associated with increased weight and position of gravid uterus which affects the axial skeleton and causes neural compression by fluid retention and hormonal changes leading to joint laxity (1). These symptoms are usually managed conservatively without specific diagnosis. However, there may be cases with severe and progressive, pain especially during the second and third trimester of pregnancy (2, 3). Transient osteoporosis of pregnancy (TOP) needs to be considered for differential diagnosis in these cases.

TOP during pregnancy was first reported by Curtis and Kincaid in 1959 (4). It is a rarely observed, self-limiting pathology of unclear etiology with severe onset of groin and/or hip pain progressive in character, mostly during the third trimester of pregnancy. The patient may not be able to walk or may present with an antalgic gait due to functional disability. The current case report presents an uncommon case of TOP during pregnancy and reviews the literature.

Case Report

A 34 year-old, nulliparous woman had severe pain of increasing intensity in the right leg at 30 weeks of gestation. The pain was aggravated by getting up, sitting down and standing up. Clinical examination revealed mild pain without restriction of motion in the lumbar area. Abduction and external rotation of her right hip were limited by severe pain and she had an antalgic gait. The right lower segment of the abdomen was very tender to palpation at examination. She did not have any motor or sensorial deficit according to the neurologic examination. Lumbar discopathy was ruled out as a cause of the pain. Laboratory tests for electrolytes, thyroid function, rheumatoid factor and antinuclear antibody were normal. Magnetic resonance imaging (MRI) scans of the hips revealed alterations at the right coccygeofemoral junction consistent with transient osteoporosis accompanied by stress fracture (Figure 1). Joint aspiration was performed due to increased synovial fluid in the hip junction. She received analgesic treatment with paracetamol and nonsteroid anti-inflammatory drugs during pregnancy. During the remainder of the

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doi:10.5152/jtgga.2010.27

pregnancy she was prescribed bed rest and avoided weight bearing. Cesarean section was recommended considering the risk of hip fracture during normal vaginal delivery. Calcitriol 0.5 mcg and calcium 1000 mg were prescribed soon after delivery. Whole body scintigraphy performed one month after delivery also demonstrated osteoporosis. Calcium 1000 mg, vitamin D3 880 IU and salmon calcitonin 200 IU/day, switched to alendronate 70 mg/week, were started for six months according to the scintigraphy findings. Her symptoms and MRI findings regressed 6 months following delivery (Figure 2).

Discussion

Physiotherapy, rheumatology and orthopedic consultations are considered for persistent, moderate to severe hip, back and/or groin pain during pregnancy. Pubic symphysiolysis, avascular necrosis, ostemyelitis, neoplasm and TOP should be ruled out in the differential diagnosis. Plain radiographs of the limb may demonstrate severe osteopenia for advanced cases of TOP. MRI is a more sensitive, safe and effective imaging technique for detecting skeletal abnormalities including TOP and hip fractures during pregnancy (5). Diffuse bone marrow edema by MRI, normal levels of markers for inflammation, severe and persistent groin and/or hip pain during the last months of pregnancy are positive findings for TOP.

Prevention or permission for only limited weight bearing is essential in order to avoid complications in TOP. Atraumatic fractures may also be observed. Hip fracture is reported during labor, therefore cesarean section is preferred in cases with TOP (6). The palliative approach to TOP includes use of non-weight bearing measures such as bed rest, wheelchairs, crutches, analgesia and physiotherapy. Hips are usually remineralized within 6-14 months postpartum (7). Antiresorptive therapy with biphosphonates, calcium and vitamin D are used as adjunctive measures during the recovery period.

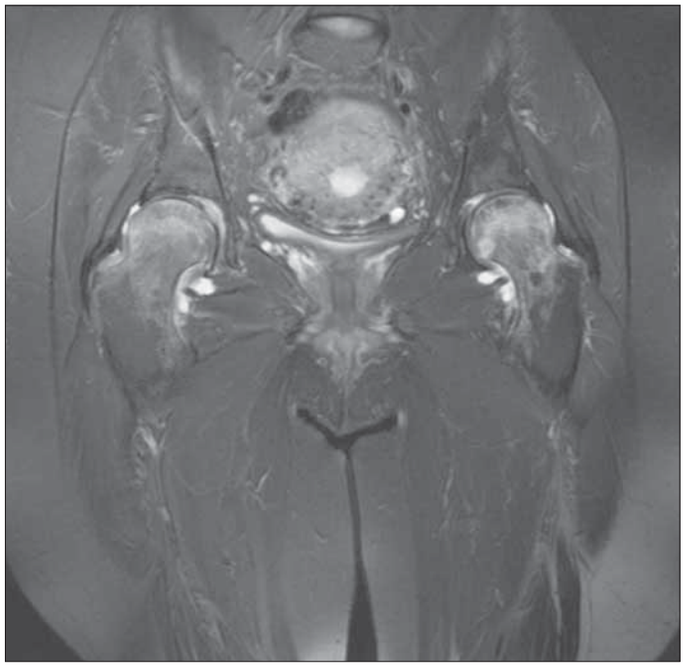

Figure 1. T2 weighted MRI demonstrates bone marrow edema in the femur head and neck and increased synovial fluid in the hip junction

