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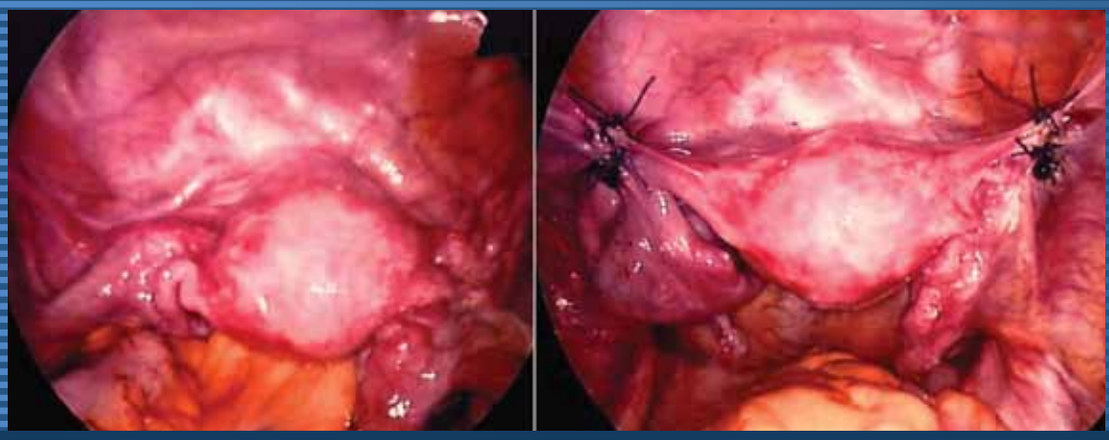

Volume 12
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September
2011

Original Investigations

Cumulus oocyte complex and IVF failure
Thomas Ebner, et al.; Linz, Austria

Thyroid dysfunction and hyperemesis gravidarum
Nermin Akdemir et al.; Ankara, Zonguldak, Turkey

Drospirenone pill in polycystic ovary syndrome
Sudhindra Mohan Bhattacharya et al.; Kolkata, India

Effectiveness of two Sperm-wash techniques
Surveen Ghumman et al.; Manipal, India

Iron, folate and vitamin B12 levels
Aysun Karabulut et al.; Denizli, Turkey

Leptin and endometrium
Ali Özler et al.; Diyarbakır, Manisa Turkey

Autologous cumulus body improve pregnancy rates
Tahsin Murad Aktan et al., Konya, Turkey

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Journal of the Turkish-German Gynecological Association Vol.: 12 Issue: 3 September 2011 Page: 135 - 198

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Cover Picture:
Laparoscopic Hysteropexy. After segmental removal an reapproximation of the round ligament, the correction of the retroversion is clearly seen (from the Arcieve of Assist. Prof. Gazi YILDIRIM).

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Aims and Scope

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

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

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

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Journal of the
Turkish-German
Gynecological Association

Contents

Original Investigations

135 Assisting in vitro fertilization by manipulating cumulus-oocyte-complexes either mechanically or enzymatically does not prevent IVF failure
Thomas Ebner, Marianne Moser, Omar Shebl, Richard Mayer, Gernot Tews, Linz, Austria

140 Thyroid dysfunction in hyperemesis gravidarum: a study in Turkish pregnant women
Nermin Akdemir, Cemil Bilir, Ankara, Zonguldak, Turkey

144 Effects of drospirenone pill in Indian women with polycystic ovary syndrome
Sudhindra Mohan Bhattacharya, Mainak Ghosh, Nupur Nandi, Kolkata, India

148 Combination of swim-up and density gradient separation methods effectively eliminate DNA damaged sperm
Surveen Ghumman, Satish Kumar Adiga, Dinesh Upadhya, Guruprasad Kalthur, Varshini Jayaraman, Satish Bola Rao, Pratap Kumar, Manipal, India

153 Iron, folate and vitamin B12 levels in first trimester pregnancies in the Southwest region of Turkey
Aysun Karabulut, Osman Şevket, Ayhan Acun, Denizli, Turkey

157 Leptin expression in proliferative, secretory and hyperplastic endometrial tissues
Ali Özler, Naci Kemal Kuşçu, Peyker Temiz, Ali Rıza Kandiloğlu, Faik Mümtaz Koyuncu, Diyarbakır, Manisa,Turkey

162 Improvement in Embryo Quality and Pregnancy Rates by Using Autologous Cumulus Body during ICSI Cycles
Tahsin Murad Aktan, Hüseyin Görkemli, Kazım Gezginç, Aslı Saylan, Selçuk Duman, Fatma Yazıcı Yılmaz, Konya, Turkey

Reviews

168 Diagnosis and treatment of deep-vein thrombosis and approach to venous thromboembolism in obstetrics and gynecology
K. Mehmet Burgazlı, Mehmet Bilgin, Ethem Kavukçu, M. Metin Altay, H. Turhan Özkan, Uğur Coşkun, Hakan Akdere, A. Kubilay Ertan, Giessen, Giessen, Wuppertal, Leverkusen, Germany, Istanbul, Turkey

176 Periodontal diseases as an emerging potential risk factor for adverse pregnancy outcomes: A review of concepts
Jyoti Bansal, Abhishek Bansal, Navneet Kukreja, Urvashi Kukreja, Mullana, India

Case Reports

181 Metastatic ovarian malignant melanoma with no obvious primary
Ateş Karateke, Niyazi Tuğ, Davut Şahin, İstanbul, Turkey

183 A prenatal tertiary trisomy resulting from balanced maternal 8; 9 translocation
Gülsüm Kayhan, Mehmet Ali Ergün, Aydan Asyalı Biri, Meral Yirmibeş Karaoğuz, Ankara, Turkey

186 Prenatal diagnosis of caudal regression syndrome without maternal diabetes mellitus
Ahmet Özgür Yeniel, Ahmet Mete Ergenoğlu, Sermet Sağol, İzmir, Turkey

189 Unicornuate uteri associated with contralateral renal agenesis and ovarian anomalies
Albana Cerekja, Kathleen Comalli Dillon, Eva Racanska, Juan Piazze, Roma, Frosinone, Italy, California, Nashville-Tennessee, USA

Medical History and Ethics

192 A sexually transmitted disease: History of AIDS through philately
Emine Elif Vatanoğlu, Ahmet Doğan Ataman, Istanbul, Turkey, Vienna, Austria

Quiz

197 What is your diagnosis?
Gazi Yıldırım, İstanbul, Turkey

Journal of the
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Gynecological Association

Editorial

Dear Colleagues,

I am proud to present you the third issue of twelfth volume of JTGGA with many interesting articles. Our journal is playing an important role in our field for twelve years, indexed by many internationally accepted databases as SIIC, Tübitak/Ulakbim Turkish Medical Index, EBSCO host, SCOPUS, Excerpta Medica (EMBASE) and DOAJ database, ProQuest, CINAHL and Index Copernicus. The submitted manuscripts are evaluated by at least two experts in the related field and the submission process are quite fast and easy for the authors and with the JournalAgent online submission and editorial system.

Some months after the successful IX. Turkish - German Gynecology Congress, with a great interest of the gynecology and obstetrics society in a total of 1450 participants from 20 different countries, we are still getting the positive feedbacks of the participants about the organization, social activities, hotel selection and the high level of scientific program. Regarding the intensive demand on getting the presentations of the congress, the PDF format presentations of the speakers who accepted to share their studies are published at our TAJEV web site. (**www.tajev.org**)

Another good news about our foundation is we are planning our fourth social responsibility project in the early new year. We are working hard to determine the best available conditions regarding the social utility and going to announce the details later on our web site and the journal. After three successful projects in South Eastern Anatolia and Eastern Anatolia regions, we would like to make our fourth project in another region, that is my only clue for our readers right now.

In one of the following pages of our journal, you will find the announcement of the ORReady project. For the readers who are wondering what is ORReady, I would like to explain this life saver project shortly. ORReady is a project to encourage hospitals and clinics around the world to do the same and improve the outcomes for all patients. It is a worldwide, multi-Specialty initiative to encourage steps that are known to improve surgical outcomes and save lives. Working together, Medical Societies and Other Organizations around the World are sharing ideas that work to help improve Outcomes for Surgical Patients. By using, analyzing and continuing to improve guidelines and procedures, including Check Lists, Time Outs and Warm Ups, it is estimated that Six Million patients around the world could have better outcomes. You can find further details at the **http://www.sls.org/outcome** web site.

I wish you all success in your studies and look forward to meeting you in the final issue of the year.

Best regards,

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

Assisting in vitro fertilization by manipulating cumulus-oocyte-complexes either mechanically or enzymatically does not prevent IVF failure

Kümülus-oosit-komplekslerinin mekanik veya enzimatik olarak manipulasyonuyla invitro fertilizasyona yardımcı olmak IVF başarısızlığını önlemez

Thomas Ebner, Marianne Moser, Omar Shebl, Richard Mayer, Gernot Tews

Landes- Frauen- Und Kinderklinik, Kinderwunsch Zentrum, Linz, Austria

Abstract

Objective: This prospective study was set up in order to analyze whether additional treatment (cutting off supernumerous cumulus cells; adding hyaluronidase) of the cumulus-oocyte-complex (COC) would help to improve treatment outcome.

Material and Methods: COCs from 50 patients were prospectively subdivided into a control group A (no manipulation of COC) and two study groups. In group B, surplus cumulus cells were cut off using syringes, and in the second study group COCs were incubated with a 1:11 dilution of hyaluronidase (final concentration 7 IU/l). Main outcome measures were fertilization rate, embryo development, as well as rates of implantation, pregnancy, and live birth.

Results: Fertilization was higher in group C as compared to the untreated control group A ($p<0.05$). However, complete fertilization failure could not be avoided by any of the modified IVF approaches. Compaction on day 4 and blastocyst quality on day 5 were significantly improved in group C as compared to group B (but not to group A). Rates of implantation, pregnancy, and live birth were not affected by any of the methods.

Conclusion: ICSI seems to be the only choice for avoiding the vast majority of fertilization failures after IVF.

(J Turkish-German Gynecol Assoc 2011; 12: 135-9)

Key words: Blastocyst, cumulus-oocyte complex, fertilization, hyaluronidase, IVF

Received: 17 March, 2011 **Accepted:** 10 May, 2011

Özet

Amaç: Bu prospektif çalışma, kümülus-oosit-kompleksine (KOK) ilave işlemin (çok sayıdaki kümülus hücrelerinin kesilip ayrılması; hiyaluronidaz eklenmesi) tedavi sonuçlarını iyileştirmede yardımcı olup olmayacağını analiz etmek için yapıldı.

Gereç ve Yöntemler: Elli hastadan elde edilen KOK'lar prospektif olarak bir kontrol grubuna (Grup A; KOK manipülasyonu yok) ve iki çalışma grubuna bölündü. Grup B'de, artık kümülus hücreleri şırıngalar kullanılarak kesilip ayrılmıştır ve ikinci çalışma grubunda KOK'lar hiyaluronidazın 1:11'lik dilüsyonu (son konsantrasyon 7 IU/l) ile inkübe edildi. Başlıca sonuç ölçümleri fertilizasyon oranı, embriyo gelişmesi yanı sıra implantasyon, gebelik ve canlı doğum oranlarıydı.

Bulgular: İşlem yapılmamış kontrol grubu A'ya kıyasla fertilizasyon, grup C'de daha yüksekti ($p<0.05$). Bununla beraber, tam fertilizasyon başarısızlığı modifiye edilmiş herhangi bir IVF yaklaşımı tarafından önlenemedi. 4. günde kompaktlaşma ve 5. günde blastokist kalitesi grup C'de grup B'ye kıyasla (fakat grup A'ya değil) anlamlı olarak iyileşmişti. İmplantasyon, gebelik ve canlı doğum oranları bu yöntemlerin herhangi birinden etkilenmedi.

Sonuçlar: IVF sonrası fertilizasyon başarısızlıklarının büyük çoğunluğundan sakınmak için tek seçenek ICSI gibi görünmektedir.

(J Turkish-German Gynecol Assoc 2011; 12: 135-9)

Anahtar kelimeler: Blastokist, kümülus-oosit kompleksi, fertilizasyon, hiyaluronidaz, IVF

Geliş Tarihi: 17 Mart 2011 **Kabul Tarihi:** 10 Mayıs 2011

Introduction

The choice of whether to use conventional in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) to guarantee fertilization is mostly based on the semen quality of the male partner. ICSI was found to be highly efficient in cases of severe male infertility which would otherwise be untreatable. The success of ICSI, however, led to extensive use (1, 2) of this rather invasive method culminating in routine ICSI (3). Avoidance of complete fertilization failure may be the most important reason for this overuse.

The risk of fertilization failure seems to be marginal if IVF is done in normozoospermic patients (4, 5). However, in patients with teratozoospermia (6) or asthenozoospermia (7) these reduced sperm parameters will affect the fertilization rate. Provided that there is a sufficient number of motile spermatozoa, higher insemination concentrations may yield adequate results in terms of fertilization (8-10). However, many patients principally fulfilling the WHO (11) criteria for normozoospermia show borderline sperm parameters. Although conventional IVF may be the method of choice, embryologists tend to split the recruited oocytes into IVF and

Address for Correspondence: *Assoc. Prof. Dr. Thomas Ebner, Landes- Frauen- und Kinderklinik, Kinderwunsch Zentrum, Krankenhausstr. 26-30 A-4020 Linz, Austria Phone: 0043 732 6923 24600 e.mail: Thomas.ebner@gespag.at*

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ICSI ones, thus rescuing approximately 10% of the cycles in which standard IVF did not work (12). This value is even higher (33%) with mild male factor infertility (13).
The question is, whether use of a rather invasive method, such as ICSI, is really indicated, in cases in which IVF (following in vivo fertilization) would yield the same results. This prospective study was set up in order to analyze whether additional treatment (cutting off supernumerous cumulus cells; adding hyaluronidase) of the cumulus-oocyte complex (COC) would help to increase fertilization rate, embryo development, and rates of implantation and pregnancy in normozoospermic patients as well as borderline patients.

Material and Methods

According to our IRB, informed consent was obtained from all persons involved. Within a period of 6 months, a total of 50 patients (32.9±4.1 years) fulfilled our inclusion criteria, namely being under the age of 40 years, not suffering from endometriosis or PCO, and having at least 9 COCs. Patients showed either a tubal factor (n=28), unexplained infertility (n=9) or a combination of more than one factor (n=12).
In preparation for follicular puncture, all patients treated in the Kinderwunsch Zentrum Linz, Austria, had their controlled ovarian hyperstimulation done using either a long protocol or an antagonist protocol. In the long protocol, down-regulation of the pituitary was achieved with the GnRH agonist buserelin (Suprecur®, Sanofi-Aventis, Frankfurt am Main, Germany). Stimulation was initiated with human menopausal gonadotrophin (Menopur®, Ferring, Kiel, Germany) or recombinant FSH (Puregon®, Aesca Pharma, Vienna, Austria). In the GnRH-antagonist protocol, recombinant FSH (Puregon®, Aesca Pharma, Vienna, Austria) was started on day 3 of the cycle. In addition, a GnRH-antagonist (Orgalutran®; Organon, Vienna, Austria) was administered after 5-6 days of stimulation, depending on the presence of a 12-13 mm follicle. In all patients ovulation was induced with 10,000 IU human chorionic gonadotrophin (hCG, Pregnyl®, Aesca Pharma, Vienna, Austria). Oocyte retrieval was carried out transvaginally under ultrasound guidance 36 hours after hCG administration. Cumulus-oocyte complexes (COC) were collected in BM1 medium (Eurobio, Les Ulis, France) and incubated for two to three hours until IVF.
Meanwhile, the ejaculate of patients was incubated in a sperm selecting chamber (Zech-selector, AssTIC Medizintechnik GmbH, Leutsch, Austria,) which accumulates an adequate number of motile sperm without exposure to centrifugation stress (14). The Zech device consists of two concentric wells overlaid by a U-ring and a cover glass. In detail, progressive motile spermatozoa migrate from the ejaculate in the outer well to concentrate in the medium-filled inner well by using a capillary bridge created by the overlying U-ring. After approximately 1-2 hours a 500μl sperm sample was taken from the central chamber, analyzed and used for insemination.
COCs were split into a control group A (no manipulation) and two study groups. In the study groups, COCs were either mechanically manipulated by cutting off their supernumerous cumulus cells with syringes (group B) or enzymatically treated (group C) by diluting the culture medium at a rate of 11:1 with 80 IU of hyaluronidase (Origio, Copenhagen, Denmark). In detail, 50μl of enzyme were pipetted into 500μl of sperm solution.
Treated and untreated COCs as well as corresponding embryos were inseminated in groups in 4-well-dishes (approximately 25-50,000 sperms per egg). From the zygote stage on, concepti were cultured in small drops (50μl) of either sequential medium (EmbryoAssist® and BlastAssist®, MediCult, Copenhagen, Denmark) or global medium (GM501 Cult®; Gynemed, Lensahn, Germany).
Fertilization was checked 18-21 hours post insemination. Two aligned pronuclei were considered to be a regular fertilization. If both pronuclei were not aligned, of uneven size and/or situated in the periphery, these types were separated since they were thought to have impaired development (15, 16). At the cleavage stage, the number and size of blastomeres were taken into account as well as the degree of fragmentation and multinucleation. On day 4, embryos were screened for signs of compaction. On day 5, survival to blastocyst stage and blastocyst quality were evaluated taking both cell lineages into account. It is noteworthy that the quality of inner cell mass and trophectoderm could only be analyzed from the full blastocyst stage onwards (17).
With adequate consideration of all prognostic markers (day 1 to day 5) a maximum of 2 embryos (n=16) or blastocysts (n=30) were chosen for intrauterine transfer. Therefore, all patients were placed in the lithotomy position during embryo replacement and neither sedation nor anaesthesia was used. Embryos were loaded into a Gynetics catheter (Gynemed, Lensahn, Germany) using <10µl of BlastAssist medium 2 or GM501 Cult and then expelled approximately 1 cm from the fundus.
A total of 9,000 IU hCG (Pregnyl®, Organon, Vienna, Austria) were injected on the day of ovum pick-up (3,000 IU), the day of transfer (3,000 IU) and day 3 (1,500 IU) and 6 (1,500 IU) post transfer to support the luteal phase. No hCG was administered in case of OHSS. In addition, progesterone was given from the day after follicular puncture until the day of the pregnancy test. This was given either in the form of house-made vaginal suppositories (400mg) or as vaginal tablets (Utrogestan®, Meda Pharma, Vienna, Austria, 300mg).
Seventeen days after intrauterine transfer, the blood concentration of hCG was measured. Clinical pregnancy was determined by visualization of at least one gestational sac with positive heart activity 4 weeks after embryo transfer. Subclinical pregnancy showed no fetal heartbeat.
The chi-square test was used to analyze nominal variables in the form of frequency tables. Ordinal variables were analyzed with the Mann-Whitney U test. Statistical significance was accepted if p-value was <0.05.

Results

A total of 592 COCs were collected in 50 patients. Unfortunately, no fertilization occurred in four patients (8%) regardless of the IVF method used. In the remaining 46 patients, 70.6% (398/564) of the oocytes showed two pronuclei on day 1 of

culture. Thirty-five zygotes were tripronuclear (6.2%), whereas only 2 zygotes showed one pronucleus (0.4%). Eighteen eggs (3.2%) were still immature (GV stage) at the time of fertilization check.

On day 2 of development, 338 embryos out of 398 zygotes (85.0%) showed regular cleavage, i.e. without multinucleation. The number and quality of embryos allowed blastocyst culture in only 30 patients (60%). In this special cohort, the overall blastulation rate was 150/280 (53.6%). The percentage of good quality blastocysts was approximately 79% (119/150).

In those patients finally having an embryo or blastocyst transfer (n=46), 24 pregnancies could be achieved (52.2%). Since 2 biochemical pregnancies occurred, clinical pregnancy and life birth rate dropped to 47.8%. In terms of multiple pregnancy six twins (25%) were observed, but in four cases a vanishing twin occurred. The corresponding implantation rate was 32.6% (30/92).

Table 1 indicates that, in group A, significantly more ($p<0.05$) GV stage ovae were found at the time of fertilization check than in the two study groups ($p<0.01$). Fertilization was higher in group C as compared to the untreated control group A ($p<0.05$). In addition, significantly fewer ($p<0.001$) 3 PN zygotes were found in the enzymatically treated group C. Embryo quality on days 2 and 3 (data not shown) were comparable between all three groups. Blastocyst formation was significantly higher ($p<0.05$) in group C as compared to group B (but not to group A). The same also holds for blastocyst quality ($p<0.05$).

Although low sample numbers and mixed transfers do not support statistical analysis, results shown in Table 2 indicate that embryos derived from enzymatically treated COCs indeed have a higher implantation potential. In detail, 16/24 (66.7%) patients having at least one embryo transferred from group C achieved pregnancy, which is higher than for groups B (50%) and A (45.5%).

Discussion

Decisions concerning the treatment option for assisted reproduction (IVF or ICSI) are usually taken after evaluation of male fertility factors, or after taking into account the results of previous IVF attempts. There are no widely accepted criteria, so decisions for couples with male subfertility are often empirical and may lead to complete fertilization failure after IVF, or to the unnecessary use of ICSI.

In principle, it is advisable to use the least invasive method of insemination, namely IVF, whenever possible. This decision is of course a difficult balancing act. One problem encountered is that no exact cut-off value for sperm parameters is available indicating to an embryologist whether to use IVF or ICSI. Naturally, sperm number and motility after processing of the ejaculate will facilitate the decision of which method to choose. However, in terms of morphology, the information from the literature is not conclusive. A report by Zollner et al. (18)

Table 2. Pregnancy outcome in control and study groups after double embryo or blastocyst transfer

n transfer	COC not manipulated	COC treated mechanically	COC treated enzymatically	PR
6	2	0	0	2 (33.3)
10	1	1	0	4 (40.0)
6	1	0	1	4 (66.7)
6	0	2	0	2 (33.3)
8	0	1	1	6 (75.0)
10	0	0	2	6 (60.0)

Values in parentheses are percentages. COC: Cumulus-oocyte complex; PR: Pregnancy rate

Table 1. Comparison of fertilization rates and embryonic development in control and study groups

	COC not manipulated	COC treated mechanically	COC treated enzymatically	p-value
n	208	184	172	
PI	0[a,b]	10 (5.4)[a]	8 (4.7)b	<0.01
2PN	144/208 (69.2)[c]	124/174 (71.3)	130/164 (79.3)[c]	<0.05
0PN	44 (21.2)	34 (18.5)	28 (16.3)	
1PN	0	2 (1.1)	0	
3PN	20 (9.6)[d]	14 (7.6)[e]	0d,e	<0.001
Cell number day 2	3.6±1.0	3.8±1.1	3.6±1.4	
Fragmentation day 2 (%)	20.7±17.1	23.8±18.1	18.9±17.5	
n blastocyst culture	102	86	92	
n compacting day 4	48 (47.1)	31 (36.1)[f]	49 (53.3)[f]	<0.05
n blastocyst	54 (52.9)	42 (48.8)	54 (58.7)	
n good quality blastocyst	42 (77.8)	29 (69.0)[g]	48 (88.9)[g]	<0.05

Values in parentheses are percentages. COC: Cumulus-oocyte complex; PN: Pronucleus/pronuclei.
[a-g]percentages marked by identical superscripts are significantly different from each other. The corresponding p-value can be found in the right column

found 8% normal spermatozoa to be a threshold significantly discriminating between fertilization (66.7% vs. 35.7%). Lundin et al. (19) suggested even lower thresholds (5%) because they neither saw an impaired fertilization rate nor an increase in abortion rate. However, since none of the patients in our prospective study showed a morphology of less than 10%, teratozoospermia was no reason for cases of failed fertilization. Unfortunately, facilitating access of the sperms to the egg by additional manipulation (groups B and C) could not prevent complete fertilization failure. Most likely, this happened due to immunological rejection.

Oocyte maturity and quality at the time of retrieval are difficult to assess as the egg is obscured by a large cumulus mass impairing adequate scoring. From the early years of IVF, it became evident that assessment of egg maturation in stimulated cycles is rather imprecise (20-23). The reported failure of adequate prognosis has more recently been confirmed by Ebner et al. (24) who noted a discrepancy between the actual appearance of the COC and the nuclear maturity of the corresponding egg. Interestingly, some 26% of presumably immature oocytes turned out to be at metaphase II, whereas approximately 11% of expected metaphase-II eggs did not show a first polar body. Since the present analysis carefully tried to equally distribute COCs according to morphology, i.e. the presence of blood clots (24) and presumed maturity, the observed difference in prophase I oocytes on the day of fertilization check must have a biological reason. It is very likely that ovae from untreated COCs (group A) have a higher potential to mature in vitro since more cumulus cells stay attached to the zona pellucida, which is not the case if surplus cumulus cells are cut off or digested (25).

This is the first report to show that prolonged incubation (overnight) with commercially available hyaluronidase has no negative effect on further outcome. Most commercially available hyaluronidases have a concentration of 80 IU/l which is only a tenth of the critical threshold above which parthenogenetic activation may occur (26). For reducing the theoretical risk of harming the oocyte, an approximately. 10-fold dilution was applied in group C (approximately 7 IU/l). On the other hand, by doing so, no benefit was observed in terms of further preimplantation development as compared to untreated COCs. The highest fertilization rate in group C primarily suggests that enzymatic digestion facilitates access of motile spermatozoa to the zona pellucida.

Nevertheless, cleavage results were significantly better than in the mechanically treated group B. This indicates that cutting off surplus cumulus cells will harm the corresponding gametes by either altering the shape of the gamete (27) or generating shearing forces via transzonal processes.

To summarize, it can be stated that, although none of the modified IVF approaches showed a clear relation to rates of implantation, pregnancy, and live birth, mechanical manipulation of the COC is associated with certain drawbacks and should be avoided. This is all the more true as it was shown that oocyte morphology cannot be rescued by cutting off suspicious tissue or blood clots from the COC (24). A tendency towards a higher pregnancy rate in group C would require a more detailed study in a larger number of patients.