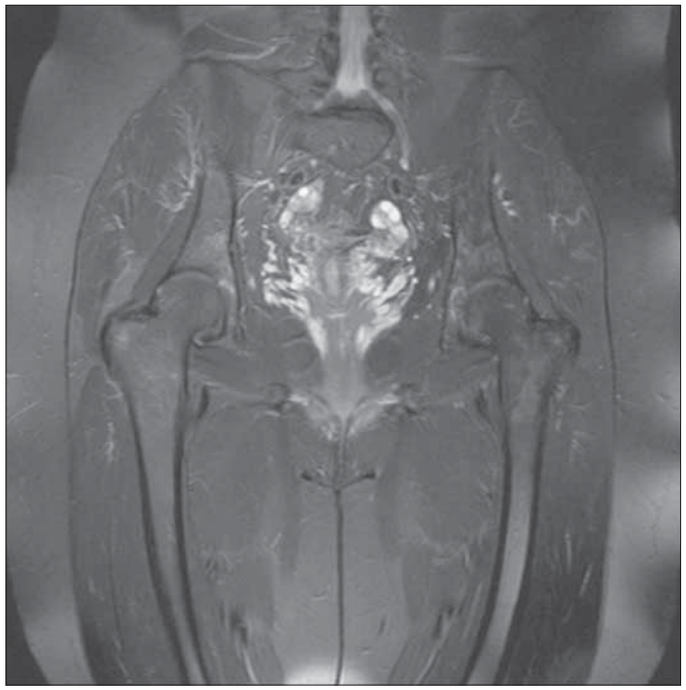

Figure 2. Fat suppressed T2 weighted sequences of the control MRI postpartum demonstrated normalization of bone marrow edema

In summary, TOP should be considered for the differential diagnosis of severe hip pain during pregnancy. Early diagnosis and treatment are essential in order to prevent its complications.

Conflict of interest

No conflict of interest is declared by authors.

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Ruptured rudimentary horn pregnancy at sixteen weeks

On altıncı haftada rüpütre rudimenter boynuz gebeliği

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Abstract

Pregnancy in a non-communicating rudimentary horn is very difficult to diagnose before it ruptures, leading to life -threatening intraperitoneal hemorrhage. A 22-year-old second gravida patient presented at the Emergency Center of the University Clinical Center of Kosova with a 16-week history of amenorrhea and acute onset of severe abdominal pain. She was resuscitated and taken for an emergency laparotomy under general anesthesia. Intraoperatively, there was a massive hemoperitoneum with a ruptured right rudimentary horn Given their rarity, ruptured rudimentary horn pregnancies are of interest.

(J Turkish-German Gynecol Assoc 2010; 11: 165-7)

Key words: Bicornate uterus, rudimentary horn, interstitial pregnancy

Received: 24 November, 2009 **Accepted:** 8 January, 2010

Özet

Rudimenter boynuz gebeliğini genellikle rüptüre olup da hayatı tehtid eden hemorajilere neden olmadıkça tanımak oldukça güçtür. Yirmiki yaşında ve 16 haftalık amenoresi olan hasta Kosova Univeristesi acil servisine akut batın yakınmaları ile müracaat etti .Resusite edilip genel anestezi ile laparotomi uygulandı. İntraoperatif masif hemoperitoneum ve rüpütre boynuz gebeliği izlendi.

(J Turkish-German Gynecol Assoc 2010; 11: 165-7)

Anahtar kelimeler: Bikornuat uterus, rudimenter boynuz, interstisyel gebelik

Geliş Tarihi: 24 Kasım 2009 **Kabul Tarihi:** 08 Ocak 2010

Introduction

Blastocysts normally implant in the endometrial lining of the uterine cavity, and implantation at any other location is called an ectopic pregnancy (1). Malformations of the uterus result from a variety of anomalies during embryogenesis from the 6th to the 17th week of development. Agenesis should be distinguished from unicornate, pseudounicornate, bicornate, septate, and communicating uteri (Musset''s classification) (2). A rudimentary horn is a developmental anomaly of the uterus, and a pregnancy in the rudimentary horn of the uterus is known as a cornual pregnancy (3). Implantation within the tubal segment that penetrates the uterine wall results in an interstitial or cornual pregnancy. Pregnancy in a non-communicating rudimentary horn is very difficult to diagnose before it ruptures, leading to life -threatening intraperitoneal hemorrhage, although rupture may not occur until up to 16 weeks (4). Ectopic pregnancy refers to the implantation of a fertilized egg in a location outside the uterine cavity, including the fallopian tubes, cervix, ovary, cornual region of the uterus, and abdominal cavity. The abnormally implanted fetus grows and draws its blood supply from the abnormal implantation site. As the fetus enlarges, it creates the potential for organ rupture because only the uterine cavity is designed to expand and accommodate fetal development (5).

Although many surgeons have attempted to distinguish interstitial from cornual pregnancy, the two are difficult to distinguish anatomically and should be classified together. Classically, cornual or interstitial pregnancies present later, because the gestational sac is surrounded by the thicker myometrial walls rather than the weaker thin-walled fallopian tube (6). This region is well vascularized because of the confluence of uterine and ovarian vessels, which can lead to the loss of a large volume of blood. Surgical treatment is usually performed by laparotomy because it is easier to stop the blood loss after incising the cornual region and resecting the cornual sac (7). However, successful laparoscopic treatment has been reported in some cases (8). Ectopic pregnancy can lead to massive hemorrhage, infertility, or death. There is severe hemorrhage because the implantation site is located between the ovarian and uterine arteries.