Most importantly, it has to be emphasized that none of the assisted IVF technologies will rescue a cycle of failed IVF. Thus, in cases of borderline sperm quality, ICSI seems to be the only choice in order to avoid complete fertilization failure.

Conflict of interest

No conflict of interest was declared by the authors.

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Thyroid dysfunction in hyperemesis gravidarum: a study in Turkish pregnant women

Hiperemezis gravidarumda tiroid disfonksiyonu: Türk gebelerde yapılan bir çalışma

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Abstract

Objective: In this study we investigate the possible relation of thyroid dysfunction and thyroid antibodies to hyperemesis gravidarum.

Material and Methods: Thirty-seven patients with hyperemesis gravidarum and 33 healthy controls have been included in this study.

Results: Thyroid dysfunction was significantly more common than in controls (38% vs 6%, p=0.002). Thyroglobulin antibodies were also significantly more common in patients with hyperemesis gravidarum than controls (54 IU/mL vs. 14 IU/mL, p=0.03).

Conclusion: Hyperemesis gravidarum can be a risk factor for postpartum thyroid dysfunction. Thyroid antibodies must be checked in the hyperemesis population in an endemic goitre region and/or iodine replacement regions. (J Turkish-German Gynecol Assoc 2011; 12: 140-3)

Key words: Hyperemesis gravidarum, thyroid function

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Özet

Amaç: Bu çalışmadaki amacımız tiroid fonksiyon bozuklukları ve tiroid antikorları ile hiperemezis gravidarum arasındaki olası ilişkiyi araştırmaktı.

Gereç ve Yöntemler: Hiperemezis gravidarumu olan 37 hasta ve 33 sağlıklı kontrol gurubu çalışmada incelendi.

Bulgular: Tiroid disfonksiyon sıklığı hiperemezis gravidarumda kontrol grubuna göre anlamlı olarak daha fazla bulundu (%38'e %6, p=0.002). Anti-tiroglobulin düzeyleride anlamlı olarak kontrol grubundan daha yüksekti (54 IU/mL'e. 14 IU/mL, p=0.03).

Sonuçlar: Hiperemezis gravidarum postpartum tiroid fonksiyon bozuklukları için bir risk faktörü olabilir. Tiroid antikor düzeyleri de özellikle endemik guatr ve/veya iyot replasmanı yapılan bölgedeki gebelerde de mutlaka bakılmalıdır. (J Turkish-German Gynecol Assoc 2011; 12: 140-3)

Anahtar kelimeler: Hiperemezis gravidarum, tiroid fonksiyonu

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Introduction

Hyperemesis gravidarum was defined as persistent vomiting accompanied by weight loss of at least 5% of pre- pregnancy body weight and/or ketonuria unrelated to other causes and requiring hospital admission for severe vomiting causing dehydration (1, 2). The pathogenesis of hyperemesis is unknown. Usually it is difficult to differentiate from the nausea and vomiting such as morning sickness from the HG, which affects 80% of pregnancies (3). Some hormonal changes determined in HG; elevated serum concentrations of estrogen and progesterone levels implicated in the pathogenesis of this disorder, serum human chorionic gonadotropin (hCG) concentration is higher in women with HG women also supports a possible etiologic role for this hormone. In addition, hCG has a thyroid-stimulating activity (4-6). Higher hCG levels in women with HG and a positive correlation between hCG levels and the severity of vomiting and degree of thyroid stimulation was established previously (7). Hyperthyroidism is possibly due to high serum concentrations of hCG which has a thyroid-stimulating activity (5). One study showed that low serum TSH concentrations are seen more often in women with HG than in normal pregnant women; TSH was suppressed in 60 percent of hyperemesis patients versus 9 percent of controls (8). In the literature there are insufficient studies which have investigated the thyroid antibodies in HG. In this study we investigate the possible relation of thyroid antibodies to hyperemesis gravidarum.

Method

Patients

Thirty-seven patients with hyperemesis gravidarum and 33 healthy controls have been evaluated in this study. We have chosen the control group as a gestational age matched control. The study protocol was approved by the Ankara Kecioren Education and Research Hospital. Written, informed consent was obtained from all patients which also adhered to the principles of the Helsinki Declaration. The study cohort has been selected from a gynecological outpatient population of a Research Hospital between June 2009 to May 2010.

Clinical Assessment

Hyperemesis gravidarum, defined as persistent vomiting accompanied by weight loss of at least 5% of pre- pregnancy

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body weight and/or ketonuria unrelated to other causes and requiring hospital admission for severe vomiting causing dehydration (2). The control group did not have any symptoms or only mild nausea without vomiting. Patients or controls with molar pregnancy, multiparity, coronary heart disease, diabetes, hypertension or any chronic disease using medication were not included in the study. Fasting (8 hours) blood samples were taken in the morning from each patient and were centrifuged for 5 min at 3.000 g and stored at -80°C until the time of analysis. Blood glucose, serum creatinine, blood urea nitrogen, aspartate and alanine aminotransferases, sodium, potassium and calcium electrolytes were measured using an automatic analyzer (Konelab 60i, Thermo Scientific, Finland). Serum thyroid stimulating hormone and free thyroid hormones were analyzed by UniCel DxI 800 Access Immunoassay System (Beckman Coulter, USA). Complete blood counts were measured using a Coulter LH 500 hematology analyzer (Beckman Coulter, USA).
Thyroglobulin antibody (Anti-Tg) and thyroperoxidase antibody (TPO-Ab) Liason analyzer systems manufacturered by Diasosin, STILLWATER, MN 55082, USA were used.
Ultrasound exams were performed by the same radiologist blinded to the clinical characteristics of the patients.
Patients admitted to the hospital were intravenously hydrated followed by reintroduction of oral intake. Dehydration was supplemented with appropriate electrolytes, vitamins and metoclopramide (5 to 10 mg) as needed. Normal values of hormones were; TSH (0.3-4.2 mIU/L), fT3 (2.2-4.2pg/mL), fT4 (0.65-1.7 ng/mL), Thyroglobulin antibody/antiTG (5-100 IU/mL) and thyroperoxidase antibody/TPO-Ab (1.16 IU/mL). Upper limits of the reference values were accepted as positive.
Thyroid dysfunctions were defined as (5, 8);

- Lower TSH values with or without abnormal thyroid antibodies and/or free thyroid hormone levels.
- Higher TSH values with or without abnormal thyroid antibodies and/or free thyroid hormone levels.

Statistical analysis
Shapiro-Wilk test was used to identify the data distribution. Normally distributed data were presented as mean (SD) for baseline and descriptive statistics, and median and interquartile range for non-normally distributed data. Data with normal distribution were analyzed using unpaired t test. Mann-Whitney U test was used for analyzing abnormally distributed data. Positive and negative values were compared by chi-square test. All *P* values were calculated as two-tailed. $p < 0.05$ was set as statistically significant. SPSS 15.0 was used for statistical calculations (SPSS Inc., Chicago, IL, USA).

Results

Baseline clinical and laboratory characteristics of the patients and controls are presented in Table 1. Groups were similar in age, weight, gestational week, hemoglobin, creatinine and glucose levels. No patients or controls had any other systemic disease or any medication. The hyponatremia frequency in HG group was 24% which was significantly higher than the 3% of hyponatremia in the control group ($p=0.005$). AST levels were significantly elevated in the HG group compared to the controls (18±7.5 u/L versus 17±3 u/L, p:0.01). ALT levels were also significantly higher in the HG group than the controls (18±14 u/L versus 14±4 u/L, $p=0.04$). Also the HG group had lower levels of TSH, p values were 0.03. HG patients had significantly higher

Table 1. Baseline clinical and laboratory characteristics of 37 patients with hyperemesis gravidarum, dysmenorrhea and 33 healthy pregnant controls

	Patients (n=37)	Controls (n=33)	p
Age, years	25.4(±5.2)	25.3(±4.5)	0.36
Gestation, week	9.7(±2.2)	10(±2.2)	0.73
Weight, kg	63(±11)	62(±9)	0.34
Creatine	0.65(±0.1)	0.61(±0.1)	0.74
Hgb, g/dL	12(±0.9)	11.7(±1)	0.33
Plt, x 10 3μL	234(±67)	226(±48)	0.056
Glucose, mg/dL	85(±8)	86(±8)	0.75
BUN, mg/100 ml	20(±6.3)	16(±3.3)	0.005
AST, u/L	18(±7.5)	17(±3)	0.01
ALT, u/L	18(±14)	14(±4)	0.04
Sodium, mEq/L	135(±2.7)	136(±1.7)	0.005
Potassium, mEq/L	3.9(±0.3)	3.7(±0.2)	0.9
TSH, mIU/l	0.95(±0.56)	1.4(±1.15)	0.03
fT3, pg/mL	2.86(±0.46)	2.6(±0.3)	0.07
fT4, ng/mL AntiTg, IU/mL, IR	1.07(±0.3)	0.89(±0.17)	0.02

Table 2. Thyroid Dysfunctions in HG and control group

	Decreased TSH (<0.3mıu/L)	Positive AntiTG (>100IU/mL)	Positive TPO-Ab (>16IU/mL)	p value (Chi-Square)
Hyperemesis (n:37)	3(8%)	9(24%)	2(5.5%)	
Control (n:33)	0	0	2(6%)	
Total (n:70)	3	9	4	0.002

levels of freeT4, but all values were within the normal reference range of our laboratory. AntiTG titers were significantly higher than controls in the HG group (54 IU/mL vs. 14 IU/mL, p=0.03) but TPO-Ab were not. In the HG group 9 (24%), patients had positive antiTG but thecontrol group did not (p=0.007). There was no significant difference between the two groups of TPO-Ab (p=0.9). Lower TSH with normal free thyroid hormone level was determined in 3 patients in the HG group. Neither hyperthyroid nor hypothyroid pregnant women had clinical symptoms so they were not given any antithyroid medication. When we evaluate the all thyroid dysfunctions (*only lower TSH with normal freeT3/T4, positive TPO-Ab with negative anti-Tg and positive anti-Tg with negative TPO-Ab)* 14 (38%) patients in the HG group and 2 (6%) in the control group (p=0.002) are also presented in Table 2. In patients who had thyroid dysfunction, thyroid stimulating antibodies were negative. There is no correlation between the anti TG, TPO-Ab, TSH, freeT3 and free T4. All the study population had normal thyroid on physical examination.

Discussion

Results from this study showed for the first time that thyroglobulin antibody concentrations are significantly higher in the HG group compared to healthy pregnant controls. Also hyperemetic pregnant women had significantly higher (38% vs. 6%, p: 0.002) thyroid dysfunctions than controls.

Thyroid functions change in pregnancy, especially within the first trimester, in general because of estrogen-induced increases in serum thyroxine-binding globulin (TBG) levels and human chorionic gonadotropin (hCG) induced increases in thyroid hormone synthesis and release (9). Most prospective studies which compared TSH and T4 levels of HG patients with the controls showed significantly lower levels of TSH and significantly higher levels of T4 titers. Also there is a relationship between hyperthyroidism and severity of HG but the exact role is not yet known (10, 11). In our study, we found 8% subclinical hyperthyroidism but there was no correlation between the TSH and HG severity. These rates are lower than previous studies carried out in other populations. Also we found hyponatremia and elevated ALT/AST significantly different, but at lower rates than the previous studies. However, this is one of several studies carried out on the Turkish pregnant population.

The prevalence of anti-thyroid antibodies (ATA) has been reported as 15-20% in normal pregnant women, and anti TPO antibodies were found to have a significant association with recurrent miscarriage. Therefore, the prognostic value of ATA remains uncertain (12, 13). Our control group had only 6% ATA but the HG group had 30% ATA. Pearce et al found that 12.4% elevated TPO-Ab in pregnant which was higher than our study population for TPO-Ab (9). The presence of measurable maternal thyroid antibodies can be a risk factor for postpartum thyroiditis, miscarriage and premature birth. In fact, findings from a recent study suggest that treatment of TPO-Ab–positive euthyroid women with levothyroxine results in improved obstetric outcomes (9, 14-17). Propylthiouracil can provide relief of symptoms of HG. In our population hyperemesis gravidarum can be a risk factor for postpartum thyroid hyperemesis, so it should be suggested that the thyroid antibodies should be checked in the HG population in an endemic goiter region and/or iodine replacement regions like Turkey.

In this study we did not follow up the pregnant patients after delivery so we could not report the pregnancy outcomes of HG patients.

In conclusion, this is the first study of Turkish HG women in whom a significantly elevated percentage of thyroid dysfunction, especially thyroglobulin antibodies, compared to controls was found.

Conflict of interest

No conflict of interest was declared by the authors.

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Effects of drospirenone pill in Indian women with polycystic ovary syndrome

Polikistik over sendromlu Hintli kadınlarda drospirenon hapının etkileri

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Abstract

Objective: To study the effects of treatment with a drospirenone pill (DRSP) (with ethinyl oestradiol, EE) in Indian women with polycystic ovary syndrome (PCOS).

Material and Methods: Fifty-one women with PCOS (Androgen excess society criteria, 2006), with preset inclusion-exclusion criteria, treated with a combination of EE 30 mcg and DRSP 3 mg cyclically in the traditional (21+7) regimen, were evaluated at baseline and after six and twelve cycles of treatment. Parameters studied were - body mass index (BMI), abdominal circumference (AC), Ferriman Galwey (FG) score, presence of acne and acanthosis nigricans, serum testosterone, sex hormone binding globulin (SHBG), fasting glucose and fasting insulin levels. Free Androgen Index (FAI) and Glucose: Insulin ratio (G: I) were calculated.

Results: Significant improvements in clinical and biochemical hyperandrogenic parameters were found at the two points of study. There were no significant changes in BMI, AC, incidence of acanthosis, or metabolic parameters studied.

Conclusion: EE/DRSP improves hyperandrogenic parameters significantly without affecting the insulin resistance adversely in Indian women with PCOS. (J Turkish-German Gynecol Assoc 2011; 12: 144-7)

Key words: Drospirenone pill, polycystic ovary syndrome, androgenic parameters, insulin resistance, Indian women

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Özet

Amaç: Polikistik over sendromu (PKOS) olan Hintli kadınlarda drospirenon (DRSP) hapı ile (etinil estradiol, EE ile birlikte) tedavinin etkilerini çalışmak.

Gereç ve Yöntemler: Önceden belirlenmiş dahil etme-dışlama kriterlerine göre seçilen, geleneksel (21+7) rejimde EE 30 mcg ve DRSP 3 mg kombinasyonu ile döngüsel olarak tedavi edilen PKOS'lu (Androjen fazlalığı topluluğu kriterleri, 2006) 51 kadın başlangıçta ve tedavinin 6. ve 12. döngüsünde değerlendirildi. Çalışılan parametreler; vücut kitle indeksi (VKİ), karın çevresi (KÇ), Ferriman Galwey (FG) skoru, akne ve akantozis nigrikans varlığı, serum testosteron, seks hormonu bağlayan globulin (SHBG), açlık glukozu ve açlık insülin düzeyleri idi. Serbest Androjen İndeksi (SAI) ve Glukoz: İnsülin oranı (G:I) hesaplandı.

Bulgular: Çalışmanın iki noktasında klinik ve biyokimyasal hiperandrojenik parametrelerde önemli iyileşmeler bulundu. VKİ, KÇ, akantozis insidansı veya çalışılan metabolik parametrelerde anlamlı bir değişiklik yoktu.

Sonuçlar: EE/DRSP PKOS'lu Hintli kadınlarda insülin direncini olumsuz olarak etkilemeksizin hiperandrojenik parametreleri anlamlı olarak iyileştirmektedir.

(J Turkish-German Gynecol Assoc 2011; 12: 144-7)

Anahtar kelimeler: Drospirenon hapı, polikistik over sendromu, androjenik parametreler, insülin direnci, Hintli kadınlar

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Introduction

Polycystic ovary syndrome (PCOS) is one of the most common gynaecological endocrinological disorders. Ethnic background of women with PCOS may affect the clinical, hormonal and metabolic characteristics of this condition. Response to treatment of PCOS in Asia may be different from those in Western countries (1). Indians are an ethnic group at particularly high risk for central obesity, type 2 diabetes mellitus and dyslipidaemia, all resulting from a state of insulin resistance (IR). Interaction of environmental factors (obesity) with the genetic factors finally results in the characteristic metabolic and menstrual disturbances and the final expression of the PCOS phenotype (2).

For a long time, combined oral contraceptives containing ethinyl oestradiol (EE) and a progestogen, have been used to treat these women. This combination can suppress the pituitary-ovary axis, increase sex hormone binding globulin (SHBG) level and can cause lowering of "free" androgen level (3).

The new progestogen drospirenone (DRSP) derived from 17-alpha-spironolactone is found to have a pharmacological profile similar to that of natural progesterone with clinically relevant antimineralocorticoid activities and antiandrogenic

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activities and has been claimed to have the potential to reduce body weight and blood pressure (BP) (4). Many studies have shown that oral contraceptives can cause deterioration of IR (5, 6).

This study was performed to evaluate the effects of EE/DRSP on various hyperandrogenic (clinical and biochemical) and metabolic parameters in Indian women with PCOS.

Materials and Methods

This was a prospective, open label, single arm study. The Ethics committee of S.C. Das Memorial medical and research center approved the study protocol and subject consent was obtained at study initiation.

Fifty-one unmarried women (age ranges 15-32 years) presenting with the complaints of oligomenorrhoea (≤ six menses per year), with clinical evidence of hyperandrogenism (hirsutism and /or acne) were studied at the gynaecology clinic of the first author. PCOS was diagnosed as per the Androgen Excess Society 2006 criteria (7). Detailed clinical and hormonal tests were done, as per the said criteria. Secondary causes of hyperandrogenism such as 21-hydroxylase deficiency, Cushing's syndrome, hypothyroidism, hyperprolactinaemia, and androgen-secreting tumors were excluded by appropriate clinical and / or laboratory tests. All patients underwent ultrasonographic examination of the lower abdomen to note the status of their ovaries. In India, transvaginal ultrasonography cannot be performed in unmarried girls. All women had an ovarian volume of more than 10 cc.

Exclusion criteria included adolescent girls with a gynecologic age (age since menarche) less than three years; those who had used oral pills in the preceding three months, those with known diabetes or hypertension or having treatment known to affect glucose metabolism such as corticosteroids.

During clinical examination, the height, weight, abdominal circumference (AC), blood pressure (BP) were recorded. The procedures followed have been reported previously (8). Body mass index (BMI) (as kg / m^2) was calculated in each case from height and weight measurements.

BP was measured in each case by Korotkoff sound (stage V was used to determine the diastolic pressure). Hirsutism scoring was done as per the modified Ferriman- Galwey score (mFG score) (9). Patients using cosmetic measures were requested not to depilate for at least one month before evaluation. To avoid interobserver error, the first author (SMB) himself graded the degree of hirsutism. The mFG score of ≥ 6 was considered as hirsutism. Presence of acne and acanthosis nigricans (AN) was noted in each case and reported as "Yes/No. The number of cases having acne and AN were expressed at each time point as % of patients with acne and AN present.

The following biochemical tests were carried out on the second/third day of a progestogen-induced bleeding -

Serum total testosterone (TT); sex hormone binding globulin (SHBG); fasting insulin and plasma glucose levels (after 8-10 hours fasting).

"Free Androgen Index" (FAI) was calculated as per the following formula

$$FAI = \frac{\text{Testosterone (nmol/l)}}{\text{SHBG (nmol/l)}} \times 100.$$

Serum testosterone was measured by Electrochemiluminescence Immunoassay, Roche Lot. No. 181371-01. Insulin was measured by Elecsys 2010, Roche Lot No. 179-202-01. SHBG was measured by ELISA technique (EIA-2996) [DRG instruments Gmbh, Germany]. Plasma glucose was measured by Glucose oxidase method. All tests were done at Ashok laboratory, Jodhpur Park, Kolkata, India. As it was an open label study, the laboratory test values were available at each point of study. After initial clinical and laboratory evaluation, each patient was advised to take a combination of EE (30 mcg) / DRSP (3 mg) (Yasmin^R, Bayer Scherring Pharma, Berlin, Germany) 1 tablet daily from the first day of her menstruation for 21 days then a 7 day gap and again for 21 days and so on cyclically. Each patient was advised to continue the tablet for 6 cycles, then to repeat the tests as done at the beginning and clinically evaluated at the first follow-up visit at the first author's clinic. The final review was performed with another set of the same tests and clinical assessments after 12 months of treatment at the second follow-up visit.

Statistical analysis

Sample size calculation: The primary outcome to be measured was a drop in the mean serum testosterone level of 0.15 ng / ml. The study assumed a standard deviation of 0.25 at baseline and 0.22 at study end, based on previous samples. Assuming a correlation coefficient of zero, for 5% level of Significance and 80% power, for a one-sided test, the study required 42 patients.

Analysis: A per-protocol analysis was done at study end using Graphpad Instat version 5.0. Repeated measures ANOVA with Tukey's post-test was used for parametric variables, Friedman ANOVA with Dunn's post-test for non-parametric variables. Dichotomous parameters were analyzed with Chi-square test.

Results

At study initiation, the participants were aged 22.2±5.4 years (mean±SD). Table 1 shows the mean±SD values of BMI in kg/M^2 (minimum 21 to maximum 35), AC (cm), presence of acne and AN (in %), systolic blood pressure (SBP), diastolic blood pressure (DBP), fasting sugar (mg%), fasting insulin (mcu /ml) and G: I ratio of the subjects at the three points of study, namely at baseline (0 month), 6 month and 12 months. It shows that there are no significant changes in the values of BMI, AC, presence of AN, SBP, DBP between 0 months and 6 months and 6 months and 12 months. However, the percentage of patients having acne was reduced significantly from baseline to 12 months ($p<0.05$).

Table 2 shows the results for FG score, serum testosterone, serum SHBG and the free androgen index (FAI). It is found that with EE/DRSP, there is significant improvement in the FG score ($p<0.005$). The testosterone level reduced significantly after 6 months of treatment and this reduction was maintained at 12 months ($p<0.001$). Serum SHBG showed a very signifi-

Table 1. Values of body mass index, abdominal circumference, systolic and diastolic blood pressure, percentage positive for acanthosis nigricans and acne, fasting blood sugar, fasting insulin and glucose-insulin ratio ratio of the subjects at various time points [Values are Mean (SD) or (%) as the case my be]

Time points	BMI (kg/M²)	AC (cm)	A.N present n (%)	Systolic BP (mm Hg)	Diastolic BP (mm Hg)	Acne Present n (%)	FBS (mg %)	Fasting insulin (mcu / ml)	G:I ratio
0 month (n=51)	26.2 (4.3)	78.3 (9.5)	32 (62.7)	122.7 (11.7)	77.8 (9.9)	28 (54.9)	89.3 (7.3)	17.3 (10.9)	8.0 (6.6)
6 month (n=51)	26.1 (4.0)	77.5 (8.8)	31 (60.8)	126.0 (8.0)	81.0 (10.5)	16 (31.4)*	87.5 (6.4)	19.6 (9.6)	5.9 (3.8)
12 month (n=51)	26.1 (4.0)	77.5 (8.6)	31 (60.8)	125.3 (8.0)	80.3 (9.8)	15 (29.4)*	87.6 (7.1)	20.1 (10.6)	5.9 (3.9)

*significant compared to corresponding value at 0 month ($p<0.05$); G: I ratio- fasting glucose- insulin ration, n=Number, BMI: Body Mass İndex, AC: Abdominal circumference, BP: Systolic and diastolic blood pressure, FBS: Fasting blood Sugar, G:I: Glucose-Insulin ratio, SD: Standard deviation

Table 2. Values of Ferriman-Gallwey score, serum testosterone, sex hormone binding globulin levels and free androgen index of the study subjects at various time-points [all expressed as mean (SD)]

Time Points	F: G score	Testosterone (ng / ml)	SHBG (nmol / l)	FAI
0 month (n=51)	6.7 (5.2)	0.51 (0.3)	31.4 (15.9)	6.4 (4.2)
6 month (n=51)	4.8 (3.1)	0.30 (0.16)**	155.1 (67.0)**	0.94 (1.2)**
12 month (n=51)	4.6 (2.9)*	0.28 (0.16)**	157.6 (68.7)**	0.81 (0.89)**

*significant comparing with corresponding value at 0 months ($p<0.05$), **very significant comparing with corresponding value at 0 months ($p<0.001$), F: G: Ferriman-Gallwey score, SHBG: Sex hormone binding globulin, FAI: Free androgen index, SD: Standard deviation

cant rise ($p<0.001$) after 6 months of treatment with EE/DRSP and remained significantly high at 12 months. The FAI similarly showed a very significant fall at 6 months ($p<0.001$) and remained suppressed at 12 months.

Discussion

In this study the authors report their clinical experience on the use of EE/DRSP in Indian women with PCOS. Yu Ng et al (1) had stressed that it is important to take into consideration the ethnic background of patients in future studies related to PCOS.

Table 2 shows that the trend in improvement in various hyperandrogenic manifestations are also maintained even after 6 months of treatment. Darney (10), Cerel-Suhl et al. (11) reported that EE/DRSP combination can help to obviate the common adverse effects found with the use of pills containing nortestosterone derivatives.

DRSP possesses a strong antigonadotropic activity per se. Muhn et al. (12, 13) in animal studies has shown that DRSP can cause significant reduction of plasma LH levels. This LH lowering effect of DRSP may be one of the mechanisms by which the hyperandrogenic manifestations are reduced. Our study has shown a significant reduction of testosterone level by 6 months of treatment and also after 12 months of treatment. Krattenmacher (14) has shown that EE/DRSP combination can inhibit the steroidogenic enzymatic activities at the ovarian level Our study has also shown significant improvement in cosmetically unacceptable signs of hyperandrogenism namely, acne, hirsutism. Hirsutism is in part ethnically determined, being more common in women with dark skin (15). Zarger et al. (16) in a study from Kashmir, India found that 10.1% had mild hirsutism (F: G score 6-9) and 0.4% had moderate hirsutism (F: G score 10-14). DRSP has been reported to have antiandrogenic effects even in the peripheral level also, by repression of androgen receptor-mediated transcription. DRSP can competitively bind to androgen receptors, because of its intrinsic molecular structure (17).

Low SHBG level in PCOS may be an intrinsic feature of the syndrome and this cannot always be explained by obesity alone because low SHBG has been reported in lean PCOS women also. Our study shows that there is a significant rise in SHBG level by 6 months of treatment and this trend is maintained even after 12 months of treatment. Consequently, FAI level shows the same trend. This stimulatory effect is due to the EE content of the medication. This shows that DRSP does not antagonize this stimulatory effect of EE on SHBG level in contrast to the progestogen derived from 19-nortestosterone. This action can also explain the significant amelioration obtained in the various manifestations of hyperandrogenism (14).

Our study did not find any change in BMI, AC, and BP (both SBP and DBP). EE activates the Rennin-Angiotensin-Aldosterone system, leading to fluid and electrolyte retention. This in turn can raise the body weight and BP. DRSP on the other hand, because of its unique antimineralocorticoid activity, induces sodium excretion and a compensatory rise in rennin secretion, plasma rennin activity, angiotensin II, and plasma aldosterone (18).

Our study found that EE/DRSP does not alter serum glucose or insulin level even after 12 months of treatment. Consequently, there is no change in fasting glucose: insulin ratio or, in other

words, there is no deterioration of insulin sensitivity. Guido et al (19), Gaspard et al. (20) stated that EE/DRSP might be considered neutral with respect to insulin resistance. Pehlivanov et al. (21) reported that in Europe, the most widely used combined oral pill in women with PCOS is a combination of EE (35 mcg) and 2 mg cyproterone acetate. However, the present study shows that the EE/DRSP can be a reasonable alternative.

Lack of a control group is a major limitation of the study. A larger study will help to establish whether this combination has any effect on insulin resistance and compare the same with similar agents. The originality of the study is that it has been carried out among the Indian population. Considering the unique Indian population structure with strictly defined endogamous and genetically homogeneous populations, the result of this present study is an enriching experience for any clinician, particularly in the Indian subcontinent interested in the treatment of the polycystic ovary syndrome. Further, this study shows that the response to treatment is the same as that in Western countries.

Conflict of interest

No conflict of interest was declared by the authors.

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Combination of swim-up and density gradient separation methods effectively eliminate DNA damaged sperm

Yüzdürme ve dansite gradient ayırma metotlarının kombinasyonu DNA hasarlı spermleri etkili bir şekilde uzaklaştırır

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Abstract

Objective: The aim of this experimental prospective study was to investigate the efficacy of single and combination sperm wash methods for their ability to isolate DNA intact spermatozoa.

Material and Methods: Sperm DNA damage was introduced by local testicular irradiation in male mice and the extent of damage was quantified by comet assay. The spermatozoa were subjected to single (swim up or density gradient method) and also a combination of sperm wash techniques. The DNA integrity in various sub-fractions of wash techniques was evaluated.

Results: The amount of DNA damaged sperm did not differ between individual fractions when single wash technique was applied. However, a combination of density gradient and swim-up techniques significantly reduced ($p<0.01$) the number of DNA damaged sperm in the final population.

Conclusion: The combination of density gradient separation and swim-up method is effective in eliminating DNA damaged spermatozoa. (J Turkish-German Gynecol Assoc 2011; 12: 148-52)

Key words: Sperm wash, DNA damage, swim- up, density gradient separation, comet assay

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Özet

Amaç: Bu deneysel prospektif çalışmanın amacı tek ve kombinasyon sperm yıkama metotlarının, DNA'sı sağlam spermatozoa izolasyon etkinliklerini araştırmaktı.

Gereç ve Yöntemler: Sperm DNA hasarı erkek farelerde lokal testiküler ışınlama ile oluşturuldu ve hasarın büyüklüğü comet assay ile ölçüldü. Spermatozoa'ya tek (yüzdürme veya dansite gradient metodu) ve ayrıca kombinasyon sperm yıkama teknikleri uygulandı. Yıkama tekniklerinin çeşitli alt fraksiyonlarında DNA'nın bütünlüğü değerlendirildi.

Bulgular: Tek yıkama tekniği uygulandığında DNA hasarlı sperm miktarı fraksiyonlar arasında farklılık göstermedi. Bununla beraber, dansite gradienti ve yüzdürme tekniklerinin kombinasyonu son popülasyondaki DNA hasarlı sperm sayısını anlamlı olarak ($p<0.01$) azalttı.

Sonuç: Dansite gradient ayırma ve yüzdürme mettotlarının kombinasyonu DNA hasarlı spermatozoa'nın uzaklaştırılmasında etkilidir. (J Turkish-German Gynecol Assoc 2011; 12: 148-52)

Anahtar kelimeler: Sperm yıkama, DNA hasarı, yüzdürme, dansite gradient ayırma, comet assay

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Introduction

Sperm chromatin is a highly organized, compact structure consisting of DNA and heterogeneous nucleoproteins. There has been increased concern regarding the role of sperm DNA integrity in male infertility (1, 2). Sperm DNA integrity is essential for accurate transmission of genetic material to the offspring (3). It has been shown that paternal DNA damage can lead to pre-implantation developmental delay and compromised post-implantation developmental potential in mice (4-6). Hence sperm preparation method for assisted reproduction techniques should aim at minimizing the potential risk caused by abnormal sperm on the outcome. The established sperm preparation techniques used in the routine assisted reproductive technique laboratory vary in their ability to separate sperm carrying DNA abnormalities (7, 8). The most common laboratory techniques used in the extraction of functionally normal spermatozoa are swim-up or sperm migration and density gradient centrifugation (9). Although density gradient centrifugation is comparable to swim-up technique in recovering spermatozoa with enhanced motility, there is still controversy about the effects of density gradient centrifugation on sperm DNA integrity (10, 11). Recently, it has been shown that semen processing by density gradient centrifugation is not generally useful in selecting sperm with higher double-strand DNA integrity (12), although others have shown that sperm DNA/chromatin integrity improves after preparation by density gradient centrifugation (13-15).

As there is still controversy and insufficient evidence concerning the ability of sperm preparation techniques for the elimination

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of DNA damaged sperm in the ejaculate, we chose to use mouse sperm to determine the efficacy of the techniques. DNA damage was introduced by local testicular irradiation in male mice and the extent of damage was evaluated before and after various sperm processing methods, including all the sub fractions of the methods used to determine the efficacy.

Methods

Animals

Eight to twelve week old healthy Swiss Albino male mice were used for the experiments. At least five animals were used for each data point. Sperm DNA damage was induced by testicular irradiation. The Institutional Animal Ethical Committee's approval was obtained before performing the experiment.

Testicular irradiation

Male mice were anaesthetized using Ketamine 50 mg/kg body weight. The whole body except the testicular area was covered using a lead shield and the animals were exposed to 3 Gy gamma radiation at a rate of 1 Gy/min from the Co^{60} teletherapy unit.

Sperm extraction

Seven days after irradiation, animals were sacrificed by cervical dislocation and spermatozoa were extracted from the cauda epididymis by squeezing the cauda epididymis in 1 ml of pre-warmed Earle's Balanced Salt Solution (EBSS, Cat No. E 2888, Sigma Chemical Co.). The sperm suspension was analyzed for DNA integrity by comet assay and the remainder of the sperm suspension was divided into three parts and used for various sperm preparation methods. The experiment was performed in duplicate.

DNA damage analysis by Comet assay

The DNA fragmentation in spermatozoa was assessed by the alkaline comet assay as described by Singh et al. (16) with minor modifications. Briefly, the spermatozoa were collected from the entire cauda epididymis in sterile phosphate buffered saline (pH 7.4) and centrifuged. Sperm density was kept constant by appropriate dilution in order to maintain the uniform distribution of the spermatozoa during electrophoresis. The sperm suspension was mixed with an equal volume of 0.8% low melting agarose (Cat No. A 9414, Sigma Chemical Co, USA) and layered on a slide pre-coated with 1% normal agarose (Cat No. 9539, Sigma Chemical Co, USA). A third coat of agarose was layered over the second layer followed by overnight incubation in lysing solution (2.5M NaCl, 100 mM disodium EDTA, 10 mM Trizma base, pH 10.1% Triton X-100, 10mM GSH, and 100 μM heparin) under alkaline conditions (pH 10) at 4°C. After sperm DNA unwinding in the electrophoresis buffer (300mM NaOH, 1mM EDTA, pH>13) for 20 minutes, electrophoresis was carried out at 25V (VcM= 0.74V/cm, 300 mA) for 20 min followed by neutralization of the slides in 0.4M Tris HCl buffer for 15 minutes. Then the slides were drained and immersed in chilled absolute alcohol for 30 minutes for dehydration and then stored in a dry area until staining.

The slides were stained with ethidium bromide (2μg/ml) and observed under a fluorescent microscope (Imager-A1, Zeiss, Germany) and images were captured under 40X objective. Each slide was coded to avoid observer bias and the images were captured by one person and analysis was carried out by another. A minimum of 50 images were obtained from each slide by scanning the different areas of the slide randomly avoiding the anode end and edges of the slides. The damaged sperms attain a shape of a comet, with the tail region consisting of fragmented DNA and head region having intact DNA. The comet evaluation (percent tail DNA and olive tail moment) of the captured images was performed using Kinetic Imaging software (Komet 5.5).

Sperm wash by swim-up

One portion of the sperm suspension was mixed with double the volume of pre-warmed EBSS medium supplemented with 0.1% bovine serum albumin (Cat No. A3311, Sigma Chemical Co. USA) and then centrifuged at 300 g for 10 min. The pellet was resuspended in the fresh EBSS medium and centrifuged again at 300 g for 10 min. The resulting pellet was overlaid with 0.3 ml EBSS medium and incubated at 37°C for one hour. The DNA integrity by comet assay was assessed in the supernatant and pellet fractions.

Sperm wash by density gradient method

Approximately 0.5 ml of sperm suspension was layered on a commercially available discontinuous two layer (40%-80%) gradient (PureCeption™, Cat. No.2040&2080, Sage) in a 14 ml tube. The tubes were centrifuged at 1000 g for 20 min at room temperature. Spermatozoa were collected from the different gradients *viz*. gradient pellet, gradient-80, gradient-40, and gradient supernatant (sample fraction), and were resuspended in EBSS medium and then assessed for DNA integrity.

Combination of density gradient and swim-up method

The final part of the sperm suspension was processed for the discontinuous density gradient as explained above. The resultant pellet was resuspended in 5 ml of EBSS medium and centrifuged at 300 g for 10 min. The washing step was repeated as above and the pellet was overlaid with 0.3 ml EBSS medium and incubated at 37°C for one hour. The DNA integrity by comet assay was assessed in the supernatant and pellet fractions.

Statistical analysis

The values were expressed as Mean$\pm$SEM (standard error of the mean). One way Analysis of Variance (ANOVA) was done to determine significance levels. A P value less than 0.05 was considered as statistically significant.

Results

A detailed investigations of sperm count, motility, and DNA integrity of pre and post wash fractions have demonstrated the efficacy of the individual methods employed. When we examined the ability of various wash techniques for the extraction of motile sperm in the improved fraction, it was

found that the mean percentage of motile sperm in both swim-up and density gradient was significantly higher than the combination of swim-up and density gradient method (Table 1). Similarly, the number of spermatozoa recovered is significantly lower in the improved fraction of the combined density gradient/swim-up technique.

We examined all the sperm wash fractions for the sperm DNA integrity by comet assay to determine which fraction effectively holds spermatozoa with aberrant DNA. The amount of tail DNA was not significantly different between the two fractions of swim-up technique (pellet and supernatant) and the unprocessed sperm (Fig. 1A). Similarly, the sperm collected from various sub-fractions after density gradient separation (gradient supernatant, gradient 80, gradient 40, and gradient pellet) did not differ significantly with respect to the tail DNA. The amount of tail DNA in the gradient pellet was almost the same as the unprocessed spermatozoa (9.92±0.53 Vs 10.29±0.52). When these pellets were further processed by swim-up method, the amount of tail DNA dropped significantly ($p<0.05$) in the swim-up supernatant (9.92±0.53 vs 7.19±0.43). Overall, the amount of tail DNA was significantly reduced ($p<0.01$) from the un-processed sperm (10.29±0.52) to the swim-up supernatant (7.19±0.43) (Fig. 2A).

We have also analyzed the Olive Tail Moment (product of the tail length and the fraction of total DNA in the tail) (17) in the above fractions. The OTM in swim-up fractions did not significantly differ from each other (Fig. 1B). However, the OTM observed in the gradient supernatant (12.38±0.68) was significantly higher ($p<0.001$) than in the unprocessed sperm (8.58±0.44) and gradient 80 fraction (7.54±0.46). Similarly the OTM of the sperm held at gradient 40 fraction (10.75±0.63) was also significantly higher ($p<0.01$) than the gradient 80 fraction. As observed in the tail DNA, the OTM was also significantly lower in the spermatozoa recovered from the swim-up fraction of combined density gradient/swim-up method when compared to gradient pellet and unprocessed sperm ($p<0.05$) (Fig. 2B).

Discussion

In this study, we have demonstrated that the combination of density gradient and swim-up method is effective in eliminating DNA damaged mouse spermatozoa. In contrast, when a single wash technique was applied, the amount of DNA damaged sperm in the final population did not differ significantly from the unprocessed population. When we tested the ability of the commonly used swim-up technique in the elimination of DNA damaged sperm, our observation did not show any significant difference in the level of DNA damaged sperm between the pellet and the swim-up fraction. This is in agreement with an earlier study where swim-up technique failed to isolate a population of sperm with a low percentage of nuclear anomalies (7).

It has been previously shown that the semen processing technique itself may either increase or not alter sperm chromatin stability (10, 11). Since the issue of iatrogenic sperm DNA damage is being debated, we made an attempt here to evaluate the nuclear integrity of sperm retained in all the sub-fractions of swim-up and density separation. Although progressive motility of spermatozoa is increased after density gradient preparation in

Table 1. Sperm count and motility in the improved fraction of various sperm wash methods

Method	Sperm count (Mean±SEM)	Sperm motility (Mean±SEM)
Swim up	2.84±1.81	25.6±9.6
Density gradient	9.52±7.66	26.4±7.76
Density gradient + Swim up	1.44±0.42	8.8±6.8

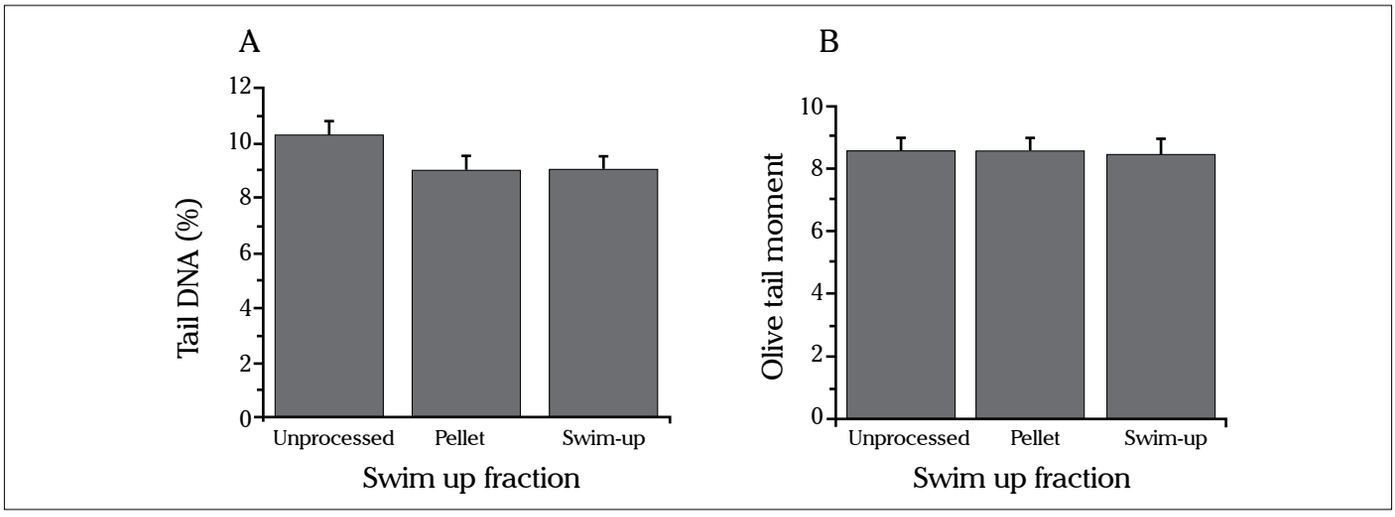

Figure 1. The incidence of sperm DNA damage in the unprocessed and various swim-up fractions A. The data representing percent tail DNA. B. Olive tail moment. P>0.05 between all the groups

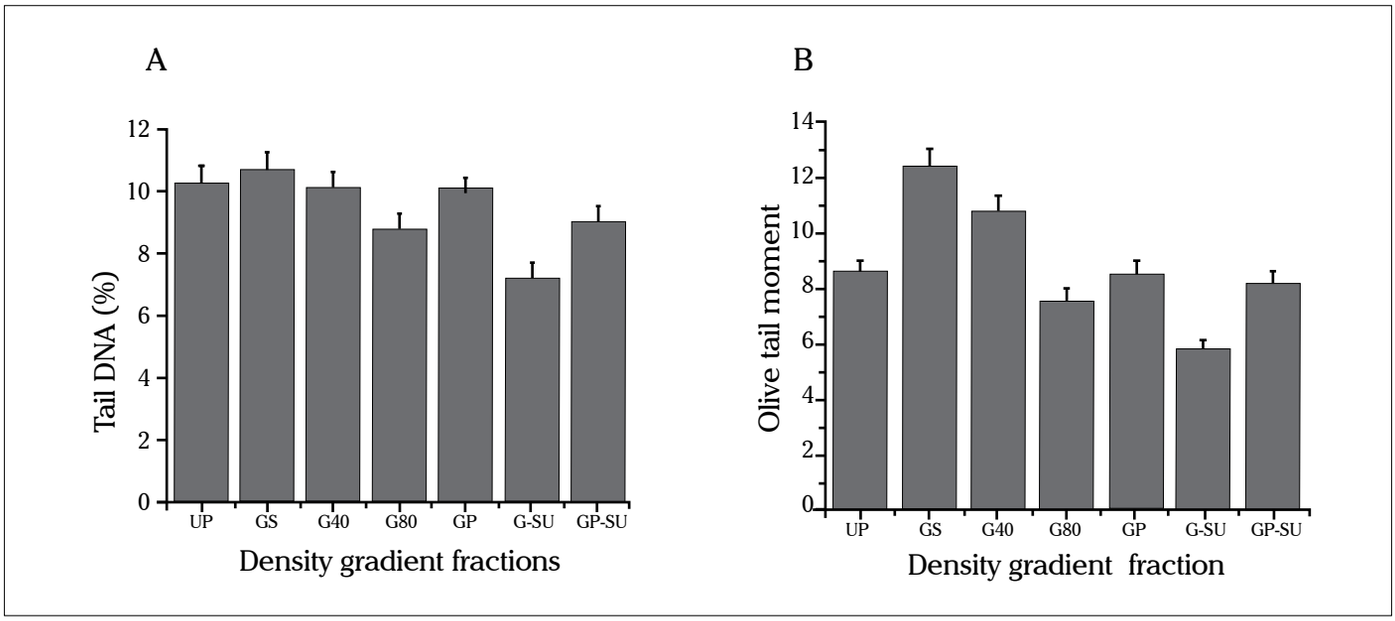

Figure 2. The incidence of sperm DNA damage in the unprocessed sub fractions of density gradient technique and combination of density gradient and swim-up technique (UP: unprocessed sperm, GS: gradient supernatant, G40: gradient 40 fraction, G80: gradient 80 fraction, GP: gradient pellet, G-SU: supernatant fraction after combined gradient and swim-up, GP-SU: pellet fraction after combined gradient and swim-up. A. The data representing percent tail DNA. The amount of tail DNA between UP and G-SU are significantly different ($p<0.01$). Similarly G-SU and GP-SU are significantly different ($p<0.05$). B. The level of Olive Tail Moment was significantly different between UP and GS, GS and G80 ($p<0.001$), G40 and G80 ($p<0.01$), GP and GP-SU, G-SU and GP-SU ($p<0.05$)

this study, the number of sperm with intact DNA in the post wash preparation remained unaltered when compared to unprocessed spermatozoa. Furthermore, we noticed a significant number of DNA damaged spermatozoa retained at gradient 40, and the level of DNA damage in these sperm was significantly higher than the unprocessed sperm. The possible explanations for this observation are either gradient 40 is quite effective in retaining the spermatozoa with nuclear abnormalities or the technique itself induces nuclear abnormalities in spermatozoa, as suggested by other authors (9, 10).

An earlier study (12) reported that semen processing by density gradient centrifugation is not generally useful in extracting sperm with intact DNA. This study used the TUNEL assay to detect DNA fragmentations, which is not a very sensitive assay as it detects fragments usually induced by endogenous nucleases (19). In contrast, flow cytometric analysis of density gradient processed samples for apoptosis revealed the superiority of this method in eliminating apoptotic sperm (8). Here we used the alkaline comet assay which detects single strand breaks, double strand breaks and alkali labile sites in the unprocessed sperm, as well as in sperm recovered from all the sub-fractions of swim-up and density gradient methods from which spermatozoa were recovered. Many different comet assay modifications are in use, including alkaline and neutral versions applying different treatments for chromatin decondensation (20). The comet results are known to vary due to a differences in techniques such as use of decondensing agents, electrophoresis time, and other assay conditions. Recent studies reported slightly high level of OTM in the irradiated mice sperm, possibly due to minor modifications in the assay condition (21, 22). The reasons for using γ-irradiated mouse sperm were:- first, to obtain a homogenous population of sperm with DNA damage as the only or dominant pathology and secondly, to thoroughly evaluate these sperm to test the efficacy of the techniques. This is not possible with human ejaculate due to heterogeneity and contamination with non spermatozoa cells.

Sperm preparation for assisted fertilization should aim to minimize the risk that abnormal sperm can have on the reproductive outcome. A reduction in the number of spermatozoa carrying DNA damage in the post-wash population is always a priority, in particular as there is concern regarding developmental delay and a compromised post implantation developmental potential of the embryos derived from the DNA damaged sperm (4). Therefore, a combination of density gradient centrifugation and swim-up technique should be the preferred method for the semen samples with higher amounts of DNA damaged sperm. However, this observation needs to be validated in a large cohort of human ejaculate.

Conflict of interest

No conflict of interest was declared by the authors.

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Iron, folate and vitamin B12 levels in first trimester pregnancies in the Southwest region of Turkey

Türkiye'nin güneybatı bölgesinde I.trimester gebelerde demir, folat ve vitamin B12 düzeyleri

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Abstract

Objective: Iron, folate and vitamin B12 play important roles in the healthy development of the fetus in pregnancy. Preconceptional levels of these micronutrients is influenced by dietary habits. The purpose of this study was to investigate the status of iron, vitamin B12 and folate in first trimester pregnancies in the southwest region of Turkey where the Mediterranean Cuisine, rich in fresh fruit and vegetables is commonly consumed.

Material and Methods: Two hundred and one low-middle income pregnant women were recruited during their first prenatal visit. Hemoglobin, ferritin, folate and vitamin B12 levels were evaluated and a structured questionnaire was given to gather information including age, gravida, parity, frequency of pregnancy, history of abortion, and intrauterine device usage. Based on WHO and international guidelines, anemia was defined as hemoglobin <11 g/dl, and iron deficiency as ferritin <15 μg/L. Serum folate and vitamin B12 deficiencies were defined as levels below 3 ng/ml and 200 pg/ml respectively.

Results: The mean age and gestational week were 26.4±5.3 years and 9±3 weeks respectively. Mean plasma concentrations were 12.8±9.7 g/dl for hemoglobin, 22.7±17.2 μg/L for ferritin, 12.2±5.6 ng/ml for folate and 266.6±100.2 pg/ml for vitamin B12. Anemia was detected in 4.5% of pregnant women, iron deficiency in 40.3%, vitamin B12 deficiency in 29.8% and folate deficiency in 0.5% of patients. In 10.9% of patients, both vitamin B12 and iron iron deficiency was detected. There was no significant difference for age, body mass index, gravida, parity, frequency of pregnancy, history of abortion, and intrauterine device usage between women with low and normal levels of vitamin B12 and Ferritin (p>0.05).

Conclusion: Iron and vitamin B12 deficiencies were relatively common in the pregnant population consuming vegetable based diets. Iron and vitamin B12 supplementation in addition to folate must be considered for the wellbeing of the fetus in pregnant women living in areas where dietary patterns are mainly vegetable based.

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Key words: Hemoglobin, ferritin, folate, vitamin B12, pregnancy

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Özet

Amaç: Gebelikte demir, folat ve vitamin B12 düzeyleri fetusun sağlıklı gelişiminde önemli rol oynamaktadır. Bu mikroelementlerin gebelik öncesi serum düzeyleri beslenme alışkanlıkları ile yakından ilişkilidir. Bu çalışmada, taze meyve ve sebzenin yoğun olarak tüketildiği, akdeniz mutfağının yaygın olduğu Türkiye'nin güney batı bölgesinde, I.trimester gebelerde serum demir, folat ve vitamin B12 düzeylerini incelemeyi amaçladık.

Gereç ve Yöntemler: I. Trimesterde başvuran, düşük-orta gelir grubunda 201gebe çalışmaya alındı. Hemoglobin, ferritin, folate ve vitamin B12 seviyeleri ölçüldü. Sorgulama formu sunularak yaş, gravida, parite, gebelik sıklığı, düşük hikayesi ve rahim içi araç kullanımı hakkında bilgi toplandı. Dünya sağlık örgütü ve uluslar arası yönergelere dayanılarak hemoglobin düzeyinin <11 g/dl olması anemi ve ferritin düzeyinin <15 μg/L olması demir eksikliği olarak değerlendirildi. Serum folate and vitamin B12 için sırasıyla 3 ng/ml ve 200 pg/ml'nin altındaki değerler eksiklik olarak kabul edildi.