Case report

A 22-year-old second gravida patient presented to the Emergency Center of the University Clinical Center of Kosova with a 16-week history of amenorrhea and acute onset of severe abdominal pain. She collapsed while being transported to the Emergency Center. There was no history of vaginal

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doi:10.5152/jtgga.2010.28

bleeding. Her menses began at age 14, occurred every 30 days, and lasted 4-5 days. There was no history of irregularity or dysmenorrhea. This patient had had a previous pregnancy with a spontaneous delivery. The presence of a rudimentary horn was not diagnosed before the current pregnancy.

During the pregnancy, she was hospitalized in a Gynecology/Obstetrics Clinic when she was 10 weeks pregnant, and transabdominal and transvaginal ultrasonography were performed. The results revealed a slightly enlarged uterus with an empty intrauterine cavity in the left cornu, and there was a gestational sac with an embryo (crown-rump length (CRL) 35 mm) with fetal movement and fetal heart activity in the right cornu. The general physical and laboratory examinations were normal and the Clinic discharged her in good health.

She presented to the Gynecology/Obstetrics clinic a second time in hemorrhagic shock An abdominal examination showed distension with generalized tenderness. The uterus was not palpable separately. There was evidence of free fluid in the peritoneal cavity. She was drowsy, but responded to a painful stimulus. A vaginal examination revealed an enlarged uterus and tenderness on rocking the cervix. Since transabdominal ultrasonography indicated a decreased uterine size and there was evidence of free fluid in the peritoneal cavity, a clinical diagnosis of a ruptured uterus with intraperitoneal hemorrhage was made. The physical examination, revealed a body temperature of 37.2°C, blood pressure of 90/55 mmHg, respiratory rate of 25/min, and pulse of 110/min. The preoperative laboratory investigation showed a hematocrit of 23%, hemoglobin of 8.7 g/dL, and 20.2×10^3 /mm^3 leucocytes.

She was resuscitated and taken for an emergency laparotomy under general anesthesia. Intraoperatively, there was a massive hemoperitoneum with a ruptured right rudimentary horn (Fig. 1), with approximately 1500 mL of blood in the peritoneal cavity. The fetus and partial placenta were lying in the peritoneal cavity (Fig. 2). The rudimentary uterine cornu in a bicornate uterus with a single cervix had ruptured (Figs. 3 and 4). The rudimentary horn was larger than the main uterus and connected by a thin fibrous band. The left fallopian tube and both ovaries were normal, which correlated with the physical examination and ultrasonography.

The right rudimentary horn was resected carefully without perforating the intrauterine cavity and the abdomen was closed after peritoneal lavage and ensuring hemostasis (Fig. 5).

Five units of blood and two of plasma were transfused perioperatively. The postoperative laboratory investigation showed a hematocrit of 30.3%, hemoglobin of 10.1 g/dL, and 8.9×10^3 / mm^3 leucocytes. The pathological examination confirmed a ruptured cornual pregnancy (Fig. 6).

She was discharged 4 days postoperatively.