Bulgular: Ortalama gebelik haftası 9±3 hafta ve ortalama yaşı 26.4±5.3 yıl idi. Ortalama hemoglobin düzeyi 12.8±9.7 g/dl, Ferritin düzeyi 22.7±17.2 μg/L, folate düzeyi 12.2±5.6 ng/ml ve vitamin B12 düzeyi 266.6±100.2 pg/ml olarak saptandı. Gebelerin %4.5'inde anemi, %40.3'ünde demir eksikliği, %29.8'inde vitamin B12, %0.5'inde folate eksikliği, %10.9'unda ise hem vitamin B12 hem de demir eksikliği tespit edildi. Ferritin ve vitamin B12 düzeyleri düşük ve normal olan gruplar arasında yaş, vücut kitle endeksi, gravida, parite, gebelik sıklığı, düşük hikayesi ve rahim içi araç kullanımı açısından istatistiksel olarak anlamlı bir fark saptanmadı (p>0.05).

Sonuç: Demir ve vitamin B12 eksikliği, sebze ağırlıklı beslenen bu bölgede folat eksikliğine göre daha yaygındı. Beslenme alışkanlığının sebze ağırlıklı olduğu bölgelerde yaşayan gebelerde sağlıklı fetal gelişim için erken gebelikte folat suplementasyonuna, demir ve vitamin B12 içeren preparatların da eklenmesi düşünülmelidir.

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Anahtar kelimeler: Hemoglobin, ferritin, folat, vitamin B12, gebelik

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Introduction

Iron deficiency is the most commonly detected nutritional deficiency in pregnant women. It was postulated to be associated with poor pregnancy outcome and preterm delivery (1). During pregnancy, there is a significant increase in iron requirements due to increased red cell mass and fetoplacental growth (1). Diet is also an important factor determining the preconceptional iron status in the reproductive age group.

Preconceptional supplementation of folic acid has been shown to reduce neural tube defects (NTD) in the fetus (2). Although some countries introduced folic acid fortification programs in time, fetal NTD still continues to affect about 6 in every 10,000 pregnancies (2, 3). There are probably other modifiable risk factors contributing to the prevalence of NTD.

Vitamin B12 shows close metabolic association with folate. It has been demonstrated that the deficiency of vitamin B12 may be an independent risk factor, almost triplicating the risk of NTD (4, 5). Vitamin B12 includes a group of cobalt-containing compounds known as cobalamins. This vitamin is involved in myelin synthesis, fatty acid degradation, and protein and DNA synthesis (6). All the natural vitamin B12 is produced by microorganisms and it is only found in foods of animal origin and vegetables contaminated with vitamin B12-synthesizing bacteria. Vitamin B12 is an animal source vitamin and deficiency is common in vegetarians due to low intake and in the elderly due to low absorption (7). Therefore vegetable based diets may lead to vitamin B12 deficiency.

In this study, we aimed to investigate the status of iron, vitamin B12 and folate in first trimester pregnancies in the southwest region of Turkey where a vegetarian based diet referred to as Mediterranean Cuisine is in common use.

Materials and Methods

This prospective study was carried out between January 2010 and June 2010. The study population was composed of patients attending the ambulatory pregnancy Clinic. The pregnant women admitted to hospital for routine obstetrical evaluation in the first trimester of pregnancy, who had not initiated any kind of vitamin preparation and had no history of neural tube defect were included in the study. Patients; (i) on vegetarian diet, (ii) with multiple pregnancies, (iii) previous history of anemia, renal disease and alcohol consumption, (iv) evidence of malabsorption, (v) BMI below 18.5 kg/m^2 and (vi) smoking patients were excluded. The study population was composed of low-middle income patients according to an income and living conditions survey of the Turkish statistical institude (8). A structured questionnaire was given to gather information including age, parity, time of previous gestation, and the history of intrauterine device (IUD) usage. Weight and height of the subjects were obtained to calculate body mass index (BMI).

Blood specimens from all subjects were obtained with a standard venopuncture technique after 8 to 10 hours of fasting. Based on WHO and international guidelines, anemia was defined as hemoglobin <11 g/dl, and iron deficiency as ferritin <15 μg/L. Serum folate and vitamin B12 deficiencies were defined as levels below 3 ng/ml and 200 pg/ml respectively. A hemoglobin measurement was performed via Photometric assay (Abbott Cell-Dyn 3700, Abbott Laboratories Abbott Park IL, USA). Ferritin, vitamin B12 and folate measurements were performed using the chemiluminescence assay (Siemens ADVIA Centaur® immunoassay, Tarry town, NY, USA).

The procedures were explained to all subjects and written informed consent was obtained. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki, as reflected in a prior approval by the institution's human research committee.

Continuous variables were expressed as mean±standard deviation (SD). To analyze the differences between groups, the independent student t test was used for continuous variables, and chi square test was used for nominal variables. Statistical analysis was performed using the statistical package for Social Sciences (SPSS 15.0, Chicago, IL) software. P values less than 0.05 were considered statistically significant.

Results

Two hundred and one patients out of 249 low-middle income women were recruited for the study. Forty eight patients were excluded from the study, since they had already started vitamin pills before admission. The mean age and gestational week of the study population were 26.4±5.3 years and 9±3 weeks respectively (Table 1). Of 201 subjects, anemia was detected only in nine (4.5%) women according to WHO criteria. However, iron deficiency was seen in 82 (40.3%) women (Table 2). There was no significant difference for age, body mass index, gravida, parity, frequency of pregnancy, history of

Table 1. Characteristics of the study population

	Mean±SD	Minimum	Maximum
Age (year)	26.4±5.3	17	42
BMI (kg/m^2)	23.9±4.3	15	38
Gestational Age (week)	9±3	5	14
Gravida	1.5±1.2	0	9
Parity	0.8±0.9	0	5
Time from last pregnancy (year)	4.9±3.1	1	20

Table 2. Hemoglobin, ferritin, vitamin B12 and folate concentrations and percentage of women with abnormal values

	Mean±SD	Minimum	Maximum	Abnormal[1] (%)
Hemoglobin (g/dl)	12.8±9.7	8.7	14.9	4.5
Ferritin (μg/L)	22.7±17.2	1.6	93.5	40.5
Vitamin B12 (pg/ml)	266.6±100.2	30.0	593.0	29.6
Folic acid (ng/ml)	12.2±5.6	3.4	24.0	0.5

[1]Abnormal is defined as hemoglobin level <11 g/dl, ferritin level <15 μg/L, vitamin B12<200 pg/ml, folate<3ng/ml

abortion, and intrauterine device usage between women with low and normal ferritin levels (p>0.05) (Table 3). Vitamin B12 deficiency was present in 60 (29.8%) patients, whereas folate deficiency was detected only in one patient (Table 2). There was no significant difference in age, body mass index, gravida, parity, frequency of pregnancy and history of abortion and intrauterine device usage between women with low and normal vitamin B12 levels (p>0.05) (Table 4).

In 22 (10.9%) women, both iron and vitamin B12 deficiency was detected. Fe^{+2} sulphate (Gynoferon® tb, Koçak Farma, Istanbul, Turkey) 80 mg orally per day and cyanocobalamin 1000 μg (Dodex® amp., Deva, Istanbul, Turkey) every other day were added to the treatment for ferritin and vit. B12 deficiency. For folate deficiency, 5mg folic acid (Folbiol® tb, I.E. Ulugay, Istanbul Turkey) perday was prescribed to the patient.

Discussion

Dietary habit is an important factor determining the micronutrient status of our body and it shows great variation according to cultural factors and geographical regions. The United Nations (UN) estimates that approximately half of pregnant women suffer from anemia worldwide (9). Anemia prevalence during pregnancy differed from 18% in developed countries to 75% in South Asia (10). Nutrition related iron deficiency is the main cause of anemia throughout the world (9). Iron deficiency and anemia during pregnancy is a global problem and is closely related with maternal and perinatal mortality (10). The high prevalence of deficiencies detected in the first trimester of pregnancy poses particular reproductive risks. In the present study, 40.3% of women had depleted iron stores and 4.5% had iron deficiency anemia. In a similar study conducted in Marmara region, low levels of Ferritin were detected in 52.3% of first trimester pregnancies (11). Karaoglu et al. detected a prevalence of iron deficiency and anemia higher as; 57% and 27.1% respectively in East Anatolia (12). In contrast to the present study, second and third trimester pregnancies were evaluated in that study. This high rate in the previous study can be attributed to the fact that anemia is more prevalent after the first trimester of pregnancy due to increased metabolic demands and physiologic hemodilution.

Table 3. Comparison of demographic characteristics of patients with low and normal ferritin levels

	Group with low ferritin level (N=82)	Group with normal ferritin level (N=119)	p value
Age (year)	26.7±5.5	26.2±5.1	NS
BMI (kg/m^2)	23.4±5.3	22.8±4.9	NS
Gestational Age (week)	7.2±4.1	7.7±3.5	NS
Gravida	1.5±0.9	1.4±1.3	NS
Parity	0.8±0.8	0.8±0.7	NS
Time from last pregnancy (year)	2.9±3.8	2.7±3.2	NS

NS; Nonspecific

Table 4. Comparison of demographic characteristics of patients with low and normal vitamin B12 levels

	Group with low vitB12 level (N=60)	Group with normal vitB12 level (N=141)	p value
Age (year)	25.9±5.26	26.4±5.26	NS
BMI (kg/m^2)	22.4±5.3	23.1±3.4	NS
Gestational Age (week)	8.5±3.6	8.2±3.5	NS
Gravida	1.4±0.9	1.5±1.3	NS
Parity	0.9±0.8	0.8±0.9	NS
Time from last pregnancy (year)	2.9±2.9	2.7±3.5	NS

NS; Nonspecific

The first trimester pregnancies without any supplementation more likely reflect the preconceptional status. Our results emphasize that, although anemia is not a major problem, iron deficiency is a common problem in this geographical region. These patients will probably develop iron deficiency anemia with the progression of pregnancy. Screening in the preconceptional period or early pregnancy seems valuable for the detection and treatment of iron deficiency and anemia.

Both folate and cobalamin deficiencies are characterized by megaloblastic anemia and elevated blood homocysteine levels, which lead to cardiovascular problems and adverse pregnancy outcomes, such as recurrent abortion and preeclampsia (13-16). It has been hypothesized that both folate and vitamin B12 could have a crucial role in folate-related NTD (17). High prevelances of deficiencies may also be seen in countries with different socioeconomic classes. A Canadian study performed in 2000 showed that the prevalence of folic acid and vitamin B12 deficiencies in pregnant women was 27% and 44% respectively (18). The same prevalences were detected as 36% and 61% for folate and vitamin B12 deficiencies respectively in Venezuela (19). In a study from Turkey, Ackurt et al. detected low levels of vitamin B12 and folate in 48.8% and 59.7% of subjects respectively among first trimester pregnant women in the Marmara region (11). In our study, low vitamin B12 levels were detected in 60 patients (30%), but low folate level was detected only in one patient. Although the regional nutritional habit is mainly vegetable based, none of the women were vegetarians and all the women included in the study implied that they ate meat. They were consuming meat, but the amount was probably insufficient. This results in iron and vitamin B12 deficiency.

Low folic acid levels were shown to be associated with increased NTD. Although there are no studies showing the prevalence of NTD in Denizli, two studies from Izmir and another city in the Aegean region sharing similar nutritional habits showed a relatively lower NTD risk compared to the northern and eastern parts of Turkey. Posaci et al. (20) and Caglayan et al. (21) found NTD in 1.5 and 1.9 per 1000 pregnancies in the Izmir region, whereas the same rates were detected as 4.4 and 5.6 in the northern and eastern parts of Turkey respectively (22, 23). Tuncbilek et al. found the overall neural tube defect

rate throughout the country to be 3 per 1000 pregnancies (24). Although the reason for the comparatively lower incidence of neural tube defects in the Aegean region of Turkey has not yet been clarified, we postulated that the diet rich in folic acid may be a factor.

Our study has a few limitations. First, it is a hospital based study with a relatively small sample size. Second, these women were not followed during pregnancy and further micronutritional status cannot be evaluated. However, this study pointed out that there are several problems which need to be clarified and evaluated in further studies regarding micronutritional supplementation during pregnancy in our country. Although the results cannot reflect the whole population, they are valuable enough to stimulate a debate for micronutritional supplementation during pregnancy in our country.

In conclusion, the present study shows that subclinical iron and vitamin B12 deficiency is a hidden risk for pregnant women living in regions where a vegetable based diet is common. Because neural tube defect is an infrequent entity, prospective longitudinal studies with larger series are required in order to evaluate the effects of nutritional habits on the developing fetus. The necessity for vitamin B12 supplementation needs to be confirmed with prospective randomized trials from different regions of our country before the introduction of a fortification program for prevention of neural tube defect. Until that time, since it plays a role in the etiology of neural tube defects, concomitant vitamin B12 supplementation may be considered preconceptionally in places where a vegetable based diet is common.

Conflict of interest

No conflict of interest was declared by the authors.

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Leptin expression in proliferative, secretory and hyperplastic endometrial tissues

Proliferatif, sekretuar ve hiperplazik endometrial dokuda leptin ekspresyonu

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Abstract

Objective: The goal of this study was to detect endometrial leptin expression in proliferative and secretory phases and then to compare the results with that of hyperplastic endometrium.

Material and Methods: Seventeen proliferative, 23 secretory phase and 18 hyperplastic endometrial tissues diagnosed in our hospital between 2002 and 2007 were included in the study. These samples were stained with leptin antibody using an immunohistochemical method. Endometrial glandular and surface epithelium and stroma were evaluated for staining distribution and intensity.

Conclusion: Staining intensity seen in early proliferative phase samples (2.33±0.51) increased significantly throughout the middle (2.40±0.54) and late phases (2.83±0.40) ($p<0.05$). Early secretory phase samples had the least staining intensity (1.42±0.53), while it increased significantly in later periods (2.38±0.51) ($p<0.05$). There was no difference in staining intensity among proliferative, secretory and hyperplastic tissues ($p>0.05$).

Conclusion: Although endometrial leptin expression was observed in a differential manner throughout the whole menstrual period, no difference was seen in endometrial hyperplasia. We consider that leptin does not play a role in hyperplastic transformation of the endometrium. (J Turkish-German Gynecol Assoc 2011; 12: 157-61)

Key words: Endometrium, hyperplasia, leptin, menstrual period, phase

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Özet

Amaç: Bu çalışmanın amacı proliferasyon fazı ve sekresyon fazı endometrium dokularında leptin ekspresyonu varlığını araştırmak ve çıkan sonuçları hiperplastik endometrium dokularından elde edilen sonuçlarla karşılaştırmaktır.

Gereç ve Yöntemler: Hastanemizde 2002-2007 yılları arasında tanı almış 17 adet proliferasyon fazında endometrium, 23 adet sekresyon fazında endometrium ve 18 adet endometrial hiperplazi dokusu çalışmaya dahil edildi. Bu örnekler leptin antikorları ile immünohistokimyasal metod kullanılarak boyandı. Endometrial bez yapıları, yüzey epiteli ve stroması boyanma derecesi ve dağılımı açısından değerlendirildi.

Bulgular: Boyanma derecesi erken proliferatif faz örneklerinde (2.33±0.51) belirgin olarak az olduğu, orta (2.40±0.54) ve geç proliferasyon fazlarına (2.83±0.40) doğru belirgin olarak arttığı gözlendi. Boyanma derecesi erken sekresyon fazı örneklerinde (1.42±0.53) en düşüktü fakat daha geç sekretuar fazlarda (2.38±0.51) belirgin olarak arttığı gözlendi ($p<0.05$). Proliferatif, sekretuar ve hiperplastik dokular arasında leptin ekspresyonu açısından istatiksel anlamda bir fark tespit edilmedi ($p<0.05$).

Sonuçlar: Adet döngüsü boyunca endometrial leptin ekspresyonu açısından anlamlı farklılıklar gözlenmesine ragmen endometrial hiperplazide anlamlı farklılık gözlenmedi. Endometriumun hiperplastik transformasyon sürecinde leptinin etkisinin olmadığını düşündürmüştür. (J Turkish-German Gynecol Assoc 2011; 12: 157-61)

Anahtar kelimeler: Endometrial hiperplazi, leptin, adet döngüsü

Geliş Tarihi: 16 Haziran 2011 **Kabul Tarihi:** 28 Temmuz 2011

Introduction

Following the discovery of the ob/ob mouse model in 1950, mutational changes were reported to cause serious obesity, hyperphagia, diabetes and reduced energy consumption at relatively early ages. The genetic defect leading to the ob/ob mouse was published in 1994 (1) and leptin, which is stimulated with 16-k Da ob gene and is mainly synthesized by adipose tissue, was introduced as an ob gene product in 1995 (2, 3). Leptin regulates body weight and energy balance by interacting with its receptors found in various tissues. Six different isoforms of leptin receptor were identified (4). In recent studies, leptin was reported to play major roles in many reproductive events such as menstruation, ovarian follicle maturation, embryo development and continuation of gestation (5-7). In addition, expression of OB-R_L mRNA was detected in human endometrium (8), which is a target organ for leptin (9). Insulin-like growth factor-1 (IGF-1) and leptin and were found to be related with proliferation and mitogenic activity in rat mammarian gland cell cultures and to play a role in the pathogenesis of breast cancer (10). Patients with endometrial hyperplasia and endometrial cancer had higher serum leptin levels when compared with controls with adjusted body mass index (BMI) values, and leptin was reported

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to have a significant effect on endometrial proliferation (11). Leptin's effect on proangiogenic molecules (VEGF, IL-1beta, LIF) and their receptors (VEGFR2, IL-1R, LIFR) was much more prominent in endometrial cancer cells compared with benign endometrial tumors, and leptin and/or a pathogenic pathway stimulated by leptin were postulated to be responsible for this malignant transformation (12). It has been considered that leptin might affect endometrial proliferation and its receptors might be regulated by some proliferative factors. Our goal in this study was to detect leptin staining in proliferative phase endometrium (PPE), secretory phase endometrium (SPE) and in tissues with endometrial hyperplasia (EH) and then to compare staining intensities by using an immunohistochemical method.

Materials and Methods

Seventeen PPE, 23 SPE and 18 EH tissues, diagnosed and kept in the Department of Pathology, Faculty of Medicine, Celal Bayar University between 2002-2007 were included in the study. Seven PPE, 6 SPE and 4 EH samples were obtained by probe curettage while 10 PPE, 17 SPE and 14 f EH samples were hysterectomy specimens. Demographic data such as age and menstrual irregularity were recorded from the patients' files. Probe curettage and hysterectomy indications were irregular bleeding and uterine fibroids. None of the patients had any additional disorders or received any medications prior to 1 month of surgical procedures. In the first step of the study, leptin expression was evaluated after PPE and SPE were individually divided into early, middle and late periods. In the second step of the study, leptin expression was evaluated in two different pathological states, simple and complex hyperplasia. Slides stained with hematoxyline and eosin were reevaluated by the pathologist who was not informed of the origin of the specimens, menstrual cycle days were determined and histopathological diagnosis was confirmed.

For immunohistochemical analysis, sections obtained from formalin-fixed and paraffin-embedded blocks were deparaffinized with xylene and rehydrated with graded ethanol. Endogenous peroxidase activity was blocked with 3% H_2O_2 for 10 min. Slides were washed with distilled water for 5 min, placed in 0.01 M citrate buffer (pH 6.0) and boiled in the pressure cooker during 3 minutes. Background blocking was performed with 1:20 normal goat serum (Dako; Glostrup, Denmark) in 0.05 M Tris-HCl buffer, 0.5 M NaCl, pH 7.6 (TBS) before incubation with specific antiserum. The tissue sections were incubated at room temperature for 20 min with a rabbit polyclonal antibody specific for human leptin (Ob (A-20) sc-843; (Santa Cruz Biotechnology, Santa Cruz, CA) diluted 1:75 in primary antibody diluent (K004, LOT G537 Pleasanton, CA). After 20 min incubation with the linker (biotin), streptavidin-peroxidase was added for an additional 20 min and the substrate-chromogen solution (DAB) was used for 5 min to stain the slides. Subsequent to each incubation step, the tissues were washed three times with PBS 50 mm Tris-HCl buffer. Counterstaining was carried out with Mayer's hematoxylin, and the slides were mounted with Entellan (Merck and Co., Berlin). A section of placental sample was used as positive control for each staining procedure. All samples were evaluated by one pathologist under standard light microscope and described as follows: Endometrial glands and surface epithelium: 0: negative, 1: minimal staining, 2: mild staining, 3: strong staining. Stroma: 0: negative, 1: mild staining, 2: strong staining.

Kruskal-Wallis and Chi-square tests were used to compare staining intensities and $p<0.05$ was accepted as statistically significant.

Results

The ages of the patients were between 30 and 74 years. Clear cytoplasmic staining of leptin is seen in the glandular and surface epithelium of the endometrium and cytoplasmic and/or nuclear staining in endometrial stromal cells. The staining intensity was significantly different among early (2.33±0.51) (Fig. 1A), middle (2.40±0.54) (Fig. 1B) and late (2.83±0.4) (Fig. 1C) PPE samples ($p<0.05$) (Table 1). Leptin expression began to increase in early PPE and reached high intensity until ovulation. In early SPE, a nadir value was observed (1.42±0.53) (Fig. 2A), and then increased with time (2.00±1.00) (Fig. 2B) and reached its maximum level (2.38±0.51) (Fig. 2C) in late SPE samples ($p<0.05$) (Table 2).

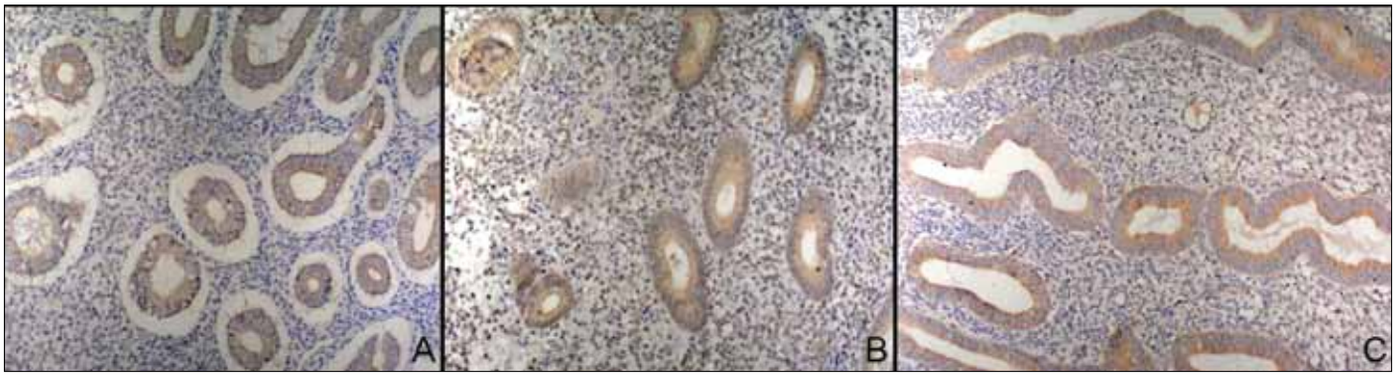

Figure 1. A: Leptin staining in endometrial gland epithelium and stromal cells in early proliferative phase (x200). B: Leptin staining in endometrial gland epithelium and stromal cells in middle proliferative phase (x200). C: Leptin staining in endometrial gland epithelium and stromal cells in late proliferative phase (x200). The strongest staining intensity is seen in this phase

Staining intensity was the same in simple and complex hyperplasia groups (2.5±0.59) (Figure 3). As the numbeof cases in complex EH was fewer and there was no difference between simple and complex groups, we joined these two groups under EH and then compared with normal PPE and SPE samples. There was no significant difference between these 3 groups (p>0.05) (Table 3).

Discussion

Obesity is accepted to be related with development, morbidity and mortality of colon, breast, endometrium, renal, pancreas and esophagus cancer (13). Increased waist circumference, commonly seen in obese patients, is a well-known risk factor for these disorders. While endometrium cancer is the fourth most common cancer in women, it is in the first place in the genital system. Endometrial hyperplasia is an important clinical entity as a precancerous lesion, with a serious potential to transform into cancer. If development of endometrial hyperplasia is prevented or cured in relatively early stages (for example at the simple hyperplasia level), endometrial cancer prevalence may be reduced.

Previous studies searching for expression of human endometrial leptin and its receptors revealed the presence of OB-R receptor mRNA while no sign was found for leptin expression (8, 9). Using Western blot analysis, the researchers suggested that the uterus was a target organ for leptin produced in adipose tissue but it was not expressed in the endometrium. However, in another study, Gonzalez et al. showed human endometrial leptin mRNA by RT-PCR and its protein by immunohistochemistry methods (5). In our present study, we revealed different levels of leptin expression in different phases of the human endometrium by using immunohistochemistry staining. Although we did not point out the leptin source, we could prove

Table 1. Leptin expression in PPE. The difference is statistically significant among the samples (p<0.05). (Numbers refer to mean±SD)

	# Patients	Staining Intensity
Early PPE	6	2.33±0.51
Mid PPE	6	2.40±0.54
Late PPE	5	2.83±0.40
Total	17	2.52±0.10

Table 2. Leptin expression in SPE. The difference is statistically significant among the samples (p<0.05). (Numbers refer to mean±SD)

	# Patients	Staining Intensity
Early SPE	7	1.42±0.53
Mid SPE	3	2.00±1.00
Late SPE	13	2.38±0.51
Total	23	2.04±0.71

Table 3. Leptin expression in PPE, SPE, EH. There was no statistically significant difference among the 3 groups (p>0.05). (Numbers refer to mean±SD)

	# Patients	Staining Intensity
PPE	17	2.52±0.51
SPE	23	2.04±0.70
EH	18	2.33±0.59
Total	58	2.27±0.64

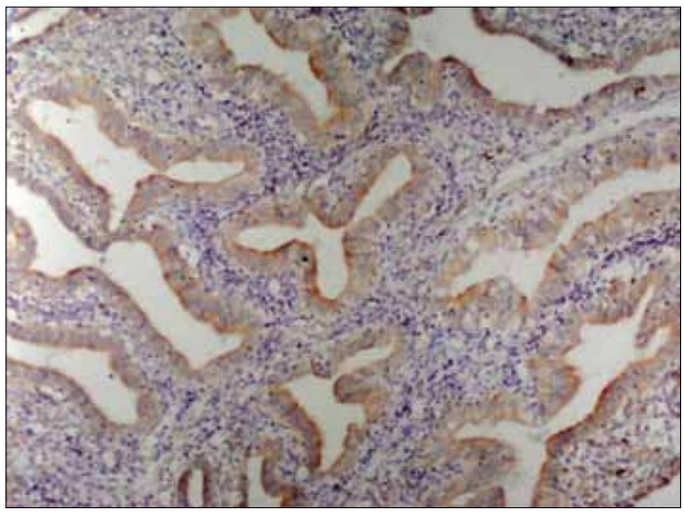

Figure 3. Leptin staining in endometrial gland epithelium and stroma in endometrial hyplerplasia (x200)

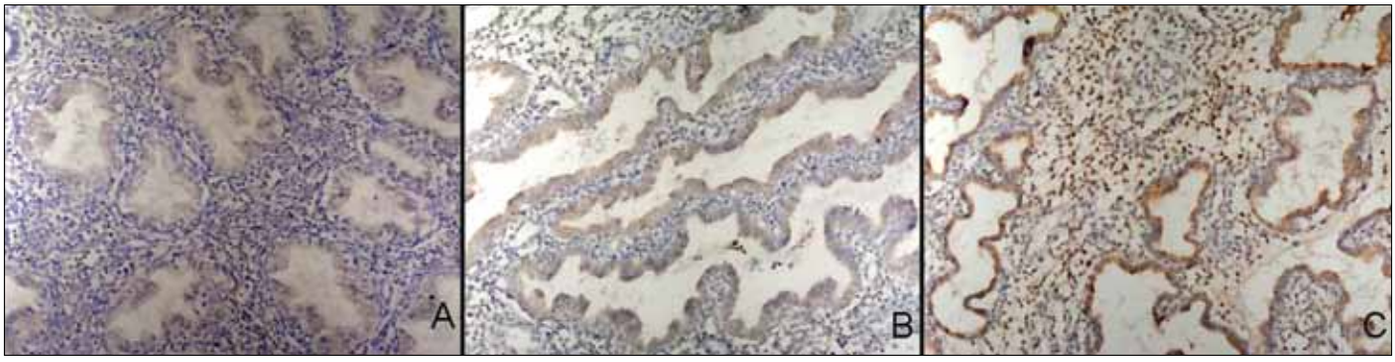

Figure 2. A: Leptin staining in endometrial gland epithelium and stromal cells in early secretory phase (x200). Mild staining in early period becomes stronger in time. B: Leptin staining in endometrial gland epithelium and stromal cells in middle secretory phase (x200). C: Leptin staining in endometrial gland epithelium and stromal cells in late secretory phase (x200)

its presence. It is accepted that leptin exists in human endometrium even though there is no consensus about whether it is produced within the endometrium or produced and secreted by adipose tissue, passes s via the vascular system and acts on the endometrium.

We found that leptin existed in an increasing level, beginning from the early to late proliferation phase and reaching a maximum level in the latter and then decreased just following ovulation in the early secretory phase. In a study investigating expression of leptin and its receptors throughout the complete menstrual period, very low OB-R_L mRNA expression was found in the beginning but it continuously increased during the remainder of the proliferative phase (9). This variable expression of the receptor is in accordance with leptin expression shown in our study. Serum leptin concentration was found to be well correlated with serum estradiol levels in a study searching for fluctation of leptin during a normal menstrual period (14). When the results of this study, which show that serum leptin increases at late proliferation and reaches a peak level at periovulatory phase are compared with our results, we can state that estradiol may act upon endometrial leptin expression and leptin may collaborate with estradiol to affect the endometrium. However, as in vitro studies have shown that estradiol does not have an acute effect on OB-R_L mRNA expression, it does not seem to take part in a direct action to increase endometrial leptin and OB-R expression. Elevation of leptin expression is in parallel to estradiol levels, however estradiol may act as a limiting factor for leptin action on endometrium by exerting no increase in receptor levels.

Following endometrial sloughing with menstrual bleeding, rapid repair commences and the endometrium prepares itself for a probable implantation. New endometrium covers 75% of the cavity on day 4, re-epithelization occurs on day 5-6 and stromal changes begin. The epithelization process accelerates with the increased vascular supply (angiogenesis). A positive correlation was determined between leptin and erythropoietin on endometrial bleeding and angiogenesis during a normal menstrual cycle (12). Proangiogenic molecules VEGF, IL-1-β, LIF and their receptors have been expressed more with leptin in both benign and malignant endometrial disorders (4). In another study, the effect of leptin on normal endometrial stromal cells was detected by the 8 Br-cAMP method (in vitro decidualization measurement system) and leptin was found to support viability and inhibit decidualization in a dose dependent manner (15). Leptin seems to play a role in differentiation of the endometrium from sloughing to implantation phases with such angiogenetic and anti-apoptotic effects reported in these studies. This result necessitates increased leptin concentration, beginning from early to late proliferative phases and a secondary increase from a nadir value at early to late secretory phase as shown in our study. The increased angiogenesis and anti-apoptotic effect in accordance with a rise in endometrial leptin expression from early to late proliferative phases elicit adequate endometrial proliferation. Embryo implantation is accepted as a phase mediated apoptosis (16, 17). Reduced leptin concentration in early SPE induces apoptosis and consequently implantation. During the late secretory phase, increased endometrial leptin expression is required for angiogenesis, which is the next step in implantation. All these relevant changes support the results of our study.

Patients with endometrial hyperplasia and cancer had significantly higher serum leptin concentration when divided into 3 groups according to their BMI levels and compared with women having normal endometrium (11). Angiogenetic and anti-apoptotic effects of leptin may be considered to participate in the pathophysiology of endometrial hyperplasia in addition to a physiologic proliferative effect on endometrium. However, when we compared staining intensities in PPE, SPE and EH groups, we could not demonstrate a significant difference in leptin expression. Although leptin may act upon cellular proliferation, it may not play a role in hyperplastic transition. A strong positive relation was reported between leptin and endometrial cancer in a case control study by Petridou et al. (18). In addition, short and long receptor isoforms of leptin were detected in endometrial cancer cells (19). In another study, proliferation and invasiveness of endometrial cancer cells were found to be enhanced by leptin (with JAK/STAT activation) (20). These studies may indicate that leptin may play a role in malignant transformation. Leptin receptor existence in cancerous tissues may give rise to leptin's role in the development of cancer even though it does not seem to participate in the hyperplasia stage. As we observed samples with established and manifest hyperplasia, we do not have information about leptin's effect during the transitional period from the normal to hyperplastic stage.

In conclusion, leptin may act upon endometrial changes seen in the proliferative and secretory phases, but as it is not expressed in different amounts in hyperplastic tissues, we consider that it does not participate in this pathological procedure. Different results may be expected in a long term, prospective study which follows obese women with histopathologically proven normal endometrium and high serum leptin levels and women having normal BMI and normal serum leptin. Such a study may t expose a probable effect of leptin on the endometrial neoplastic process with more objective criteria.

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Conflict of interest

No conflict of interest exists among the authors for any particular subject.

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Improvement in embryo quality and pregnancy rates by using autologous cumulus body during icsi cycles

Icsı sikluslarında otolog kumulus kitlesi kullanımı ile embriyo kalitesinde ve gebelik oranlarında artış

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Abstract

Objective: To determine whether the addition of intact cumulus cell mass (ICM) to both embryo culture (EC) and embryo transfer (ET) improves embryo quality and pregnancy rates.

Material and Methods: A total of 133 infertile couples were included, of which 67 received ICM (study group) and 66 did not (control group). The ICM was obtained from a simple cutting of the cumulus corona oocyte complex (CCOC). A case control study design was used.

Results: The clinical characteristics of the two groups before the embryo culturing step were similar with respect to age, estradiol level on the day of hCG and endometrial thickness on the day of embryo transfer (p>0.05). On the other hand study group with ICM had higher number of high quality embryos (3.1±1.4 vs 2.4±1.1, p=0.03), higher implantation rate (53.7% vs 34.8%, p=0.02) and higher ultrasound confirmation of gestational sac and fetal heart beat as ongoing pregnancy rates (44.7% vs 27.2%, p=0.04) compared to the control group without ICM.

Conclusion: Addition of ICM improves embryo quality and pregnancy rates. This is a cost-and time-effective simple procedure that shows great promise for the improvement of infertility treatment.

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Key words: Autologous Cumulus Body, ICSI Cycles, Embryo Quality, Pregnancy Rates

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Özet

Amaç: Bütünlüğü bozulmamış kumulus hücre kitlesi (BBKHK) hem embriyo kültürüne hem de embriyo transfer aşamalarında kullanımının gelişen embriyonun kalitesine ve gebelik oranlarına bir katkı sağlayıp sağlamadığını araştırmak.

Gereç ve Yöntemler: Çalışmaya kısırlık sorunu ile başvuran ve ICSI işlemine alınan toplam 133 çift dahil edildi, 67 tanesine BBKHK ilavesi yapıldı (çalışma grubu) ve 66 tanesine standart tedavi yapıldı (kontrol grubu). İşlemde kullanılan BBKHK'nın elde edilmesi için sadece kumulus korona oosit kompleksinden basit bir kesme işlemi yapıldı. Çalışma kontrollü olgu şeklinde dizayn edildi.

Bulgular: Çalışmaya dahil edilen her iki grubun yaş, östrodiol seviyeleri ve transfer günü endometrium kalınlığı özellikleri aynı idi (p>0.05). Çalışma grubu kontrol grubu ile karşılaştırıldığında embriyo kalitesi (3.1±1.4 vs 2.4±1.1, p=0.03), implantasyon oranları (53.7% vs 34.8%, p=0.02) ve kalp atımı ultrasound teyidi ile devam eden gebelik oranlarında (44.7% vs 27.2%, p=0.04) anlamlı farklılıklar ortaya çıktı.

Sonuçlar: Embriyo kalitesi ve gebelik oranları BBKHK ilavesi ile arttırılabilir. Bu işlem zaman ve maddi maliyet artışı getirmeden sadece basit bir girişim ile kısırlık tedavisine önemli katkıda bulunmaktadır.

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Anahtar kelimeler: Otolog cumulus kitlesi, ICSI siklusu, Embriyo kalitesi, Gebelik Oranları

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Introduction

Assisted-reproduction laboratory protocols are based on the use of parental gametes to develop an embryo and achieve pregnancy. The clinical application of assisted-reproduction technologies began with the birth of the first successful test-tube baby achieved by Robert Edwards in 1978. Since then, extensive research aimed at improving assisted-reproduction technologies has been performed to obtain higher conception rates. In addition to the development and improvement of clinical techniques, new techniques for hormone induction and embryo culture and manipulation are continuously being developed in the laboratory. Culture media development is aimed at supporting embryo development. Culture media are designed to mimic the biochemistry of human tubal fluid, and their development is limited by the spectrum of current technology. Technological progress in physical and chemical sciences may redefine the analyses of oviduct fluid composition, which may change our knowledge of the interaction of the embryo with the tubal and uterine tissues and enable the emergence of new concepts (1, 2). However, this knowledge will always be limited by the spectrum of analytical technologies.

In the present study, we compared the development and implantation of embryos and pregnancy rates between embryos developed in a co-culture system and transferred with cumulus mass addition and those not developed in a co-culture system and transferred without cumulus mass addition. In total,1232 embryos (625 embryos in the study group and 607 control embryos) were evaluated.

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Materials and Methods

Couples were informed about the study and those who volunteered were included in the study. Our study performed using autologous materials was approved by the Faculty Ethical Committee. Patients selected using the first set of exclusion criteria were further separated into groups according to the order of their inclusion in the study. The first set of exclusion criteria included an anovulatory cycle, sperm obtained by biopsy, and endometriosis (unpredicted). The second set of exclusion criteria eliminated a certain number of patients, and the numbers were compensated by inclusion of more patients from the waiting list. Exclusion using the second set of criteria was performed during the stage after oocyte pickup and progress of the co-culture, and it included ovarian hyperstimulation syndrome (ET was abandoned), unpredicted severe sperm problems, unsuccessful erythrocyte removal from cumulus mass, and other problems such as fertilisation failure or cleavage failures resulting in unsuccessful development of embryos.

Of the 192 couples who were initially included in this case control study, 15 were eliminated after applying the first set of exclusion criteria; thus, of the 177 couples included for the oocyte pickup and co-culturing stage, examination for the second set of exclusion criteria disqualified 31 couples from the study. Finally, 133 couples completed this case control study, as shown in Figure 1.

After ovarian stimulation, oocyte pickup was performed (3), follicular fluid samples were inspected, and CCOCs were collected using a stereomicroscope and placed in human tubal fluid (HTF) (Quinn's Advantage™ Protein Plus Fertilization HTF Medium, SAGE®; CooperSurgical Company, USA). CCOCs were rinsed in HTF, those belonging to group 1 were collected in a different HTF pool (300 μL), and an intact cumulus mass (ICM) was separated from the CCOC using insulin injector needles under the stereomicroscope. The separated ICMs were collected in cleavage medium (Quinn's Advantage, Cleavage Medium; SAGE®; CooperSurgical Company, USA). After ICSI, 2-3 oocytes were placed in a droplet (80-90 μL) containing 1 ICM. Pronucleus control was performed at 18-22 hours after injection. Oocytes were placed on a new droplet with the same ICM. When incu-

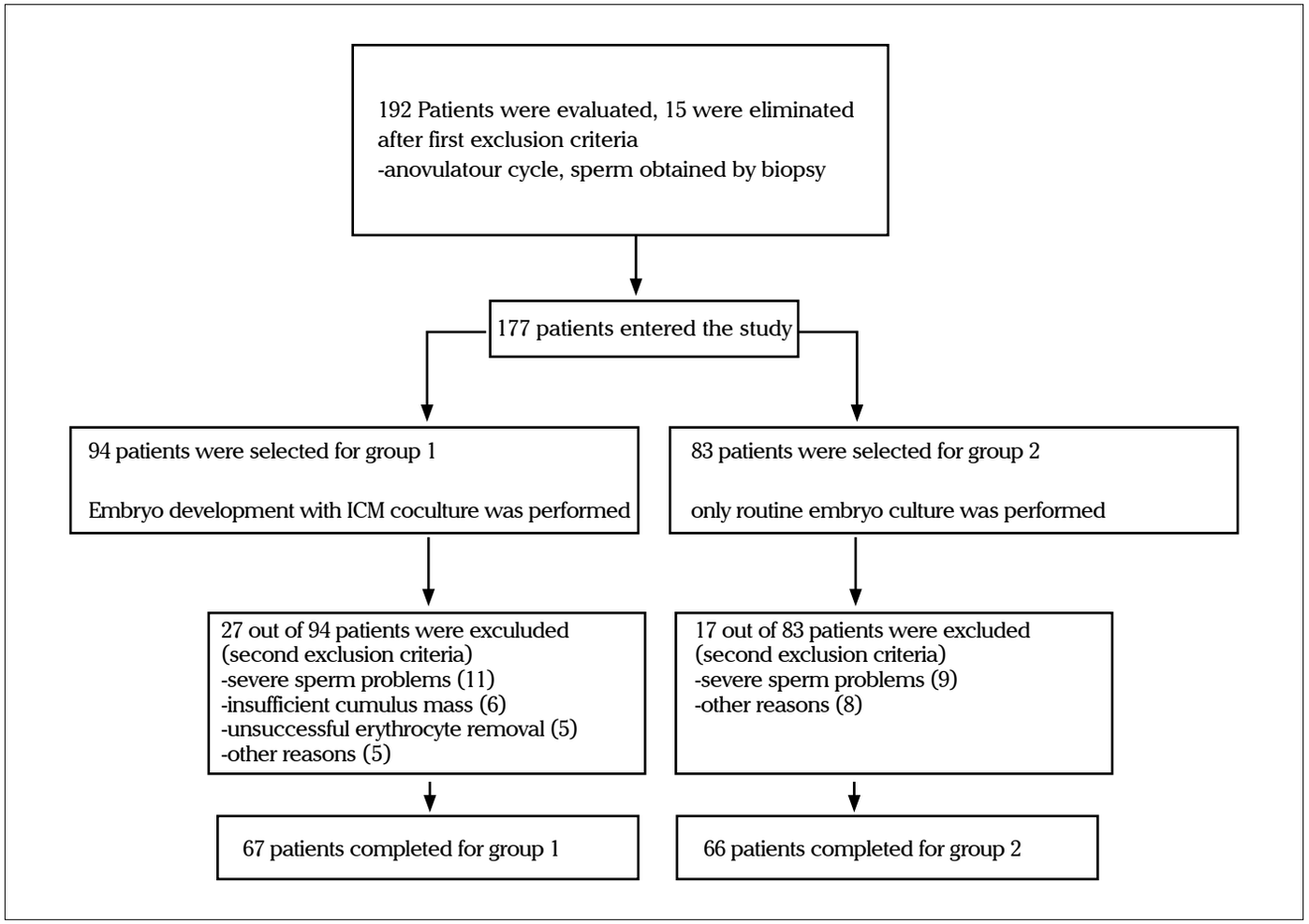

Figure 1. Patient flow chart. The first set of exclusion criteria was applied before the oocyte pickup (OPU) stage, and the second set of exclusion criteria, after OPU. The most frequent 'other reasons' for second exclusion were ovarian hyperstimulation syndrome, which indicated delay of embryo transfer. Remaining reasons included fertilisation failue or cleavage arrest. Abbreviation, ICM: Intact Cumulus Mass

bation was extended for 3, 4, or 5 days, oocytes were placed in a blastocyst medium (Quinn's Advantage, Blastocyst Medium SAGE®; CooperSurgical Company, USA) on the morning of day 3.

Embryo Loading Procedure

For group 1, cultured embryos that were ready for transfer to the uterus were loaded onto the catheter together with the ICM. In cases in which a cumulus mass was embedded with more than the desired number of embryos, separation was performed with the help of insulin injector needles under the stereomicroscope. Although no standard amount of cumulus mass was used, it was generally 10-15 times larger than the transfer embryo mass.

Samples of group 2 were not treated with the addition of cumulus mass and were subjected to routine embryo loading.

Embryo Grading

Embryo grading was performed according to the criteria of Veeck (4), with minor modifications due to development dates and Grade 1 representing the best morphology, so that Grade 1: Embryo with blastomeres of equal size; no cytoplasmic fragments, Grade 2: Embryo with blastomeres of equal size; minor cytoplasmic fragments or blebs, Grade 3: Embryo with blastomeres of distinctly unequal size; no cytoplasmic fragmentation, Grade 4: Embryo with blastomeres of equal or unequal size; major cytoplasmic fragments and numerous blebs and Grade 5: Embryo with few or no recognizable blastomeres; major cytoplasmic fragmentation.

Embryos were also evaluated for the numbers of blastomeres on the basis of the timing of the evaluation, as shown in Table 1, where a grade of 5 represents the highest quality and 1 represents the lowest quality.

The percentage of high quality embryos was calculated according to the number of retrieved metaphase 2 (MII) oocytes per patient (for example, if 8 MII oocytes were retrieved from a patient and only 2 of them reached a high grade form just before embryo transfer, the percentage of high quality embryos was 25%).

Statistical analysis of continuous variables of patient and oocyte characteristics was performed using independent sample T-tests. Implantation rates and positive embriyo cardiac activity confirmed via ultrasound were compared using Pearson chi-square analyses. Statistical significance was determined by a probability (p) value less than 0.05.

Table 1. Expected blastomere number/characteristics according to days. Modifications were introduced when blastomere number was lower than expected on the observation date; a number was subtracted from quality estimation (such as minus 1 or minus 2) as a missed blastomere number

Observation day	Blastomere number/status
1	0-2
2	2-4
3	4-8
4	8-Precompact
5	Precompact-Hatched

Results

The characteristics of the patients and collected oocytes are described in Tables 2 and 3, respectively. The data show similar characteristics among control and study groups with respect to evaluation of infertility treatment and properties of the collected oocyte.

Table 2. Details of patient characteristics (NS: Non-Significant)

Characteristic	G1 With Cumulus	G2 Without Cumulus	P Value	Significance
Number of Patients	67	66		
Age	32.8 (5.2)	31.4 (5.1)	0.128	NS
Thickness of endometrium				
During transfer (mm)	10.8 (1.8)	11.1 (1.6)	0.347	NS
Estradiol level during hCG administration (pg/mL)	1725.8 (580.6)	1855.9 (646.7)	0.224	NS
FSH Admin.	1.9560 (508)	1.9182 (423)	0.642	NS
Body Mass Index	24.1 (2.8)	23.3 (2.6)	0.139	NS

Table 3. Details of collected oocyte characteristics (NS: Non-Significant)

Characteristic	G1 With Cumulus	G2 Without Cumulus	P Value	Significance
Number of Patients	67	66		
Mean (SD) No. of Retrieved M2 Oocytes per patient	9.3 (3.9)	9.1(3.7)	0.845	NS
[Mean (SD)]	86.4%	85.8%		
No. total retrieved M2 oocytes	(624/722)	(602/701)		
No. total retrieved oocytes				

Table 4. Comparison of embryo development and pregnancy properties of G1 and G2

Characteristic	G1 With Cumulus [Mean (SD)]	G2 Without Cumulus [Mean (SD)]	P Value	significance
Number of Patients	67	66		
Fertilization rate [Mean (SD)]	78.2 (19.1)	79.5 (7.3)	0.681	NS
Percent of high quality embryos	35.3 (14.8)	27.6 (14.5)	0.03	S
Number of high quality embryos	3.1 (1.4)	2.4 (1.1)	0.03	S
No. of embryos transferred	2.4 (0.6)	2.3 (0.5)	0.281	NS
Day of embryo transfer	3.2 (0.8)	3.4 (0.8)	0.268	NS
Implantation	36 (53.7%)	23 (34.8%)	0.028	S
Ultrasound confirmation of gestational sac	30 (44.7%)	18 (27.2%)	0.047	S
Twin Pregnancy	3	3		
Triplet Pregnancy	1	0		

S: Significant, NS: Non-Significant

Table 5. Main components of the ECM of CCOC (6-9)

hyaluronan
proteoglycans
serum-derived hyaluronan-associated proteins
heparan sulphate
condroitin sulphate
inter-α-trypsin inhibitor
cumulus matrix
hyladerin
fibronectin
tenascin-c
laminin
Serum-derived HA-associated protein
collagen IV
versican
TSG-6
PTX-3

PTX-3: pentraxin-3; TSG-6: TNF-α-stimulated gene/protein 6; HA: hyaluronic acid

There were no differences between the groups concerning fertilization rates of 78.2 (19.1) and 79.5 (17.3) of group 1 (G1) and group 2 (G2), respectively.

The percent values of high quality embryos were 35.3%±14.8% and 27.6%±14.5% for the cumulus co-cultured and without cumulus cultured groups, respectively. There were significant differences (p=0.03) between groups with regard to high quality embryo development. The numbers of high quality embryos were also significantly different between the 2 groups, with 3.1±1.4 and 2.4±1.1 embryos in the study and control groups, respectively (p=0.03).

The number of transferred embryos and transfer dates showed similar properties in both groups. Statistically, the biochemical implantation and ongoing pregnancy rates confirmed by positive cardiac activity via ultrasound were significantly different (p=0.028 and p=0.047, respectively) in the 2 groups. Implantation rates in study and control groups were 53.7% and 34.8%, respectively. Ongoing pregnancy rates based on cardiac ultrasound confirmation were 44.7% and 27.2% in the study and control groups, respectively. There were 3 sets of twins in each of the 2 groups, while 1 triplet pregnancy occurred in the study group. Our results are described in Table 4.

Discussion

The present study showed that the co-culture of embryos with ICM results in a higher percent of high quality embryos. Furthermore, transfer of the co-cultured embryo to the uterus with ICM results in a significant increase in the implantation and pregnancy rates. The present technique does not involve additional effort during oocyte manipulation, and only adds 45-90 seconds to the procedure for the cutting of cumulus bodies. A higher pregnancy rate can be achieved with no extra cost to the patient.

Our preliminary study (5), in which the properties of embryos generated with and without CCOC were evaluated in 100 infertile couples (50 in the study group and 50 controls), revealed positive results with regard to embryo development and ongoing pregnancy rates (36% for the control group and 46% for the CCOC-added group), when cumulus cells were added to the culture environment. In this initial study, cumulus cell addition was not introduced during the ET procedure. The results of this preliminary study stimulate further research aimed at assessing the effects of cumulus co-culture and developing improved techniques.

During the natural development of the embryo and fertilization in the oviduct, the oocyte is surrounded with cumulus cells and a special ECM composed mainly of hyaluronan, cross-linking proteins, and proteoglycans (6-9). A partial list of the components of the ECM of CCOCs is shown in Table 5.

The interaction and close contact between the cumulus cells and developing oocyte are characterized by the presence of secreted factors (10). The interaction between the oocyte and surrounding cumulus cells is controlled by the oocyte, and this interaction ultimately determines oocyte quality (11), while the cumulus cells play a significant role in the protection of the developing embryo (12). In addition, there is a T cell population detected inside the cumulus cell mass which enhances embryo development (13). Considering that the effects of fertility treatments may not be exactly the same in natural and ovarian-stimulated cycles (14), the present study used subjects that had received hormone treatment for controlled ovarian stimulation.

Based on our observations, the relationship between the embryo and the ICM can take several forms:

- Embryos may be embedded in the ICM, and in this case, the embryos were detached from the cell culture dish and surrounded by cumulus cell layers and extracellular matrix, and a gel-like substance may surround the embryo;
- ICM may be dispersed and the cumulus cells might attach to the dish surface in a monolayer, with small cumulus cell clusters floating in the droplet, while the embryo remains in contact with the culture dish surface; or
- Embryos and ICM stay separated and are not in contact. Various intermediate forms between the above-described 3 situations were found, and while some droplets were clearly defined, others existed in an intermediate form.