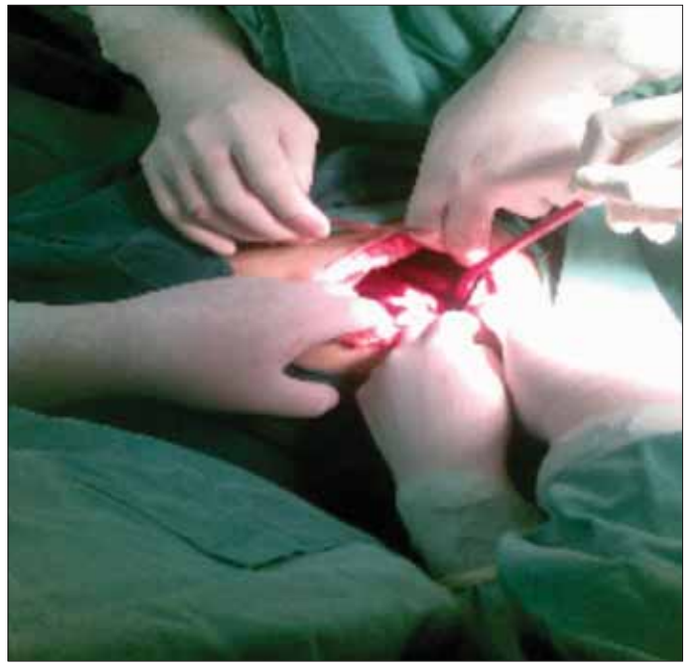

Figure 1. **Massive hemoperitoneum with a ruptured right rudimentary horn**

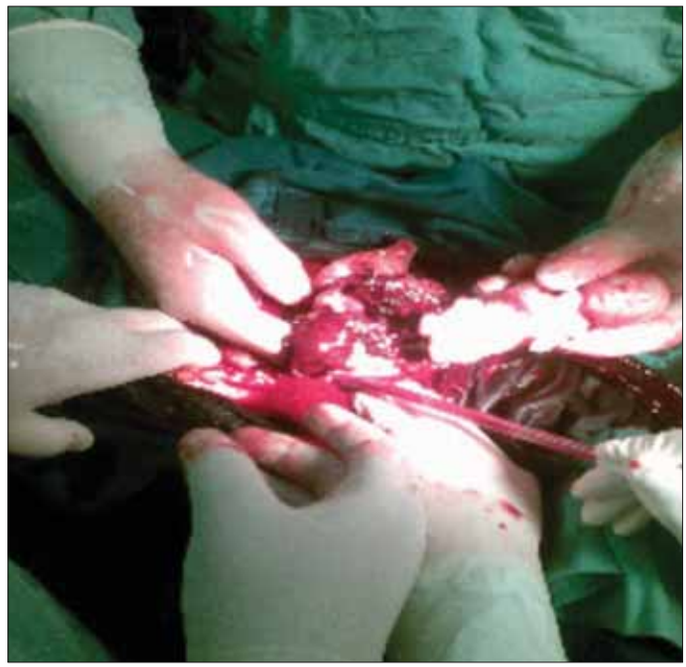

Figure 2. **The fetus and parts of the placenta lying in the peritoneal cavity**

Discussion

Pregnancy in a rudimentary horn is possible only if a spermatozoon travels up the normal fallopian tube and fertilizes an ovum that subsequently enters the fallopian tube of the rudimentary horn. The usual termination of pregnancy in the rudimentary horn is by rupture at 4-5 months gestation because of the poorly developed muscular and mucosal layers. Rupture may occur at any stage depending on the rudimentary horn anatomy, and sometimes not until midterm (9).

Cornual (interstitial) pregnancy poses a significant diagnostic and therapeutic challenge and carries a greater maternal mor-

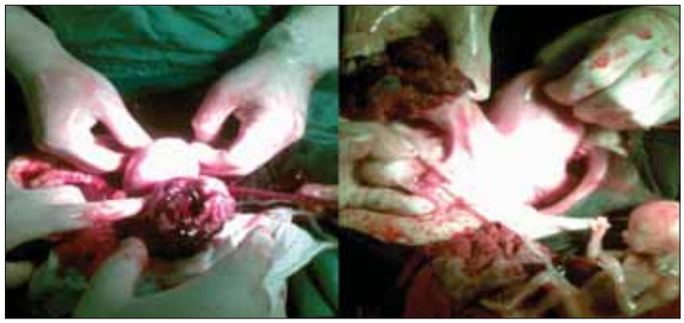

Figure. 3 and 4. The rudimentary uterine horn in a bicornate uterus with a single cervix had ruptured

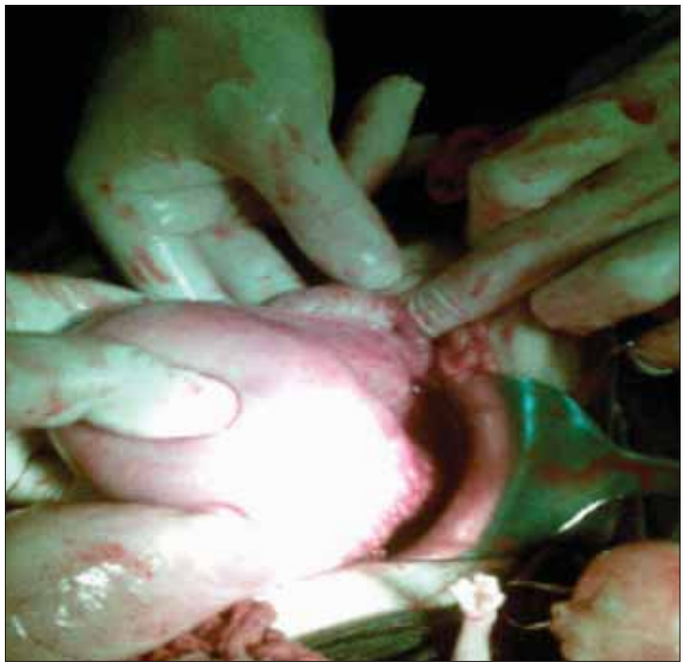

Figure 5. The rudimentary right horn was resected

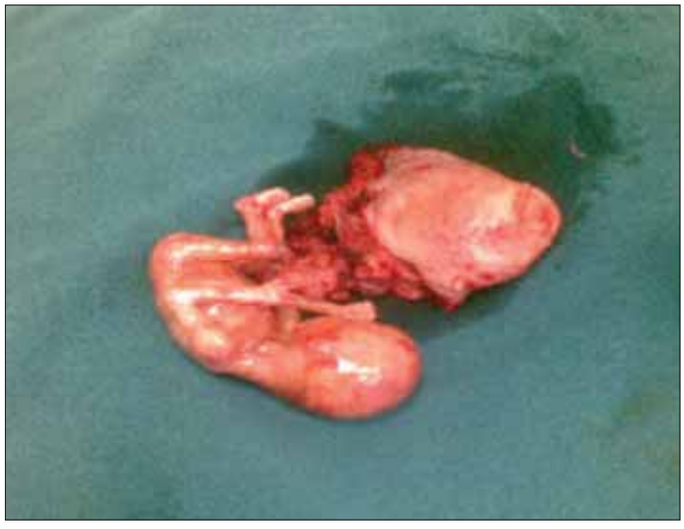

Figure 6. Pathological examination confirmed a ruptured cornual pregnancy

tality risk than ampullary ectopic pregnancies. Cornual pregnancies tend to present relatively late due to myometrial distensibility (10). The diagnosis can be made with transabdominal or transvaginal ultrasound, using three criteria: an empty uterus, a separate gestational sac <1 cm from the lateral-most edge of the uterine cavity, and a thin myometrial layer surrounding the sac (11).