Stereomicroscopic observations of the embryos embedded in the ICM showed that embryos were inside a gel-like mass. This gel-like substance was mainly composed of hyaluronan. A limited number of excess embryos (surplus embryos after transfer) embedded in ICM were stained with Toluidine blue (0.05%), and some local polychromatic areas (light magenta) were observed in the extracellular environment (unpublished data).

One limitation of the present study was the visualization of embryos in the study group using stereo- and inverted microscopes due to the surrounding layer of cumulus cells, which raised the embryos above the surface of the dish, making focusing difficult and delaying the inspection of droplets.

Signalling interactions between the granulosa, cumulus cells, local extracellular matrix, and oocytes have been well documented in studies on humans, rat, mouse, bovine, and swine. In most of these studies, a genetic site for this interaction was revealed (15), and some of the benefits of this relationship were found to be due to the antioxidant effect of cumulus cells (12). There is a study in the literature (16) using cumulus cells for co-culture and embryo transfer, but in their study they separated cumulus cells with hyaluronidase enzyme treatment and so used monolayer cultivation. In our study we gave special importance to avoiding enzyme contact with the cumulus mass, because cumulus cells with their intact extracellular matrix seem more physiological.

Signalling factors can activate or inhibit processes, and they can affect the expression of specific receptors at a certain time or due to the activation of a different process (17).

The specific definition of granulosa, cumulus, and oocyte interactions or mechanisms is beyond the scope of this study, and these parameters depend on the developing technology, which can change our understanding of these processes.

The results of the present study suggest that the development of a microinjection technique where cumulus cell layers are not removed may result in the improvement of the applications of assisted-reproduction techniques.

Conflict of interest

No conflict of interest was declared by the authors.

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Diagnosis and treatment of deep-vein thrombosis and approach to venous thromboembolism in obstetrics and gynecology

Kadın hastalıkları ve doğum alanında venöz tromboembolizmine yaklaşım ve derin ven trombozu tanı ve tedavisi

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Abstract

Deep vein thrombosis (DVT) is a common condition in which the approach to its diagnosis has evolved over the years. Currently, an algorithm strategy combining pre-test probability, D-Dimer testing and compression ultrasound imaging allows for safe and convenient investigation of suspected lower-extremity thrombosis. Patients with low pre-test probability and a negative D-Dimer test result can have proximal DVT excluded without the need for diagnostic imaging. The mainstay of treatment of DVT is anticoagulation therapy, whereas interventions such as thrombolysis and placement of inferior vena cava filters are reserved for special situations. The use of low-molecular-weight heparin (LMW) allows for outpatient management of most patients with DVT. The duration of anticoagulation therapy depends on whether the primary event was idiopathic or secondary to a transient risk factor. More research is required to optimally define the factors that predict an increased risk of recurrent DVT to determine which patients can benefit from extended anticoagulant therapy. DVT is also a serious problem in the antenatal and postpartum period of pregnancy. Thromboembolic complications are the leading cause of both maternal and fetal morbidity and mortality. The incidence of venous thromboembolism during normal pregnancy is six-fold higher than in the general female population of childbearing age. The treatment of DVT during pregnancy deserves special mention, since oral anticoagulation therapy is generally avoided during pregnancy because of the teratogenic effects in the first trimester and the risk of fetal intracranial bleeding in the third trimester. LMW heparin is the treatment of choice for DVT during pregnancy. If acute DVT occurs near term, interrupting anticoagulation therapy may be hazardous because of the risk of pulmonary embolism. In this situation, placement of a retrievable inferior vena cava filter must be considered. However, there is no consensus as to what the appropriate dose should be and whether anti-Xa levels need to be monitored.

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Key words: Venous thrombosis, heparin, low- molecular- weight: heparin, anticoagulants, partial thromboplastin time, thromboembolism in pregnancy

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Özet

Derin ven trombozları (DVT), teşhis yaklaşımlarının yıllar içinde büyük değişimler ve gelişmeler gösterdiği sık karşılaşılan bir durumdur. Son zamanlarda, pre-test probabilite, D-Dimer testi ve kompresyon ultrason görüntülemesini kombine eden bir algoritma stratejisi, alt ekstremite trombozlarından şüphelenilen olgularda hem güvenli hem de kullanışlı araştırma imkanı sağlamaktadır. Pre-test probabilitesi düşük, D-Dimer testi negatif olan hastalarda diagnostik görüntülemeye ihtiyaç olmadan, proksimal DVT dışlanabilir. DVT tedavisinin dayanak noktası antikoagülasyon tedavisidir, bununla birlikte tromboliz ve inferiyor vena cava filtreleri gibi girişimler özel durumlara mahsus tedavilerdir. Düşük moleküler ağırlıklı heparin kullanımı DVT'li pekçok hasta için ayaktan tedavi imkanı sağlar. Antikoagülasyon tedavisinin süresi primer olayın idiopatik ya da geçici bir risk faktörüne sekonder oluşuyla ilişkilidir. Tekrarlayan DVT'ler için yüksek risk taşıyan faktörlerin, hangi hastaların uzun süreli antikoagülasyon tedavisinden fayda görebileceğinin optimal olarak belirlenebilmesi için daha çok araştırmaya ihtiyaç vardır. DVT hamileliğin antenatal ve postpartum peryodlarında da ciddi bir problemdir. Tromboembolik komplikasyonlar hem maternal hem de fetal morbidite ve mortalitenin başlıca nedenlerindendir. Normal bir hamilelikte tromboemboli insidansı, doğurganlık yaşlarındaki genel bayan popülasyonuna oranla altı kat daha fazladır. Hamilelik döneminin ilk trimesterindeki teratojenik etkilerinden ve üçüncü timesterde fetal kafa içi kanama riskinden ötürü hamilelikte oral antikoagülan tedaviden kaçınılması nedeniyle, hamilelik döneminin DVT tedavisi özel önem arz eder. Düşük moleküler ağırlıklı heparin hamilelikte DVT tedavisinde tercih edilen tedavidir. Term dönemde akut DVT gelişmişse, pulmoner emboli gelişme riskinden ötürü antikoagulasyon tedavisinin kesilerek ara verilmesi sakıncalı olabilir. Bu durumda erişilebilir inferiyor vena cava filtresi uygulanımı mutlaka dikkate alınmalıdır. Ne var ki, uygun dozun ne olması gerektiği ve anti-Xa seviyelerinin monitorizasyonunun gerekliliği gibi konularda tam bir konsensus mevcut değildir.

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Anahtar kelimeler: Venöz tromboziz, heparin, düşük moleküler ağırlıklı heparin, antikoagülan, parsiyel tromboplastin zamanı, hamilelikte tromboembolizm

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Definition

Deep vein thrombosis (DVT) is a systemic disease which can be seen in any location in the venous system. In 1856, Virchow described the major causes of DVT as, venous stasis, hypercoagulation and intimal damage. Factors increasing venous stasis such as; long lasting immobilization, varicosities, obesity, atrial fibrillation, factors increasing hypercoagulability; factor V Leiden mutation, homocystinuria, protein C or S deficiency, pregnancy, surgery, malignancy, hyperlipidemia and factors increasing the incidence of intimal damage; post operation, intravenous drug abuse, venous catheter insertion cause increased risk for DVT. Clinical findings and symptoms are sometimes insufficient to diagnose a venous thromboembolic event; so some objective tests may be needed for diagnosis. Risk factors, clinical findings, diagnostic methods, complications and treatment modalities of deep vein thrombosis are described in this review.
Underdiagnosis of DVT or pulmonary embolisation (PE) causes a high risk of short-term and long-term morbidity and mortality as well as DVT progression, new PE and long-term tissue damage, especially in the lower leg. A postphlebitic syndrome (PPS) occurs in one third of distal DVT, in contrast to one half in cases of proximal DVT (1).
DVT has an estimated annual incidence of 67 per 100000 among the general population (2, 3). DVT can lead to complications such as postphlebitic syndrome, pulmonary embolism and death. Despite adequate therapy, 1% to 8% of patients developing PE will die, (4, 5) whereas others will experience long-term complications such as postphlebitic syndrome (40%) and chronic thromboembolic pulmonary hypertension (4%) (6). Although anticoagulant therapy decreases the risk of recurrent thrombosis, the treatment also increases the risk of major hemorrhage. Before 1995, the approach was to image all patients with suspected DVT and to repeat tests 1 week later if results were negative (7, 8). This approach was inefficient, since only 10%-25% of patients with suspected DVT were found to actually have the disorder, and results of serial tests were usually negative (8, 9). Over the last 10 years, new strategies for diagnosing and treating suspected DVT have been introduced.

Diagnosis

Single symptoms (edema, pain, sensation of tension, cyanosis and increased protuberance of veins) and classical clinical signs (Homans, Sigg, Payr, Bisgaard, et al.) show a sensitivity of 60-90% for DVT in outpatients but are not very specific for DVT (9).

Imaging tests

In recent years the most important diagnostic technique in DVT is Doppler ultrasonography. Compression ultrasonography (CUS) is now the imaging test of choice for diagnosing DVT. Lack of compressibility of a venous segment is the diagnostic criterion used, but the addition of Doppler (including colour flow) can be useful for accurately identifying vessels and confirming the compressibility of a particular segment.
CUS is the method of choice for detection or exclusion of a symptomatic DVT. In contrast to phlebography (gold standard), CUS has been shown to reach a sensitivity of 95% and specificity of 94% for proximal veins (iliofemoral and popliteal veins) (10). Sensitivity for distal veins (paired lower leg veins, calf muscle veins) was lower. This data has to be reviewed with caution because familiarity with the CUS method has increased during recent years and further new comparison between CUS and phlebography are lacking.
In many centres, ultrasound testing is limited to proximal veins (from the common femoral vein caudally to the region of the calf veins where they join the popliteal vein), for which the sensitivity is 97% for DVT. For DVT in the calf veins, sensitivity is only 73% (11).

Phlebography

Ascending contrast venograhy is the traditional gold standard test for diagnosing DVT, but is rarely used in clinical practice because it is labour-intensive, requires injection of contrast dye, and is uncomfortable for the patient.

Clinical prediction rules

Although none of the symptoms or sings of DVT is diagnostic in isolation, it has been well established that a clinical prediction rule taking into account signs, symptoms and risk factors can accurately be applied to categorize patients as having low, moderate or high probability of DVT. Alternatively, the same rule can be used to categorize cases as “DVT likely” or “DVT unlikely.” (12). Over 14 studies have demonstrated the reproducibility of this model (13). Patients who are found to have low pretest probability can have DVT safely excluded on the basis of a single negative ultrasound result (9). Thus, serial ultrasound testing can be avoided in this subgroup of patients. The incorporation of plasma D-Dimer testing into diagnostic algorithms can indentify patients who do not require ultrasonography (12).

D-Dimer testing

D-Dimers are end products of the proteolysis of fibrin which was created from fibrinogen by thrombin and then cross-linked by factor XIII. D-Dimers function as a marker for fibrinolysis as well as a marker for increased coagulation activity because coagulation leads to simultaneous activation of fibrinolysis to sustain physiological homeostasis. The site where coagulation takes place cannot be displayed by D-Dimers, as markers are increased with any coagulation in the body. Increased D-Dimers are not always a sign of a clinically important clot and coagulation is also an accompanying phenomenon of many pathophysiological states in the body, e.g. inflammation, trauma, operation, pregnancy, cancer or severe bleeding. Due to this fact, proof of elevated D-Dimers cannot be equated with a thromboembolic disease (14).
When the D-Dimer test is compared to the DVT symptom, it is said to be an important test. D-Dimer is a degradation product of a cross-linked fibrin blood clot. Levels of D-Dimer are typically elevated in patients with acute venous thromboembolism, as well as in patients with a variety of nonthrombotic conditions (e.g., recent major surgery, hemorrhage, trauma pregnancy or cancer) (15). D-Dimer assays are, in general, sensitive but nonspecific markers of DVT. The value of the D-Dimer assay resides

with a negative test result that suggests a lower likelihood of DVT, thus making it a good "rule out" test with the appropriate pretest probability. If applied properly, incorporation of D-Dimer testing into diagnostic algorithms simplifies the management of a patient presenting with suspected DVT.

Diagnostic algorithm for DVT

Diagnostic algorithms combine single methods to an examination sequence which is able to establish a reliable basis for therapeutic decisions with the least effort. The first steps in the examination sequence have a high sensitivity whereas the later steps have a high specificity (16). Selection of method includes special test characteristics, their local availability and their validity. Above mentioned methods (clinical prediction rules, D-Dimer testing, CUS and phlebography) can be combined to a logical algorithm. Figure 1 displays a recommendation. A diagnostic test for thrombophilia during an acute DVT is rarely indicated as the results will have no impact on the immediate therapeutic decision (17-19). Only in a very few cases can results of thrombophilia diagnostic influence the duration of anticoagulation therapy. In an acute descending thrombosis, local reasons for thrombosis shall be exploited. Reasons could be a tumor or anatomical variants and anomalies in vein formation in young patients.In cancer diseases, there is an increased rate of venous thromboembolism, and around 15 % of cases with an acute DVT have a previous cancer diagnosis at the time of DVT diagnosis. In the case of an idiopathic venous thrombosis, cancer diagnostic work-up is indicated because of its high coincidence, especially after the fifth decade of life (16).

Patients with symptoms compatible with DVT should initially have a determination of pretest probability, using an established prediction model (Figure 2). It is important to take the history first and carry out a physical examination. The model should be applied only if DVT remains a diagnostic possibility. After the clinical pretest probability is determined, a D-Dimer test should be performed. We are using a scoring system for suspected DVT as described in the reference number 20. In our centre, a score of less than 1 (unlikely DVT) by our current model, which incorporates previously documented DVT as a new variable, is sufficient to exclude DVT in patients with a negative moderately sensitive D-Dimer level without ultrasound imaging (20). No D-Dimer assay should be used to exclude DVT in patients having high pretest probability. Clinical assessment and D-Dimer testing have the further advantage of enabling the management of patients with suspected DVT, presented at times when radiographic imaging is not routinely available. Patients with a moderate or high clinical suspicion of DVT may receive an injection of LMW heparin in doses designed to treat acute DVT. Diagnostic imaging can then be arranged on a more elective basis the following day. Since LMW heparin therapy is safe and effective for patients with proven DVT, it provides adequate protection for patients with a suspicion of DVT (20, 21). For patients with low risk of DVT (as determined either by means of a clinical diagnostic model or sensitive D-Dimer test), diagnostic imaging may be delayed for 12-24 hours without the need for anticoagulant coverage (9). The clinical prediction rule was developed and validated predominantly in studies involving outpatients. Pregnant women were not included in these studies. Furthermore, the utility of the D-Dimer test in patients admitted to hospital often with other accompanying comorbidities (e.g., infection, postoperative symptoms) is less, since the D-Dimer assay rarely yields negative results. Finally, if DVT is not a diagnostic possibility, D-Dimer test should not be done, because a positive result may cause the clinician to unnecessarily investigate for DVT rather than investigating the actual cause of leg symptoms. The ideal strategy for diagnosing DVT in patients with previous DVT in the symptomatic leg is still debated. However, results of a randomized trial demonstrated the safety of combining clinical probability, D-Dimer and ultrasound imaging in these patients. The biggest concern with this patient population is false-positive ultrasound results. It is helpful to recognise that acute DVT is usually occlusive, not echogenic, and it tends to be continuous. When it reveals thrombosis that is echogenic, nonocclusive or discontinuous, then chronic DVT should be considered. Serial testing or venography can help to clarify the issue. Previous ultrasound results are helpful for comparison, when available. An increase in clot diameter by 4 mm suggests recurrence, as does extension (22).

Phlebography is not generally available anymore. Due to this lack of availability, qualities of results are sometimes not satisfying. However, in an idealistic case, phlebography is able to detect very small clots, evaluate the status of calf muscle veins, display collateral cycles in total and exclude a thrombosis with high certainty (23-25). Another advantage of phlebography is its objectivity and its comprehensive documentation, which also displays the total anatomy. Disadvantages of phlebography are invasiveness, radiation exposure, possible allergic reactions to the contrast medium and its lack of information on differential diagnosis compared to ultrasonography. Due to these disadvantages, the first line diagnostic tool is ultrasonography. Phlebography is used as a second line when ultrasonography is not able to reliably detect the case of recurrent thrombosis and during preparation of restitution surgery.

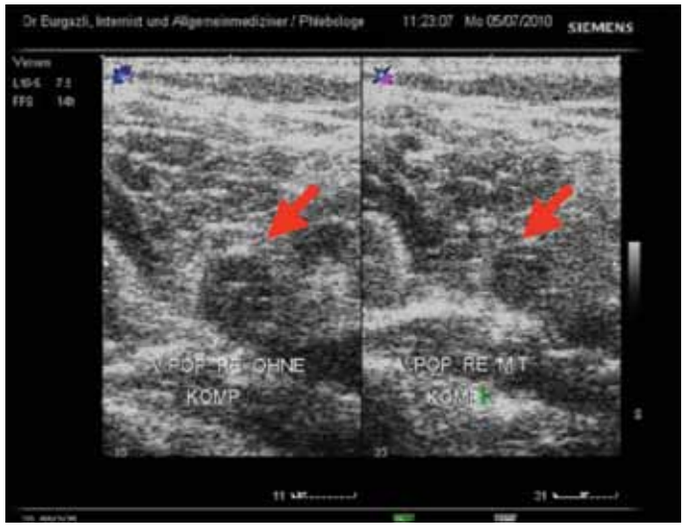

Figure 1. Image with and without compression of right V. popliteal Deep Vein Thrombosis with B mode ultrasonography

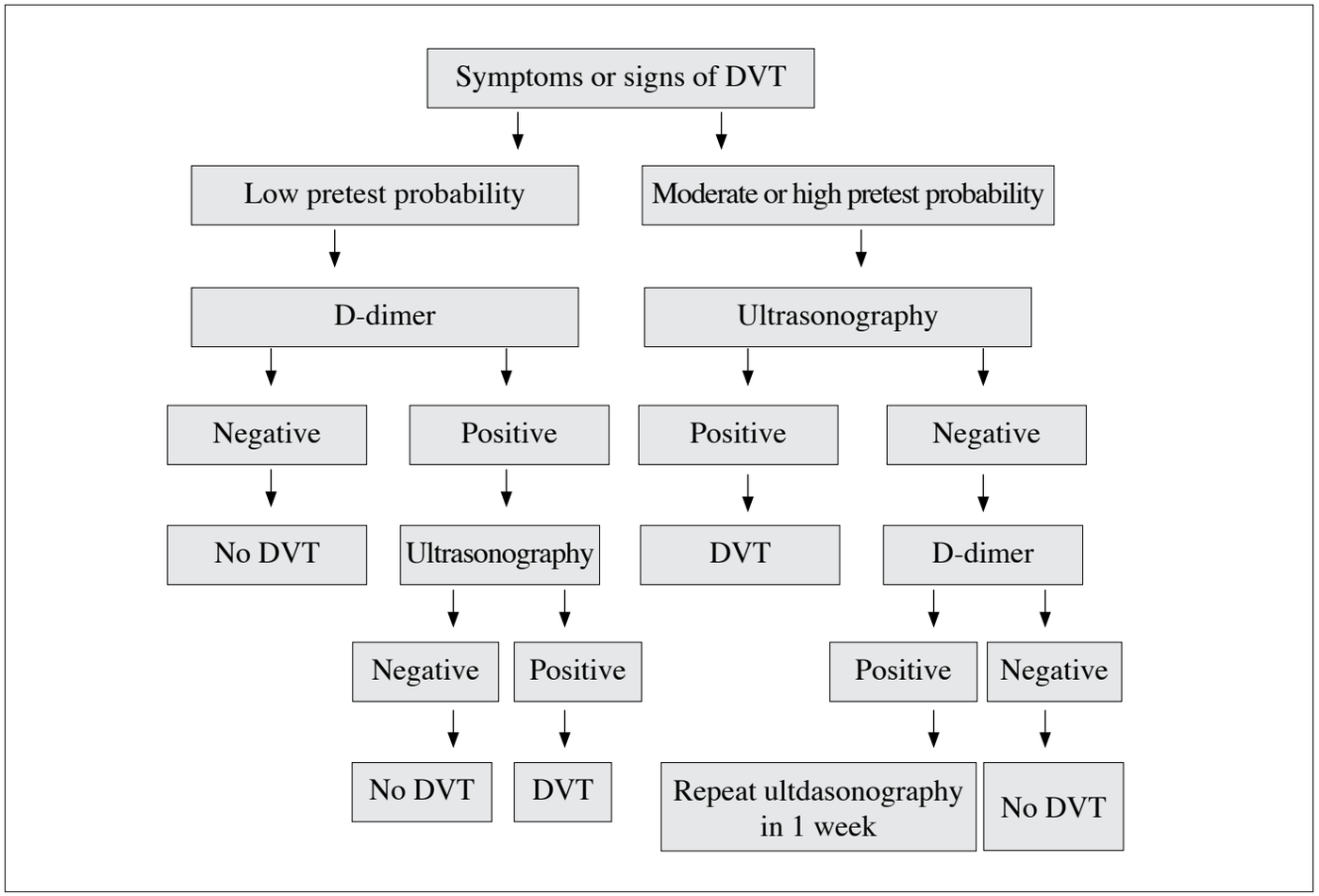

Figure 1. Diagnostic algorithm using D-Dimer testing and ultrasound imaging with suspected DVT

Most diagnostic and treatment studies of DVT have excluded pregnant women, and therefore it is difficult to formulate evidence-based recommendations for this population. Although serial impedance plethysmography has been demonstrated to safely rule out DVT (26), it is not widely used. Results of a small pilot study suggest that a strategy involving serial CUS combined with a moderately sensitive D-Dimer assay is effective in excluding DVT in pregnant women (27). D-Dimer levels are often positive in the later stages of pregnancy, (28, 29) lowering the utility of this test to rule out DVT. Research to develop algorithms to diagnose DVT in pregnant women is ongoing. D-Dimer levels show higher increases in preeclampsia-eclampsia, but these increases are not related with adverse pregnancy outcomes (30).

Treatment

The aim of DVT treatment is to avoid PE and postphlebitic syndrome. Anticoagulation with LMW heparins in therapeutic dosage should start immediately after diagnosis. In case of severe renal failure (creatinin clearance ≤30 ml/min) or during vascular angioplasty procedure, non-fractionated heparin is the medication of choice. LMW heparins cause significantly less probability of a heparin-induced thrombocytopenia (HIT) type 2 than non-fractionated heparins. In case of a contraindication for heparin (e.g. because of previous HIT type 2) LMW heparins can be used instead heparin.

Initial treatment of DVT with parenteral anticoagulation (heparin or LMW heparin) should be performed for at least 5 days combined with the administration of a vitamin K antagonist therapy between day one and two after diagnosis until an INR >2.0 for at least 24 hours.

A compression therapy with compression stockings is reasonable because it is able to decrease frequency and severity of the postphlebitic syndrome. Long-term effects show a 50% decreased incidence of the postphlebitic syndrome at compression pressures between 30 to 40 mmHg.

The goal of the therapy for lower-extremity DVT is to prevent the extension of the thrombus and PE in the short-term and to prevent recurrent events in the long-term. Based on extensive research evaluating the risk of recurrent DVT, guidelines have been established for the duration of anticoagulation therapy. LMW heparin therapy has changed the landscape of treatment of DVT by enabling home treatment and by providing an alternative long-term anticoagulant in cases where warfarin is less effective or contraindicated. The following applies in the treatment of proximal lower -extremity DVT, since there is little evidence to formulate recommendations for isolated DVT in calf veins.

Cancer patients with thrombosis should be treated with LMW heparin for 3 to 6 months instead of vitamin K antagonist. Type and duration of the following anticoagulation therapy depends on the cancer disease activity and the bleeding risk. Secondary prevention with vitamin K antagonists is complicated by interactions between vitamin K antagonists and chemotherapeutical cancer treatment, by liver dysfunction, transient thrombocytopenia and accompanying infections and their treatment. Due to these interactions, LMW heparins appear to have a greater benefit. Further, it is reported in randomized studies that venous thromboembolism risk is halved by LMW heparins in comparison to vitamin K antagonist without an increased bleeding risk (31).

Initial choice of anticoagulation

Initial therapy must involve therapeutic doses of either unfractionated heparin or LMW heparin. Initial treatment with oral anticoagulant therapy alone is unacceptable (32). The ease of administration and efficacy of LMW heparin makes it the preferred choice of anticoagulant, whether given on an outpatient or inpatient basis. In a meta-analysis comparing the effectiveness of LMW heparin at a fixed dose with unfractionated heparin at an adjusted dose, significantly fewer deaths, major hemorrhage and recurrent venous thromboembolism were reported to occur with LMW heparin (33).

Thus, the current standard of care is to administer weight-adjusted LMW heparin once daily, for 5-7 days as the initial treatment. It remains unknown whether it is better to administer LMW heparin once or twice daily. The results of a meta-analysis suggested that hemorrhage and recurrent venous thromboembolism were less likely to occur with twice daily dosing, but the 95% confidence interval on the odds ratio crossed 1.0 (34). Since LMW heparin is predominantly excreted by the kidneys, unfractionated heparin should be used in patients with significant renal dysfunction. A newer agent is the synthetic pentasaccharide fondaparinux, which is at least as effective and safe as LMW heparin in the treatment of DVT (35). Fondaparinux can be considered as an alternative agent for the treatment of DVT with the added benefit that, to date, heparin-induced thrombocytopenia has not been reported with this agent.