The presence of the rudimentary horn was not diagnosed until it ruptured. Significant maternal hemorrhage leading to hypovolemia and shock can rapidly result from cornual rupture. Clinically, risk factors are similar to other types of ectopic pregnancy (12). Different surgical intervention modalities for cornual gestation have been reported. Traditionally, laparotomy with cornual resection is performed for a ruptured cornual pregnancy when the patient is hemodynamically unstable. Recently, more conservative operations have been developed, and operative laparoscopy provides yet another management option. Laparoscopic cornuostomy and cornual resection have been reported for the treatment of interstitial ectopic pregnancy (13). The accepted treatment is to remove the gravid rudimentary horn and leave the normal one.

Currently, there is insufficient evidence to recommend any single treatment modality for cornual gestation, and the decision should be based on factors such as clinical presentation, the surgeon's expertise, side effects, overall cost, and patient preference

Conflict of interest

No conflict of interest is declared by authors.

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Ectopic pregnancy following levonorgestrel emergency contraception: a case report

Levonorgestrelli acil kontrasepsiyon sonrası ektopik gebelik: Olgu sunumu

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Abstract

Hormonal contraceptive pills containing 750 microgram levonorgestrel are the most commonly used postcoital contraceptive method because of their high efficacy and fewer side effects. Emergency contraceptive pills containing levonorgestrel present their effects by several mechanisms, including delayed tubal transport of the ovum. A delay of tubal transportation of an ovum is also one of the possible etiologic factors of tubal ectopic pregnancies. There are limited data on the risk of ectopic pregnancy following levonorgestrel treatment as an emergency contraception. Here, a case of tubal pregnancy associated with the use of levonorgestrel containing emergency contraceptive pills has been presented along with discussion of the relevant literature. (J Turkish-German Gynecol Assoc 2010; 11: 168-9)

Key words: Emergency contraception, extrauterine pregnancy, levonorgestrel

Received: 17 November, 2009 **Accepted:** 3 January, 2010

Özet

750 Mg levonorgestrel içeren hormonal kontraseptif haplar, yüksek etkinlikleri ve düşük yan etki profilleri ile en yaygın kullanılan kontraseptif yöntemlerdir. Levonorgestrelli acil kontraseptif haplar, ovumun tubal transportunun gecikmesini de içeren çeşitli mekanizmalarla gebelikten koruyucu etki gösterirler. Ovumun tubal transportunun gecikmesi aynı zamanda tubal ektopik gebelikler için de bir risk faktörüdür. Levonorgestrelli acil kontraseptif hap kullanımı sonrası ektopik gebelik riski ile ilgili sınırlı bilgiler mevcuttur. Bu yazıda acil kontrasepsiyon amacıyla levonorgestrelli hap kullanımı sonrası oluşan tubal ektopik gebelik olgusu sunulmuş ve konu ilgili literatür bilgileri eşliğinde tartışılmıştır. (J Turkish-German Gynecol Assoc 2010; 11: 168-9)

Anahtar kelimeler: Acil kontrasepsiyon, ekstrauterin gebelik, levonorgestrel

Geliş Tarihi: 17 Kasım 2009 **Kabul Tarihi:** 03 Ocak 2010

Introduction

Either elective or criminal termination of unwanted pregnancies is still a major health problem in developing countries. Emergency contraception (EC) has been practiced in the last 3 decades in order to prevent unwanted pregnancies and to protect women against unsafe abortions (1, 2). Levonorgestrel (LNG) is a synthetic derivative of progestogen. Use of 750 μg levonorgestrel containing emergency contraceptive pills (ECP) in 2 doses at 12-hour intervals as EC is a safe and effective measure for preventing an unwanted pregnancy. After long-term and widespread use, its efficacy and safety have been confirmed in two large series in the multiple clinical trial by World Health Organization (WHO) (1, 2). Possible mechanisms of action of LNG containing ECP are inhibition of ovulation, decreased tubal motility, and certain changes in the endometrial secretory pattern (3). Tubal mucosal damage secondary to either pelvic infections or previous surgery and tubal motility changes due to the use of hormonal contraceptive methods are the known causes of tubal ectopic pregnancy (4). Beyond the theoretical relationship between the LNG-ECP and ectopic pregnancy, several case reports of extrauterine pregnancies have been published after using LNG as EC (5). Here, we present a patient with ectopic pregnancy after using LNG for EC.

Case Report

The patient was a 24-year-old unmarried female, gravida 1, parity 0, and a normal menstrual period of 28 days. After a single unprotected intercourse in her follicular phase (day 11), she had taken 750 μg levonorgesterol (Norlevo™) 16 hours and 28 hours later. Three weeks later, on the 15th of May, 2009, she was first seen in our hospital. Her complaints were lower abdominal pain and light vaginal bleeding. The patient's level of serum β-human chorionic gonadotropin (β-hCG) was 2980 mIU/ml. Transvaginal ultrasound showed a complex structure of 3x3x4.3 cm in diameter in her left tube, with no evidence of intrauterine pregnancy. A diagnosis of ectopic pregnancy was made. the patient was given the necessary information and written informed consent of the patient was obtained. Since the patient had a history of chronic duodenal peptic ulcer, we excluded methotrexate as a treatment option for our patient. At laparoscopy, there was an unruptured ectopic pregnancy in the ampullar region of the left Fallopian tube, and laparoscopic left salpingostomy was performed. In addi-

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doi:10.5152/jtgga.2010.29

tion, endometrial sampling was performed. Histology of the endometrial sample confirmed arias-stella reaction. Tubal ectopic pregnancy was proven by the presence of chorionic villi in the left tubal tissue.

Discussion

Unwanted pregnancy can cause many social problems. In countries where safe abortion is not available, termination of pregnancy may lead to serious medical complications and maternal mortality. EC methods are safe and effective for preventing unwanted pregnancies. Yuzpe regimen and only LNG containing pills are the most commonly used contraceptive methods for EC worldwide. In the latter, 750 μg LNG should be taken in 2 doses at 12-hour intervals within 72 hours after unprotected intercourse. However, it is now recommended to take 1,5 mg in one dose instead of two 750 μg doses (6). The use of levonorgestrel for EC was first reported by Ho and Kwan, as an alternative method to the Yuzpe regimen with its fewer side effects such as nausea and vomiting (7).

In the multicenter study of the World Health Organization (WHO), LNG-ECP was found to be more successful than the Yuzpe regimen (failure risks 1.1% and 3.2%, respectively) (2).

In their clinical study in 2003, Trussell et al reported a pregnancy rate of 5.2% after use of LNG-ECP (8).

The exact mechanism of action of LNG as a postcoital contraceptive is unknown. It is thought to work mainly by interruption of follicular development, inhibition of ovulation due to suppression of gonadotropins in the preovulatory period; and prevention of fertilization via decreased tubal motility and inhibition of implantation in the post-ovulatory period (3). Altering the capacity of sperms and making cervical mucus of a consistency to prevent sperm motility into the cervical canal are also common effects of LNG-ECP (1-3). The most effective period of LNG for EC has been reported to be in the preovulatory follicular phase (3, 5). Use of LNG-ECP after the ovulatory period and an increased interval between the unprotected intercourse and beginning of treatment have been described as main causes of failure (5). The patient presented here had used the treatment in the follicular phase as suggested previously as the most efficient period of the method. It is well known that a high level of progesterone causes a decrease in tubal motility and ciliary functions, when the risk of tubal ectopic pregnancy increased (3-5). Impaired tubal peristalsis may contribute to a delayed arrival of the egg in the endometrial cavity and implantation of the fertilized ovum in the tubes. There are few but increasing data regarding the possibility of an extrauterine pregnancy, in case of failure of LNG-ECP. Pereira et al reported two cases of ectopic pregnancies after the use of levonorgestrel close to the ovulatory period, and warned that attention should be paid to the risk of tubal pregnancy in case of failure of EC (5). Farkas et al reported a much higher risk (6.4%) of ectopic pregnancy in users of levonorgestrel (9). Similarly, Sheffer-Mimouni et al reported three cases of ectopic pregnancies after levonorgestrel use close to the ovulatory period (10).

Although the data on the relationship of ectopic pregnancy and LNG-ECP are limited, patients who have used LNG after unprotected intercourse and have a positive pregnancy test should be monitored closely in order to rule out a possible ectopic pregnancy.

Conflict of interest

No conflict of interest is declared by authors.

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Reply to comment on the article "The characteristics of sexual behaviour and extent of condom usage among sexually active Croatians from Eastern Croatia" carried out by Miskulin et al. and published in the J Turkish – German Gynecol Assoc 2009; 10: 142-7

J Turkish-German Gynecol Assoc 2009; 10: 142-7 de yayınlanmış olan "The characteristics of sexual behaviour and extent of condom usage among sexually active Croatians from Eastern Croatia" adlı makalenin yorumuna yanıt

Maja Miskulin

Department of Public Health, University of Josip Juraj Strossmayer, Osijek, Croatia (Hrvatska)

Dear Sir,

This is a reply to the Letter to the Editor published in The Journal of the Turkish German Gynecological Association, 2010(2):118. As authors we are very pleased that our work has received the interest of your readers and distinguished colleagues. As they have very well noticed we did not include the answers to question 12. "I have NEVER in my lifetime involved myself in sexual activities combined with drug or alcohol abuse without condom: a) yes b) no" in the text of our article. The reason for this is that the main goal of our research was to determine the extent of condom usage as a method of protection from sexually transmitted infections (STIs) in the studied population (sexually active Croatians from Eastern Croatia, mean age 31.31±8.42 years) during each and every possible situation, not only in situations such as risky circumstances connected with drug or alcohol abuse that were found to be related to inconsistent or non-use of condoms and increased difficulty to implement condom use (1). In an attempt to show our data in the most concise and clear way, we simply left out some findings from the result section and aimed to discuss these separately.

Considering the data regarding the hepatitis B virus vaccination we are very well aware that hepatitis B is classified as a sexually transmitted infection (2). That was the reason why we included this question in our questionnaire in the first place. However,after we analysed our data we saw that, as we expected, only 22/278 (7.9%) study subjects had received hepatitis B vaccination, 11/22 (50%) of them from the youngest 18-24 age group. Of 62 subjects reporting sexual relations with two or more partners during the one-year period, five (8.1%) had received hepatitis B vaccination. Bearing in mind the allowed length of the manuscript and our attempt to make our investigation and our results as clear as possible, we omitted these results from the text. Due to the interest of that aspect of our work shown by your readers this is maybe the appropriate place to comment on results regarding the hepatitis B vaccination and the reasons for our lack of surprise at such a low prevalence of vaccinated persons in our sample. Actually, the hepatitis B vaccination was not mandatory for the entire population in Croatia until 1999 when this vaccination became part of the Croatian Vaccination Programme, and all children in the sixth grade of elementary school have been vaccinated. Since 2007, vaccination against hepatitis B became mandatory vaccination for all new –borns in our country also (3, 4). Taking all these into account ,it is clear that in future we will have more vaccinated persons in the general population and we will be able to investigate the sexual behaviour and preferences of hepatitis B immune or non immune people more accurately.