Long-term treatment

The long-term treatment in DVT prevents new attacks. For the majority of patients with DVT, oral therapy with vitamin K antagonists (e.g. warfarin) is very effective for long-term prevention of recurrent thrombosis (36). Although the initial treatment of DVT is similar for most patients, the duration of long-term treatment varies depending on the perceived risk of recurrent DVT. The risk can be classified into the following 5 categories:

- First proximal DVT occurs in the context of a transient risk factor (e.g. surgery or trauma).In this situation, the risk of recurrence is very low and a limited duration of therapy (3 months) is adequate (37, 38).
- First DVT occurs in the context of active malignant disease, which is an ongoing risk factor. Patients with malignant disease have a higher incidence of recurrent thrombosis and bleeding complications while receiving oral anticoagulation therapy following a first thrombotic event (39, 40). This is probably due to the prothrombotic state associated with cancer and to the difficulty of managing oral anticoagulant therapy with concomitant drugs, erratic oral intake and liver dysfunction. Researchers with the CLOT trial (31) have shown that long-term anticoagulation therapy with LMW heparin is more effective than warfarin in preventing recurrent venous thrombosis without a statistically significant increase in bleeding risk.
- First DVT occurs in the context of a thrombophilic defect. These defects include factor V Leiden, prothrombin gene mutation, deficiencies in protein C, protein S and antithrombin, increased factor VIII levels, hyperhomocysteinemia and elevated antiphospholipid antibody levels. Many of these defects are associated with an increased risk of first DVT. Patients with persistently elevated antiphospholipid antibody levels determined by either ELISA or clotting assays have a 2-fold higher relative risk of recurrence within 4 years after stopping anticoagulation therapy for a first DVT than those without this thrombophilia (41).
- Recurrent DVT. After a second recurrence of DVT, the risk of further thromboembolic events following the discontinuation of anticoagulation therapy is felt to be excessive if only 6 months of oral anticoagulation therapy is administered (42).
- First DVT occurs in the absence of temporary or identifiable on going risk factors for thrombosis (idiopathic). Six months is considered a minimum duration for anticoagulation therapy in these patients, while continuing for longer is effective in preventing thrombosis. However, the risk of recurrent venous thromboembolism in the first year after stopping anticoagulation therapy is about 10%, regardless of when the therapy is stopped after 6 months (43).

In addition to the thrombophilic defects described previously, two factors have been shown to increase the risk of recurrence after stopping anticoagulation therapy. Residual thrombosis (seen on a follow-up ultrasound scan 3 months after an initial event) increases the risk of recurrence (odds ratio 2, 4) (44). One-third of the recurrences occur in the initially unaffected leg, which suggests that residual DVT is a marker of systemic hypercoagulability In one study, elevated D-Dimer levels 1 month after stopping anticoagulation therapy were associated with an elevated risk of recurrent thrombosis in all but cancer-related thrombosis (45).

Intensity of anticoagulation therapy

The standard intensity of oral anticoagulation therapy is an international normalized ratio (INR) of 2 to 3. In patients who have antiphospholipid antibody-related thrombosis, it has long been considered that higher intensity anticoagulation therapy is needed to prevent recurrence (46). However, results of two randomized controlled trials showed that standard anticoagulation therapy is as effective as high-intensity treatment, even in the subgroup of patients (47, 48). Therefore, high-intensity anticoagulation therapy is not recommended in any patient with DVT: Maintaining good INR control will decrease the risk of postphlebitic syndrome (49). There has also been debate on the usefulness of long-term low-intensity anticoagulation therapy (INR

1.5-1.9) in preventing recurrent thrombosis, while reducing the risk of bleeding. A large randomized trial has shown that low-intensity anticoagulation therapy is less effective than standard anticoagulation therapy in preventing recurrent thrombosis and does not lower the risk of bleeding (50). Therefore, low-intensity therapy is not recommended.

Upper-extremity DVTs

Upper-extremity DVTs can be subdivided into catheter- and noncatheter-related thrombosis. There is a risk of pulmonary embolism with this condition, and therefore treatment with anticoagulation therapy is generally recommended.

Other interventions

Although anticoagulation therapy is the mainstay of treatment of DVT, thrombolysis and placement of an inferior vena cava filter are the two interventions worthy of mention.

The addition of systemic thrombolysis to standard anticoagulation therapy leads to earlier patency of an occluded vein, however, it does not affect the rate of PE. There is a definite increase in the risk of major hemorrhage; including intracranial hemorrhage, with thrombolysis. Catheter-directed thrombolysis has also been associated with increased risk of bleeding complications. It is unclear whether the earlier recanalization observed with thrombolysis translates into lower rates of the postthrombotic syndrome in the long term (14, 51). Thrombolysis is not generally recommended except in the case of massive DVT, which leads to phlegmasia cerulean dolens and threatened limb loss.

Placement of an inferior vena cava filter in addition to anticoagulation therapy has not been found to prolong survival among patients with DVT: While preventing PE, insertion of a filter increases the risk of recurrent DVT (52, 53).

A retrievable filter is indicated when there is a contraindication to anticoagulation therapy (recent hemorrhage, impending surgery) in patients with newly diagnosed proximal DVT. It remains to be determined whether a retrievable filter in patients at higher risk of death (e.g. limited cardiopulmonary reserve) will lead to a reduction in pulmonary embolism-related death.

Postphlebitic syndrome is a frequent complication of DVT and a major public health issue that has been underresearched. It is unclear who is at highest risk and how best to prevent and treat this complication.

Thrombolytic therapy and surgical embolectomy

Compared with heparin, thrombolysis improves vein patency and reduces the risk of post-thrombotic syndrome, but increases the risk of bleeding, and there is no evidence of a net clinical benefit. Thrombolysis and surgical embolectomy have been used as a limb-saving therapy in patients with extensive proximal DVT and circulatory compromise or venous gangrene (16).

Vena cava filter

Inferior vena cava filters are indicated to prevent PE in patients with DVT who experience embolism despite adequate anticoagulation. Filters do not obviate the need for anticoagulation because they are associated with an increased risk of recurrent DVT. However, the optimal duration of anticoagulation which is deemed safe is uncertain (16).

Deep vein thrombosis in pregnancy

Deep vein thrombosis (DVT) is a serious problem in the antenatal and postpartum periods of pregnancy. Thromboembolic complications are the leading cause of both maternal and fetal morbidity and mortality. The incidence of venous thromboembolism during normal pregnancy is six-fold higher than in the general female population of childbearing age (26).

Pathophysiology

During normal pregnancy there are substantial changes in the hemostatic system, many of which are procoagulant and are thought to be in preparation for the hemostatic challenge of delivery. Normal hemostasis requires a balance between coagulation and fibrinolysis to maintain the integrity of the vasculature. The complex physiological changes evident during pregnancy appear to ensure a constant coagulation-fibrinolysis balance. The balance is maintained, at least partially, by an increase in fibrinolytic activity, but decreases in other factors such as factor XI, and monocyte tissue factor expression may also serve to counter- balance procoagulation changes.

Anticoagulant therapy

Anticoagulant therapy is the standard treatment for DVT but is mostly used in non-pregnant patients. In pregnancy, unfractionated heparin (UFH) and LMW heparin are commonly used. Warfarin therapy is generally avoided in pregnancy because of its fetal toxicity.

LMW heparin has been shown to be as safe and effective as UFH for the treatment of acute venous thrombosis and non life-threatening PE. LMW heparins are anticoagulants of choice, when a rapid anticoagulant effect is required. LMW heparin has several advantages over UFH, including a longer plasma half life, higher bioavailability and a predictable dose response, which enable once or twice-daily dosing, and a more convenient route of administration (16).

The non hemorhagic adverse effects of UFH include heparin-induced thrombocytopenia (HIT) and osteoporosis, which is less with LMW heparin. HIT is a life threatening condition that is associated with the development of antibodies, activating platelets and the coagulation system in the presence of UFH or LMW heparin (54).

The safety of LMW heparin administration for the mother and fetus has not been well established. Enoxaparin has been shown not to cross the placenta and therefore appears safe for the fetus. It is uncertain whether weight adjusted dosage regimens without laboratory monitoring can be used in pregnant women (16).

Diagnosis

Therapy is usually initiated following a clinical diagnosis of venous thrombosis. Therapy is continued while awaiting diagnostic tests such as Doppler ultrasound (compression or duplex

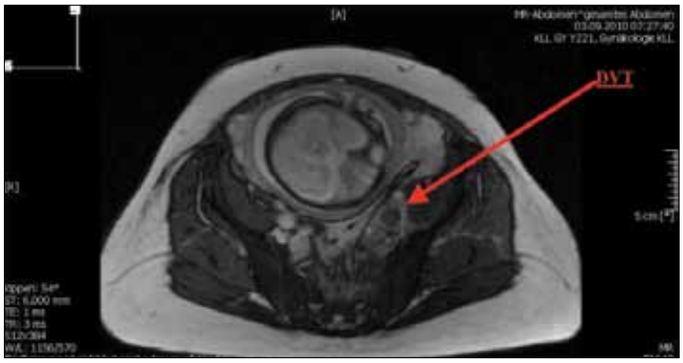

Figure 3. MRI image showing the DVT in pregnancy

scanning) and MRI (Figure 3), which are the best diagnostic methods in pregnancy. D-Dimer, a screening test for DVT with high negative predictive value, is used in non- pregnant patients. Its use in pregnancy is still controversial.

MR-Phlebography is a promising alternative especially in the proximal thigh and pelvis area. Diagnosis of a venous thrombosis during pregnancy should be interdisciplinary. So far there is no algorithm tested for thrombosis during pregnancy but any suspicion should be traced up to exclusion (55).

Mode of administration of anticoagulant

The mode of administration influences the effectiveness of anticoagulant therapy, UFH is subsequently administered subcutaneously. Intravenous administration has a more rapid onset and is therapeutically more reliable than subcutaneous administration. However, intravenous administration is difficult to maintain on a long-term basis.

Subcutaneous injection of LMW heparin leads to less pain due to a smaller injection volume. However, the patient may still suffer from bruises.

Warfarin therapy is easier to administer but is usually avoided in pregnancy due to its teratogenic effect.

Monitoring of anticoagulant therapy

Patients receiving anticoagulant therapy are monitored to observe the therapeutic efficacy as well as side effects. Hemorrhagic complications are clinically assessed. Patients receiving UFH, LMW heparin and warfarin are routinely monitored with activated partial thromboplastin time (APTT), anti-Xa activity and INR respectively. Platelet count is occasionally assessed especially in patients receiving UFH and LMW heparin. Patient knowledge of proper injection technique, prevention and recognition of complications should be considered during follow up.

Conflict of interest

No conflict of interest was declared by the authors.

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Periodontal diseases as an emerging potential risk factor for adverse pregnancy outcomes: A review of concepts

Olumsuz gebelik sonuçları için yeni ortaya çıkan potansiyel bir risk faktörü olarak periodontal hastalıklar: kavramların gözden geçirilmesi

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Abstract

Preterm birth is the leading perinatal problem with subsequent morbidity and mortality in developed as well as developing nations. Among the various possible environmental, genetic, demographic, psychosocial and obstetric risk factors responsible for premature labor, poor oral health with periodontal infection has also emerged as a potential and modifiable risk factor for preterm low birth weight babies. The infected periodontium is regarded as a reservoir for periodontopathic bacteria, mainly gram negative anaerobes that serve as a source of endotoxins and lipopolysaccharides, proinflammatory cytokines and prostaglandins that enhance uterine muscle contraction leading to preterm low birth weight. Also, the progression of periodontal disease during pregnancy appears to increase the fetal growth restriction, irrespective of baseline periodontal disease status. Thus, identification and treatment of periodontal disease should be considered an important intervention strategy as a part of prenatal care to reduce adverse pregnancy outcomes. (J Turkish-German Gynecol Assoc 2011; 12: 176-80)

Key words: Adverse pregnancy outcomes, periodontal diseases, preterm low birth weight

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Özet

Hem gelişmiş hem de gelişmekte olan ülkelerde, preterm doğum ile bunu izleyen morbidite ve mortalite önde gelen perinatal problemdir. Prematür doğumdan sorumlu çeşitli olası çevresel, genetik, demografik, psikososyal ve obstetrik risk faktörleri arasında, periodontal enfeksiyonla birlikte olan kötü ağız sağlığı da preterm düşük doğum ağırlıklı bebekler için potansiyel ve değiştirilebilir bir risk faktörü olarak ortaya çıkmıştır. Enfekte olmuş periodontium, preterm düşük doğum ağırlığına yol açan uterus kas kasılmalarını artıran endotoksinler, lipopolisakkaridler, proinflamatuvar sitokinler ve prostaglandinler için bir kaynak görevi gören başlıca gram negatif anaeroplar olmak üzere periodontopatik bakteriler için bir rezervuar olarak görülmektedir. Aynı zamanda, gebelik sırasında periodontal hastalığın ilerlemesi başlangıçtaki periodontal hastalık durumuna bakmaksızın fötal büyüme sınırlamasını artırıyor görünmektedir. Sonuç olarak, adverse gebelik sonuçlarını azaltmak için prenatal bakımın bir parçası olarak periodontal hastalığın tanımlanması ve tedavi edilmesi önemli bir müdahale stratejisi olarak göz önünde bulundurulmalıdır. (J Turkish-German Gynecol Assoc 2011; 12: 176-80)

Anahtar kelimeler: Advers gebelik sonuçları, periodontal hastalıklar, preterm düşük doğum ağırlığı

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Introduction

Gingivitis and periodontitis are two periodontal conditions of significance during pregnancy. Gingivitis is an infectious and inflammatory condition of the gingiva with prevalence estimates during pregnancy ranging from 30% to 100% (1). Periodontitis is a more severe condition that results in the destruction of tooth-supporting structures affecting 5% to 20% of pregnant women (2).

A mother giving birth “early” due to sudden trauma or stress is a worldwide problem in all population groups. Birth weight is the most important determinant of the chances of survival, growth and development of a newborn infant. The international definition of low birth weight adopted by the Twenty-ninth World Health Assembly in 1976 is a birthweight of ‘‘less than 2500 g’’. Birth weights are considered to be very low when less than 1500g, and extremely low if less than 1000g. Low birth weight also results from both a short gestational period and retarded intrauterine growth. The normal gestation for humans, full term is 40 weeks. The World Health Organization defines preterm birth as any live birth at less than 37 weeks of gestation. Delivery at less than 32 weeks is termed as very preterm and delivery at less than 28 weeks is extremely preterm. It is generally said that the majority of preterm births are also low birth weight (3).

Birth weight has long been a subject of epidemiological investigations concerning the associated risk factors and public health interventions. The primary cause of low birth deliveries is premature rupture of membranes (PROM). On health grounds, preterm babies suffer from respiratory distress syndrome, periventricular hemorrhage, periventricular leucomalacia, necrotizing enterocolitis, sepsis, patent ductus arteriosus, cerebral palsy, retinopathy of prematurity, mental retardation and cardiovascular malformations.

Recent evidence suggests that maternal periodontal disease is associated with adverse pregnancy outcomes including early pregnancy loss, preterm birth, low birth weight, and preeclampsia (4). Over the last decade, great interest has been generated to support the hypothesis that subclinical infection,

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including maternal periodontal infection, is an important cause of preterm labor. Therefore, the present literature summarizes the concepts and mechanisms concerning periodontal infection and adverse pregnancy outcomes.

Risk factors for preterm and lowbirth weight

Among the various possible environmental, genetic, demographic, psychosocial and obstetric risk factors responsible for premature labor, poor oral health with periodontal infection has also emerged as a potential risk factor during the past decade (Fig. 1). However, it is not clear how these predictors are interrelated, or if multiple factors act synergistically to affect the risk for preterm birth. Since there is a strong potential for confusion, especially in smaller epidemiological studies, it becomes difficult to identify a true risk factor responsible for preterm birth.

Risk factors can be considered primary if they are present before the pregnancy, or secondary if they develop during the course of the pregnancy. Primary risk factors could be black race, young mothers, low socio-economic status, stress or depression, cigarette smoking, low body mass index, low maternal weight gain before pregnancy, previous history of preterm birth or abortion, chronic lung disease, hypertension, diabetes and renal disease. Secondary factors could be no or inadequate prenatal care, in vitro fertilization, pre-eclampsia, elevated fetal fibronectin, alkaline phosphatase, early contractions, vaginal bleeding in the first or second trimester, bacterial vaginosis, especially early in pregnancy, chorioamnionitis, placenta previa and multiple foetuses (5). Probably the most consistent predictor of preterm birth is the history of previous preterm birth (6).

Physiology of labor at term

The mechanisms involved in the initiation of labor in women are not fully understood, although prostaglandins appear to play a crucial role, and prostaglandin E2 can be used to induce human labor. The earliest identified events during labor are increase in the bioavailability of prostaglandin F2a (PGF2a) and the receptors for the hormone oxytocin. Oxytocin is one of the most potent agents that stimulate uterine contractions. Also, increase in oxytocin receptors during labor, the stretching of the cervix and myometrium have been thought to initiate a neurogenic reflex to the neurohypophysis of the pituitary gland, which acts as a positive feedback for oxytocin production (7).

The obligatory precursor for prostanoid synthesis is free arachidonic acid that increases in the amniotic fluid during spontaneous labor. Fetal membranes contain phospholipids and a significant amount of arachidonic acid which is esterified on these phospholipids. These membranes also contain phospholipase A2, which can split arachidonic acid from the phospholipids. It has been suggested that bacterial sources of phospholipase A2 may be significant in the intiation of premature labor (8).

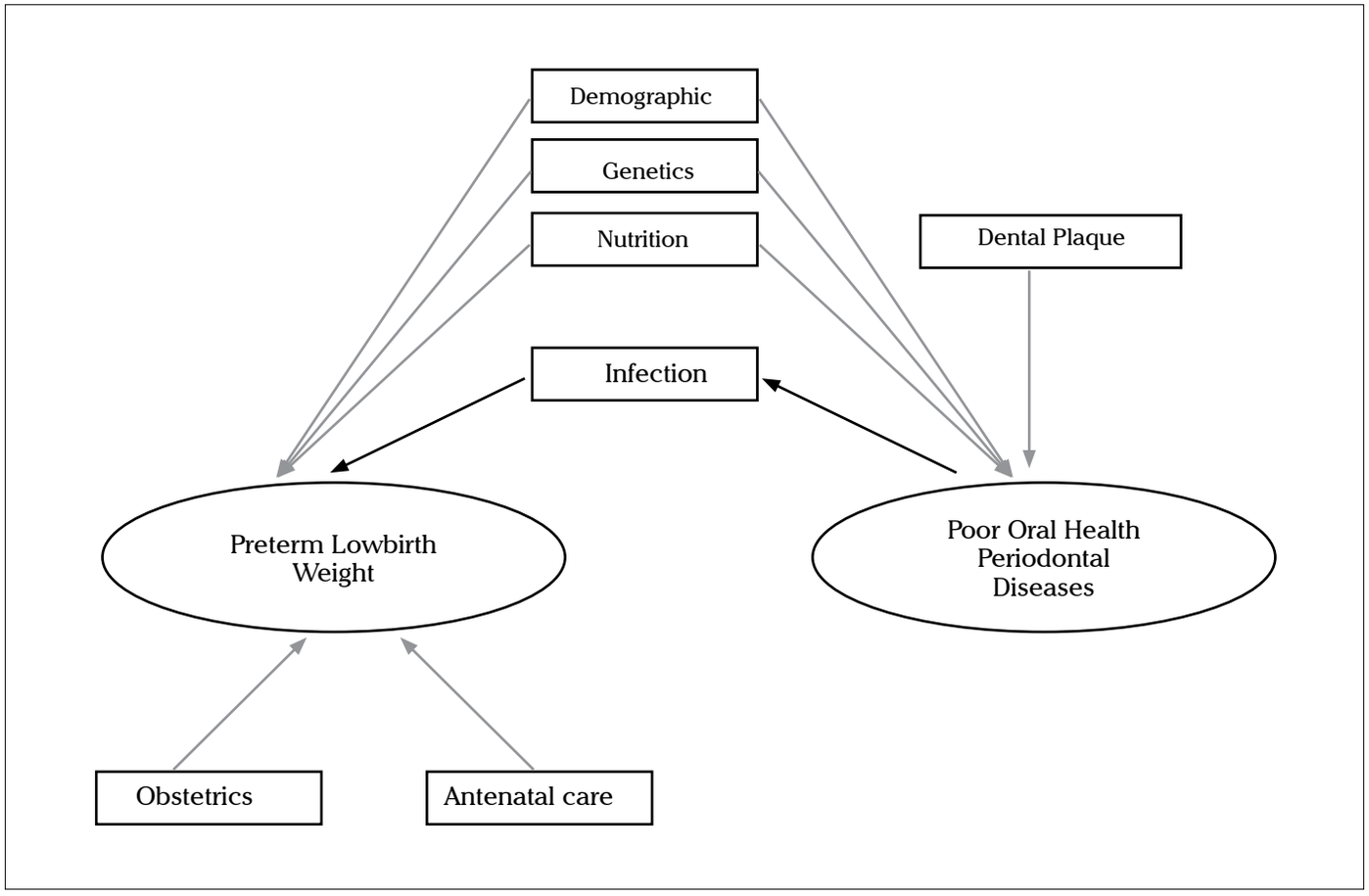

Figure 1. Multifactorial nature of risk factors for preterm labor and their interactions

Mechanism of preterm labor

The possible mechanisms for preterm labor are infection, inflammation, placental ischaemia, hemorhage and stress that interplay to cause preterm rupture of the membranes. (Fig. 2)

There is now evidence linking maternal infection with preterm delivery. Maternal infection could be subclinical or nondetectable cases of bacterial vaginosis, prenatal events unrelated to infection and maternal periodontal infections. Bacterial vaginosis and genitourinary tract infection have been linked with a 60% increase in the risk of preterm delivery. Subclinical infection has also been shown to be linked to preterm birth (9).

Markers of infection in preterm labor and periodontitis

The primary site of infection is the membranes or decidua and not the amniotic fluid during infection induced premature labor. Recent studies have sought potentially more sensitive markers of infection. These markers have been identified in women presenting with risk for preterm labor and periodontitis. The various biochemical markers of infection for preterm birth and periodontitis are:

1. Prostaglandins.
2. Phospholipase A_2 produced by genital tract anaerobes responsible for prostaglandin sysnthesis.
3. Inflammatory cytokines such as IL-6 and tumor necrosis factor. IL-6 is reported to stimulate prostaglandin release by human amnion and deciduas in women with preterm labor associated with infection (10) as well as women who experience spontaneous abortion (11).
4. Bacterial products such as endotoxins that stimulates uterine muscle contractions.
5. Increased fetal adrenal cortisone production.

Thus, combinations of increased prostaglandins production, increased fetal cytokine and chemokine production, bacterial toxins and fetal adrenal cortisone production may lead to myometrial contractions, membrane rupture, cervical ripening and preterm delivery (12).

Periodontal infection and adverse pregnancy outcome

The association between maternal periodontal disease and preterm birth was first reported by Offenbacher in 1996 (13). He conducted a case control study enrolling 124 pregnant or postpartum mothers with and without the presence of periodontal disease. The study concluded that preterm or low birth weight mothers had significantly worse periodontal conditions than respective normal birth weight controls. In a meta-analytic systematic review of 17 articles, Vergnes and Sixou (2007) found a statistically significant association between periodontitis and adverse pregnancy outcomes (14). Guimaraes AN conducted a study to address the possible association between maternal periodontal disease and preterm or extreme preterm birth. The results of the study indicate that periodontal disease in the mothers, besides being associated with preterm birth, as indicated in most literature reports, is also related to extreme preterm birth (15).

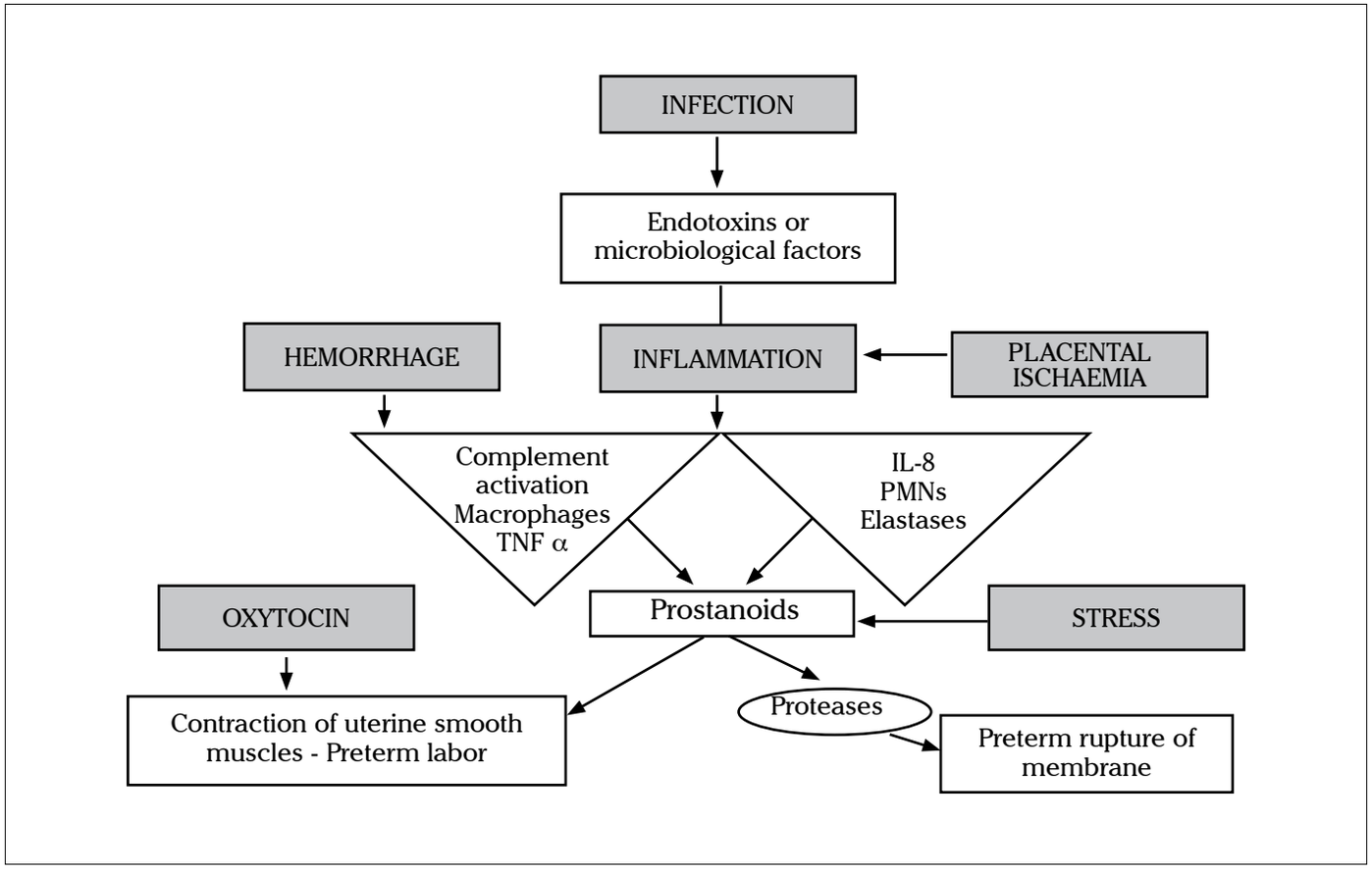

Figure 2. Possible mechanisms of preterm labor

Current understanding of the biological events surrounding preterm labor strongly suggests that prostaglandins and proinflammatory cytokines play a pivotal role in the initiation process. In a later study, Offenbacher (16) explained the key mechanism through which periodontal infection is linked to preterm low birth weight. He proposed that alterations in the levels of these inflammatory mediators resulting from the normal host response to an infectious agent enhances maternally or fetally derived proinflammatory cytokines, polymorphonuclear leukocytes and phospholipase A2 production enhances prostaglandin production or directly to uterine muscle contraction. The second hypothesis regarding the spread of infection could be either through microbes or their toxins that enter the uterine cavity either by an hematogenous route via transient bacteremia or they could be introduced into the vagina by the ascending route from the lower genital tract. In either case, a key issue which is unresolved but is highly pertinent to the potential role of periodontal infection and preterm low birth weight involves the site of action of the infectious challenge and resulting inflammatory response. It has also been suggested that mechanisms such as genetic predisposition for a hyperinflammatory response may be responsible for periodontitis and adverse pregnancy outcomes (17).