It is true that we did not define what should be considered risky sexual behaviour in our manuscript because we addressed educated readers and we thought that considering the allowed length of the manuscript it was not necessary to give this definition (which we certainly had in mind when writing the article) within the text of the manuscript. We are however very grateful to our distinguished colleagues who made such an effort and emphasized the main determinants

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doi:10.5152/jtgga.2010.30

of risky sexual behaviour (5) in order to make our article clearer and easily understood by the wide audience of your journal

Finally, we wish to express our sincere gratitude to the distinguished colleagues who have shown an interest in our study, considering our data to be valuable for them and all of your readers.

We look forward to further successful collaboration with your Editorial Office and active interaction with your readers.

Sincerely yours,

Conflict of interest

No conflict of interest is declared by authors.

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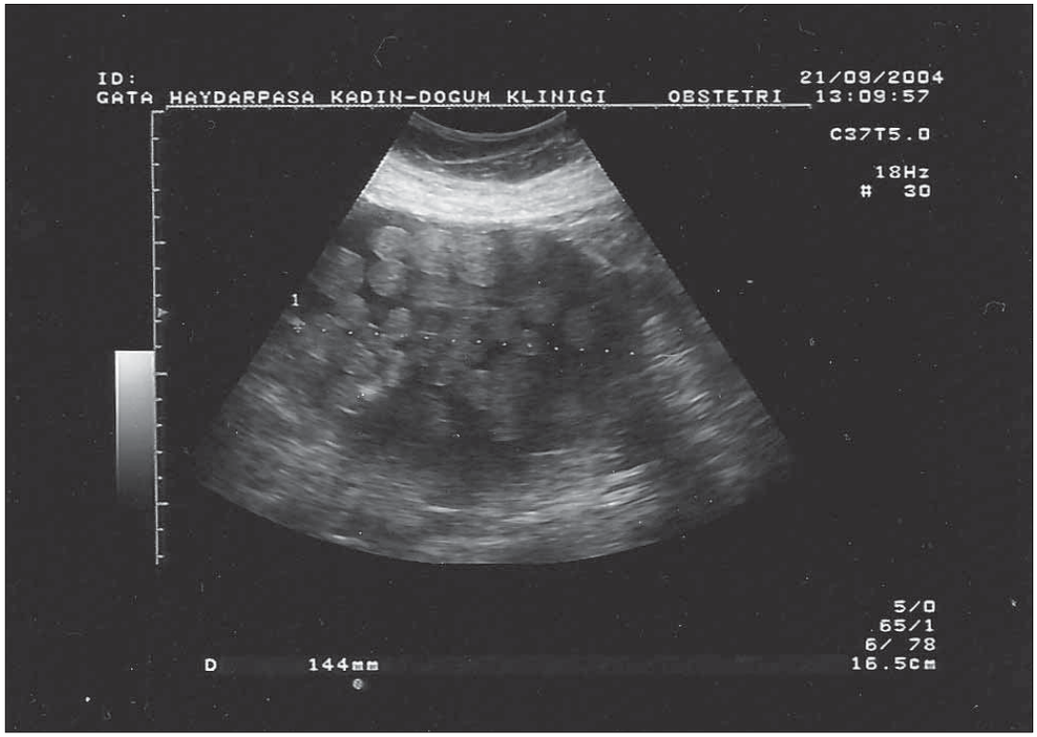
Quiz Case: A Right Adnexal Mass in a Postmenopausal Patient



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doi:10.5152/jtgga.2010.31

Answer

Introduction

Dermoid cysts are true hamartomas occurring during 15-40 years of age and are usually asymptomatic. 10-15% are bilateral tumors. Their prevalence is not well-known. They are covered by a thick dermislike wall that consists of tissues ectoderm, endoderm, and/or mesoderm (1). Treatment for dermoid cyst is complete surgical removal and is usually easy to excise. The prognosis of the disease is usually very good, but malignant transformation may be seen as a rare complication. The aim of this study is to report a rare case of dermoid cyst of differential complex appearance.

Case

A 52 year old postmenopausal woman was referred to our unit to investigate and treat a right pelvic mass. The patient had no complaints. On transvaginal ultrasonographic a 6x5 cm complex mass in the right ovary was seen. There were abundant and small circular solid parts within the pelvic mass (Figure 1). The remainder t of the abdominal contents were normal., There were no remarkable features in her medical and surgical histories. In addition to the routine laboratory findings, tumor markers such as CA125 and cervical cytology were performed in the preoperative period and these were normal. Total abdominal hysterectomy and bilateral salpingo-oophorectomy under general anesthesia were performed in order to remove the cysts. Pathologic diagnosis showed the presence of benign dermoid cysts.

Discussion

The most frequent germ cell tumor derived from the ovaries is the dermoid cyst and is mostly benign. Malignancy potential is more common in patients over 40 years of age. Dermoid cysts are bilateral in 12-13% of cases and they are rarely symptomatic (1).

In the diagnosis of dermoid cyst ultrasound, magnetic resonance imaging (MRI) and computed tomography (CT), respectively can be used. Ultrasonography for diagnosis of ovarian malignancy has a sensitivity of 62 to 100% and a specificity of 77 to 95%. They have a characteristic ultrasound appearance (2). Although in the diagnosis of our case we used transvaginal ultrasound, we were not exactly sure of the diagnosis, because there were abundant and small circular solid parts within a 6x5 cm complex mass. If a complex cyst or solid mass is present on ultrasound, the patient should undergo definitive diagnosis with fine needle aspiration biopsy (FNAB), core needle biopsy, or excisional biopsy. In our case total abdominal hysterectomy and bilateral salpingoophorectomy under general anesthesia was performed.

In conclusion, dermoid cysts may be present with different ultrasonic appearances.

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CONGRESS CALENDAR

12-16 September 2010	**19th SLS Annual Meeting and Endo Expo 2010 Sheraton New York Hotel and Towers,** *New York, USA* http://www.laparoscopy.blogs.com
12-16 September 2010	**20th World Congress on Fertility & Sterility (IFFS)** *München, Germany* http://www.iffs2010.com
25-29 September 2010	**12. Jinekolojik Onkoloji Kongresi** **Gloria Hotels & Convention Center,** *Antalya, Turkey* http://www.jineonko2010.org
26-29 September 2010	**7. Ulusal Maternal Fetal Tıp ve Perinatoloji Kongresi (TMFTP)** **Hilton Convention Center,** *Istanbul, Turkey* http://tmftp.org
29 September-2 October 2010	**19th Annual Meeting of the European Society for Gynaecological Endoscopy (ESGE),** *Barcelona, Spain* http://esge.org
6-9 October 2010	**17th Annual meeting of the Middle East Fertility Society (MEFS)** **Omayyad Palace,** *Damascus, Syria* http://www.mefs.org
7-10 October 2010	**TSRM (4th) Sungate Port Royal Resort,** *Kemer, Antalya, Turkey* http://www.2010.tsrm.org.tr
10-13 October 2010	**5th World Congress of the World Association of Reproductive Medicine (WARM),** *Moscow, Russia* http://warm2010.ru
11-14 October 2010	**Hands on Course on In Vitro Maturation Kocaeli University,** *IVF Center* dreraycaliskan@yahoo.com
11-14 October 2010	**20th World Congress on Ultrasound in Obstetrics and Gynecology (ISUOG),** *Prague, Czech Republic* http://www.isuog.org/WorldCongress/2010
23-27 October 2010	**ASRM (66th),** *Denver, Colorado, USA* http://www.asrm.org
4-7 November 2010	**The 13th World Congress on Controversies in Obstetrics, Gynecology & Infertility (COGI) Maritim Hotel,** *Berlin, Germany* http://www.comtecmed.com/cogi/berlin
8-12 November 2010	**39th Congress On Minimally Invasive Gynecology (AAGL)** *Las Vegas, Nevada, USA* http://www.aagl.org/annual-meeting
2-5 December 2010	**International Consensus Meeting on Embryo Transfer** *Lavasa, India* http://www.sisab.net/et2010
9-10 December 2010	**Intensive Laparoscopic Suturing, Knot Tying, and Laparoscopic and Robotic Hysterectomy The Rosevelt Hotel,** *New York, NY, USA* www.chpnet.org/cme

CONGRESS CALENDAR

10-13 February 2011	**SLS AsianAmerican Multispecialty Summit Laparoscopy and Minimally Invasive Surgery Hilton Hawaiian Village® Beach Resort & Spa, Honolulu,** *Hawaii, USA* http://www.laparoscopy.blogs.com/asianamerican_summit
24-26 March 2011	**World Symposium on Endometriosis InterContinental Hotel,** *Atlanta, GA, USA* http://www.endometriosisatlanta.com
2-6 March 2011	**10. Uludağ Jinekoloji ve Obstetri Kış Kongresi** *Uludağ, Bursa, Turkey* http://www.uludagkadindogum.org
6-10 April 2011	**5th International Congress On Minimally Invasive Gynecology (AAGL & JED) Swıssotel The Bosphorus,** *Istanbul* www.tsge2011.org
7-9 April 2011	**Excellence in Female Surgery (NESA Days)** *Florence, Italy* http://www.nesaflorence2011.org
13-16 April 2011	**13. Ulusal Perinatoloji Kongresi&43rd International Meeting of Gestosis Organisation,** *İstanbul, Turkey* http://www.perinatoloji2011.org/
23-25 April 2011	**7. Ulusal Üreme Sağlığı ve Aile Planlaması Kongresi** *Ankara, Turkey* www.uremesagligi2011.org
4-8 May 2011	**TAJEV (9th) Susesi De Luxe Resort ve Cornelia Diamond Resort,** *Antalya, Turkey* http://www.tajev2011.org
25-29 May 2011	**19th International Pelvic Pain Society Annual Scientific Meeting** *Istanbul, Turkey* http://www.ipps2011.org
3-6 July 2011	**27th Annual Meeting of ESHRE Stockholm,** *Sweden* http://www.eshre.eu/home
1-4 July 2012	**28th Annual Meeting of ESHRE** *Istanbul, Turkey* http://www.eshre.eu/home