The infected periodontium is regarded as a reservoir for periodontopathic bacteria, mainly gram negative anaerobes that serve as a source of endotoxins and lipopolysaccharides, proinflammatory cytokines and prostaglandins that have been shown to be increased in subjects with periodontitis (18). Boggess and colleagues (19) have also confirmed that risk for preterm birth may be highest when the foetus is exposed to periodontal bacteria that generate an inflammatory response. They measured levels of C-reactive protein, 8-isoprostane, prostaglandin E2 and IgM specific against Campylobacter rectus, Peptostreptococcus micros, Prevotella nigrescens, Prevotella intermedia, or *F. nucleatum* in cord blood samples from preterm and full-term births. The risk of preterm birth (<35 weeks) was highest when both detectable levels of IgM against at least one pathogen and elevated levels of any of the inflammatory mediators were present. Furthermore, animal experiments where pregnant hamsters challenged with *Porphyromonas gingivalis*, a gram-negative bacterium frequently associated with periodontitis, have shown significant association between increasing levels of both prostaglandin E2, tumor necrosis factor α and fetal growth retardation or reduced fetal birth weight (20). Recently, a study conducted by Adriaens et al. investigated the impact of pregnancy on subgingival microbiota. The authors concluded that although subgingival levels of A. actinomycetemcomitans, P. gingivalis, *T. forsythia*, and *T. denticola* did not change, elevated levels of *P. gingivalis* and *T. forsythia* were associated with bleeding on probing at week 12 of pregnancy (21).

Offenbacher (22) conducted a five year prospective study on oral conditions and pregnancy (OCAP) to find out an association between periodontitis and the risk of preterm birth and fetal growth restriction. The study concluded with the hypothesis that untreated moderate to severe maternal periodontal disease is an independent risk factor for preterm birth (less than 37 weeks), low birth weight (less than 2500 gms) and fetal growth restriction (small weight for gestational age) after adjusting for traditional obstetric risk factors and covariates. The progression of periodontal disease during pregnancy appears to increase the fetal growth restriction, irrespective of baseline periodontal disease status. In the second part of his study, preterm birth resulting from systemic dissemination of the maternal periodontal infection was assessed in the absence of protective antibodies. The study concluded that mothers who did not mount a strong IgG response to certain periodontal pathogens experienced the highest percentage of preterm births (23).

Jeffcoat (24) analyzed multiple epidemiological studies to suggest cause and effect relationships between the maternal periodontal health and preterm birth. To address cause and effect association, periodontal disease must be present prior to preterm birth and the treatment of periodontal infection in pregnant females can lower the risk of adverse pregnancy outcome. If conventional periodontal therapy reduces the risk of preterm birth, then it needs to be established what type of therapy and at what trimester of pregnancy periodontal therapy must be delivered to achieve the most efficacious reduction in the risk of adverse pregnancy outcomes. Mitchell-Lewis et al. (2001) investigated the effect of periodontal treatment on preterm births and/or low birth weight in a cohort of young 74 pregnant women compared with 90 post-partum women. Although the incidence of adverse pregnancy outcomes was higher in women without periodontal treatment, this difference was not statistically significant (25).

In contrast, López et al. observed a significant reduction in the rate of preterm births and/or low birth weight in women that had received periodontal treatment before the 28th gestation week when compared with women who had not received any treatment (26). Later in a pilot study by Offenbacher et al. (2006), it was observed that periodontal treatment significantly reduced the incidence of preterm births, in spite of the small size of the sample (53 women) (27). Therefore, large scale longitudinal epidemiological and intervention studies are necessary to validate the causal relationship of periodontal diseases to adverse pregnancy outcomes.

Conclusion

The magnitude of effect of periodontal infection on adverse pregnancy outcome suggests that periodontitis is a significant emerging risk factor but clearly insufficient to establish a cause and effect relationship. As the periodontal disease is preventable and treatable, therefore, future prospective studies are required to ascertain the potential effects of periodontal therapy on the rate of prematurity. Thus, identification and treatment of periodontal disease should be considered an important intervention strategy as part of prenatal care to ensure the best possible start to life by reducing the adverse pregnancy outcomes.

Conflict of interest

No conflict of interest was declared by the authors.

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Metastatic ovarian malignant melanoma with no obvious primary

Primer odağı belli olmayan metastatik Over melanomu

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Abstract

The differential diagnosis of metastatic ovarian malignant melanoma from primary ovarian tumors is a significant challenge, particularly if there is no obvious primary site. A 39-year-old patient with bilateral ovarian malignant melanoma presented as stage IV primary ovarian tumor, with metastases in the omentum and spleen. She underwent a total abdominal hysterectomy and bilateral salpingo-oopherectomy with infracolic omentectomy and splenectomy. The diagnosis on examination of frozen sections was inconclusive. The final diagnosis was made by immunohistochemistry. The sections showed positive staining with HMB-45, vimentin, S-100, and no staining for cytoceratin, inhibin, calretinin and caldesmon. After the operation, the MRI at the 14th postoperative day revealed metastatic lesions in the vertebrae, sacrum, bilateral humerus and femur and in the cerebral cortex, together with edema and hemorrhagic foci. The patient stayed in the intensive care unit for 12 weeks until her death due to cardio-respiratory arrest. This case highlights the clinical fact that metastatic malignant melanoma may mimic primary ovarian tumor with an occult or regressed primary. Both the standard pre-operative imaging modalities (such as CT, MRI) and the histo-pathologic examination of the frozen sections may be inconclusive in the differentiation of ovarian melanoma from epithelial ovarian malignancies. The final diagnosis could be established by immunohistochemistry. Intra-abdominal debulking surgery would not prolong the survival of metastatic ovarian melanoma because of the occult or rapid metastasis to the extra abdominal sites of the tumor. (J Turkish-German Gynecol Assoc 2011; 12: 181-2)

Key words: Melanoma, ovary, metastatic melanoma, amelanotic melanoma

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Özet

Metastatik overyan malign melanomun primer over tümörlerinden ayırt edilmesi, özellikle primer odağın belli olmadığı olgularda oldukça zordur. 39 yaşında bilateral overyan malign melanomu olan hasta, dalak ve omentumda metastazları mevcut evre IV primer over tümörü olarak prezente olmuştur. Hastaya total abdominal histerektomi, bilateral salpingo-ooferektomi, infrakolik omentektomi ve splenektomi yapılmıştır. Frozen seksiyonda tanı şüpheli olup kesin tanı immünohistokimyasal boyama sonrası konulabilmiştir. Kesitlerde HMB-45, vimentin ve S-100 müsbet, sitokeratin, inhibin, kalretinin ve kaldezmon menfi boyanma göstermiştir. Post-operatif 14. günde yapılan MRI incelemesinde vertebra, sakrum, bilateral humerus ve femur ve serebral korteksde metastatik lezyonlar, ödem ve hemorajik lezyonlar saptanmıştır. Hasta 12 hafta boyunca, burada kalp-solunum yetmezliği sonucu hayatını kaybedinceye kadar yoğunbakım ünitesinde kalmıştır. Bu olgu, primer odağı gizli yada regrese olmuş metastatik malign melanomun primer over tümörlerini taklit edebileceğini vurgulamaktadır. Hem tomografi ve MRI gibi standart pre-operatif görüntüleme yöntemleri, hemde frozen seksiyon incelemesi overyan melanomun epitelyal over malignensilerinden ayırt edilmesinde yetersiz kalmaktadırlar. Kesin tanı immünohistokimyasal incelemeyle konulabilir. İntra-abdominal debulking cerrahi metastatik ovaryan melanomda, tümörün ekstra-abdominal bölgelere gizli veya erken metastaz yapması sebebiyle yaşam süresini uzatmamaktadır.
(J Turkish-German Gynecol Assoc 2011; 12: 181-2)

Anahtar kelimeler: Melanom, over, metastatik melanom, amelanotik melanom

Geliş Tarihi: 26 Temmuz 2010 **Kabul Tarihi:** 05 Eylül 2010

Introduction

Melanoma in the ovary is a rare condition often found at autopsy as a part of extensive multi-systemic spread of the disease. Primary ovarian melanomas usually arise from a benign cystic teratoma. In the majority of metastatic ovarian melanomas, the primary lesion is in the skin and may present clinically even after long periods of remission. Presentation as solitary organ tumors has also been reported. Diagnosis of the metastatic ovarian melanomas is difficult, especially if the primary lesion is not prominent (1-3). The reported case is a bilateral amelanotic metastatic ovarian melanoma presenting as a primary ovarian tumor with no obvious primary site.

Case Report

A 39 year old multiparous female with a complaint of abdominal mass was referred to our clinic. Her personal history for cancer was unremarkable. She had bilateral ovarian solid masses completely occupying the recto-uterine space and ascites. MRI and CT revealed enlarged pelvic lymph nodes on the right side and metastatic lesions in the omentum and spleen. No lesion was observed in the thorax CT. Among tumor markers, CA125 was 404 IU/L while others were normal. Pre-operative diagnosis was stage IV ovarian cancer and optimal debulking surgery was performed.

Gross examination of the excised material revealed soft, hemorrhagic, necrotic white-yellow tumor tissue. The spleen

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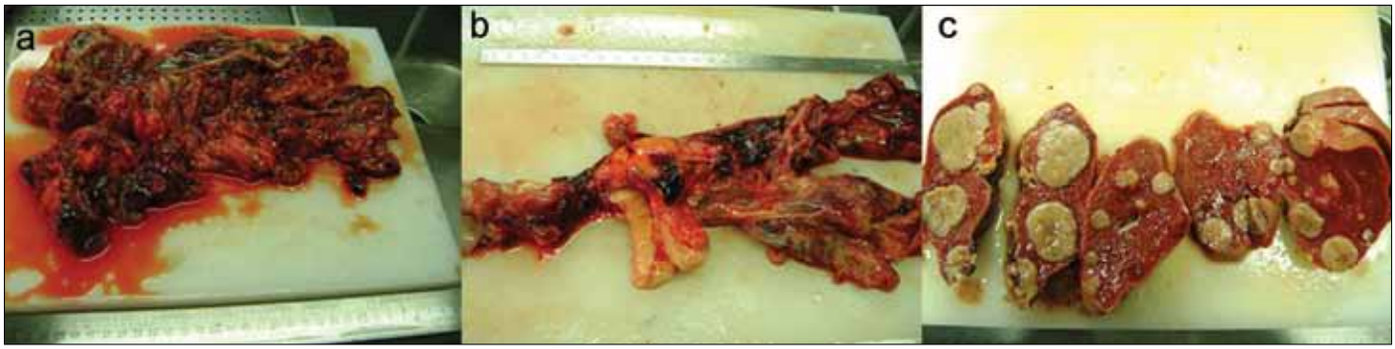

Figure 1. Macroscopic view of the resected specimens: a) Hemorrhagic, necrotic gross appearance of the tumor resected from the abdomen b) hysterectomy material c) spleen

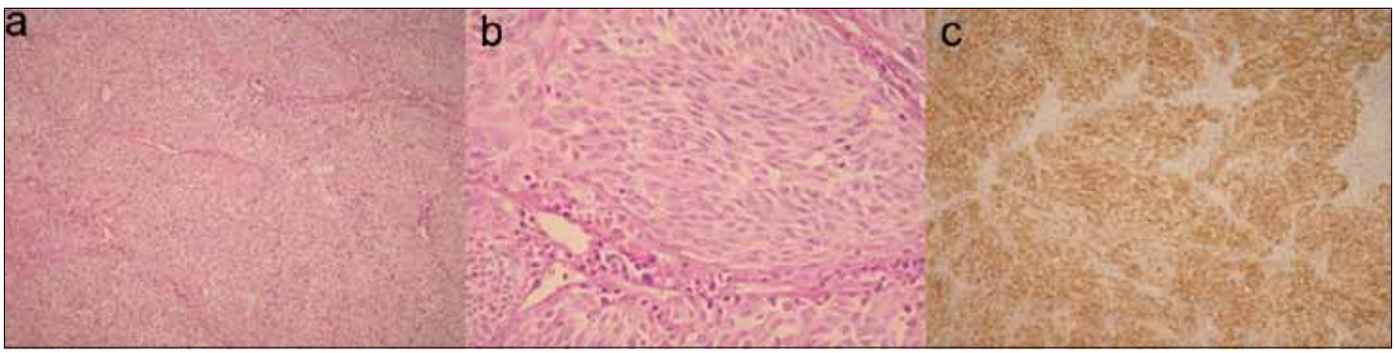

Figure 2. Microscopic examination of the tumor a) nested architecture of the tumor (HE. x 100) b) Spindle shape tumor cells with prominent nucleolus (HE. x 400) c) Immunoreactivity for HMB-45 (x 100)

contained white metastatic lesions (Figure 1a-c). On frozen sections, microscopy revealed a predominantly diffuse growth pattern with focal nested architecture, coagulation necrosis and gland-like structures. The tumor was composed of large epithelioid cells with eosinophilic cytoplasm and spindle-shaped cells, with prominent nucleoli. The diagnosis according to the examination of frozen sections was malignant tumor of unknown origin.

The final histo-pathologic diagnosis was metastatic malignant melanoma based on the observation of positivity for S-100, HMB-45 and Masson-Fontana and negativity for cytoceratin, inhibin, calretinin and caldesmon and the absence of a cystic teratoma.

No peri-operative surgical complication occured but a pleural effusion developed on the 2nd postoperative day. MRI on the 14th postoperative day revealed many metastatic lesions in the vertebrae, sacrum, humerus, femur, and cerebral cortex. The patient stayed in intensive care unit for 12 weeks until her death.

Discussion

Preoperative assessment of ovarian melanoma should involve sensitive screening modalities to assess metastases because ultrasonography and tomography usually fail to characterize these lesions. MRI may be helpful if there is a considerable amount of melanin deposited in the lesions but the majority of ovarian melanomas are amelanotic (4). Positron Emission Tomography scans have been shown to be sensitive and specific in detecting metastases of the melanomas (5).

This case highlights the clinical fact that metastatic malignant melanoma may mimic a primary ovarian tumor with an occult or regressed primary. A regressed cutaneous melanoma or a primary site of mucosal surfaces might be an explanation for a possibly undetected primary origin. Both the standard pre-operative imaging modalities (such as CT, MRI) and the histo-pathologic examination of the frozen sections may be inconclusive in the diagnosis of ovarian melanoma. The final diagnosis could be established by immunohistochemistry. Intra-abdominal debulking surgery would not prolong the survival of metastatic ovarian melanoma because of the occult or rapid metastasis to the extra abdominal sites of the tumor.

Conflict of interest

No conflict of interest was declared by the authors.

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A prenatal tertiary trisomy resulting from balanced maternal 8; 9 translocation

Annenin dengeli 8; 9 translokasyonu sonucu oluşan prenatal tersiyer trizomi

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Abstract

An additional derivative chromosome 8 was found in the cytogenetic analyses of the chorionic villus biopsy specimen of a balanced reciprocal translocation carrier mother. This was a 3:1 segregation of the unbalanced product of the balanced maternal 8:9 translocation. The chromosomes of the carrier of the balanced reciprocal translocation pair with their matching homologous segments at meiosis I, a quadrivalent figure is formed and chromosomes segregate from this configuration. Increased nuchal tranaslucency was also determined on fetal sonography at the 13rd week of gestation. The final karyotype was 47,X Y,+der(8)t(8;9)(q11.2;p22) mat, and the parents were informed about this tertiary trisomy. After genetic counseling, the parents decided to terminate the pregnancy. The presented case is a reminder of the probability of the unbalanced products of the 3:1 segregation, rather than the common 2:2 segregation.

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Key words: Derivative chromosome 8, nuchal translucency, reciprocal translocation, tertiary trisomy, 3:1 segregation

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Özet

Dengeli resiprokal translokasyon taşıyıcısı annenin, koryon villüs biyopsi materyalinden yapılan sitogenetik analizinde, ek olarak derivatif 8. kromozom belirlenmiştir. Bu dengesiz durum, 8. ve 9. kromozomlar arasında dengeli resiprokal translokasyon içeren annenin, 3:1 gamet ayrışımından kaynaklanmaktadır. Resiprokal dengeli translokasyon taşıyıcılarının kromozomları, mayoz 1'de homologları ile eşleşebilmek için quadrivalen yapıyı oluşturmakta ve buradan kromozomların segregasyonu sözkonusu olmaktadır. Gebeliğin 13. haftasında yapılan fetal ultrasonografide ayrıca nukkal kalınlık artışı saptanmıştır. Fetüsün karyotipi 47,XY,+der(8)t(8;9)(q11.2;p22) mat olarak rapor edilmiş ve tersiyer trizomi hakkında aileye bilgi verilmiştir. Aile genetik danışmanlık sonrası gebeliğini sonlandırmaya karar vermiştir. Burada sunulan olgu, resiprokal translokasyon taşıyıcılarının dengesiz gamet oluşumunda 2:2 segregasyonun yanısıra, daha az oranda da olsa 3:1 segregasyon olasılığının da olduğunu hatırlatması açısından önemlidir. (J Turkish-German Gynecol Assoc 2011; 12: 183-5)

Anahtar kelimeler: Derivatif 8. kromozom, nukkal kalınlık, resiprokal translokasyon, tersiyer trizomi, 3:1 segregasyon

Geliş Tarihi: 17 Ağustos 2010 **Kabul Tarihi:** 09 Eylül 2010

Introduction

Reciprocal translocations are structural chromosomal abnormalities commonly seen in humans, with a frequency of 1:500. The phenotype of the balanced translocation carriers is usually normal; however, they have significant risks of unbalanced progeny or spontaneous abortion (1-3). At meiosis I, the translocated chromosomes pair with their matching homologous at a quadrivalent formation and imbalanced gametes result from the disjunction of these chromosomes for the segregation models i.e. adjacent 1, adjacent 2, 3:1, 4:0 (1, 2).

Offspring of carriers often have 46 chromosomes, including one of the derivative chromosome, so are partially trisomic and partially monosomic for the translocated chromosomes. Nevertheless, a karyotype with 47 or 45 chromosomes due to 3:1 segregation is often complicated by the presence of two partial trisomies or regular and partial monosomies; namely tertiary trisomy/monosomy and interchange trisomy/monosomy. Here, we presented a prenatal tertiary trisomic case with the partial trisomy 8p (and also including a tiny part of 8q; 8q11.2) and the partial trisomy 9p due to 3:1 segregation derived from balanced maternal 8;9 translocation.

Case Report

A 33 year old balanced reciprocal translocation carrier, was referred to us at the 14th week of her third gestation for prenatal cytogenetic analyses. The parent was informed and then signed the informed consent for the invasive prenatal sampling and cultivation of the chorionic villus biopsy specimen. Her first pregnancy had ended with spontaneous abortion at the 7th week and they had a 2-year-old healthy boy from the second pregnancy. Chromosome analyses of the parent had been performed in our laboratory after the first pregnancy and the mother was diagnosed as a balanced reciprocal translocation carrier [t(8;9)(q11.2;p22)] (Figure 1). In this progeny at the 13rd week of gestation, a thickened nuchal fold (10 mm) was also found on fetal sonography. G-banding

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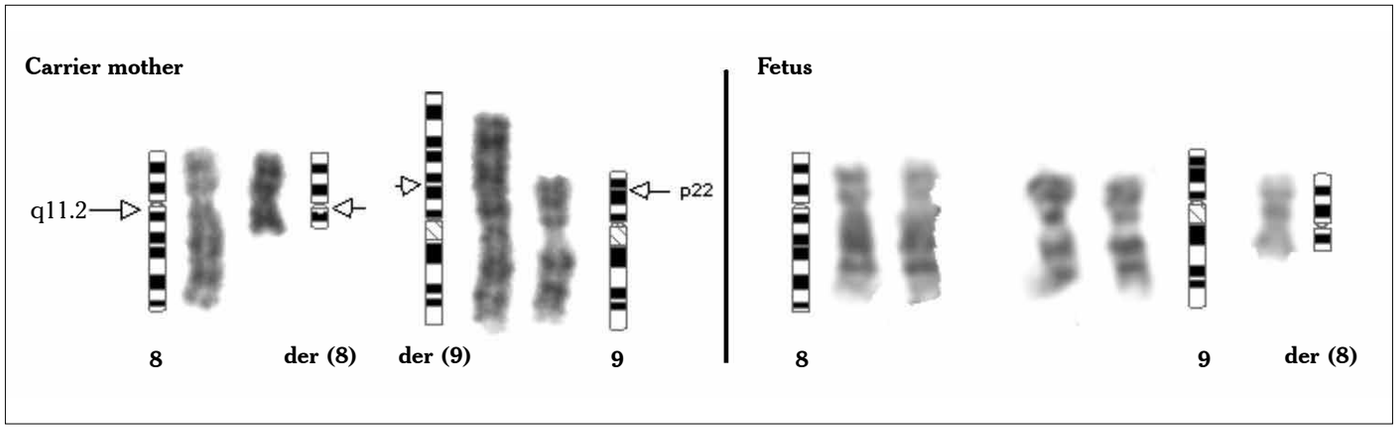

Figure 1. GTG banded partial karyotype and ideogram of carrier mother (left) and the imbalance fetus (right). The arrows indicate the breakpoints

for short-term and long-term tissue cultures and Fluorescence *in situ* hybridization (FISH) technique were performed on the chorionic villus biopsy specimen. FISH was informative for the common aneuploidies but not for the reciprocal translocation because of the lack of the specific probes for the chromosomal rearrangement. We could not obtain a qualified chromosome from short-term tissue culture and the long-term tissue culture result was 47,XY,+der(8)t(8;9)(q11.2;p22)mat (Figure 1). Breakpoints of the derivative chromosome involved the same bands; 8q11.2 and 9p22, as the mother. This was a tertiary trisomy for the whole short arm and a small part of the long arm of the chromosome 8 (8pter→8q11.2) and the partial short arm of the chromosome 9 (9pter→9p22) resulting from the 3:1 segregation of the balanced maternal 8;9 translocation.

The pregnancy was terminated in the 15th week of gestation at another center. We could not evaluate the physical properties, but only learnt from the information obtained from the mother that the fetus had frontal bossing and hypertrichosis.

We could not evaluate the physical properties, only the information obtained from the mother that the fetus had frontal bossing and hypertrichosis.

Discussion

Balanced reciprocal translocations are usually harmless rearrangements for the carriers, but the derivative chromosomes and their matching homologous form a quadrivalent figure at meiosis I and 2:2, 3:1, and 4:0 segregation models yield mostly unbalanced gametes. The rates of segregant distributions are not a significant distinction in each sex, except the 3:1 category and a predisposition for 3:1 segregation in oogenesis can be confirmed (4-7). The factor for the possibility of segregant models produced by Daniel A (1979), is the percentage of the total haploid autosomal lentgh (HAL) (8). As a viability conceptus, if one of the translocated or derivative chromosomes is small, similar to our case, 3:1 segregation is more likely than the other segregation models (9, 10).

The living patients with tertiary trisomy are mostly reported for chromosome 9p (11, 12). The distal half of the short arm of chromosome 9p (9pter-9p21), approximately the same segment as our case (9pter-9p22) (Figure 1), is responsible for the major clinical features of the syndrome; growth and mental retardation, ear anomalies, hypertelorism etc. (12). The reported cases with trisomy 8p, are generally results of the inversion duplication and of the reciprocal translocations, and their clinical features are hypotonia, neonatal feeding problems, mental retardation, brain and orthopedic abnormalities and specific facial features (13, 14). As far as we know, there is no report which met all the criteria of the translocated segments of our case. A case of trisomy 8p12-pter, quite similar to our case (trisomy 8q11.2-pter) resulting from 8;15 balanced maternal translocation, had similar findings of the other trisomy 8, however, since our case was detected prenatally, we could not evaluate the clinical findings except for the increased nuchal translucency on fetal sonography (14). Nuchal translucency thickness shows an increased risk for trisomies 13, 18 and 21, this also may be true for other regular trisomies and/or tertiary trisomies as well (15). This information can be helpful in genetic counseling and for decision making. The recurrence risk of unbalanced infants is the second important parameter for the reciprocal translocation carriers and their proceeding gestations. The risk from 3:1 disjunction is similar to that for other imbalanced offspring; only the abortion rate for 3:1 disjunction is higher than others (2).

In conclusion, in the couples with chromosomal rearrangement and/or with abnormal fetal sonography findings, cytogenetic analyses and chromosome specific FISH paintings should be performed in the prenatal period. While providing genetic counseling, not only the unbalanced gametes due to 2:2 segregation but also the risk of unbalanced progenies from 3:1 disjunction should be considered and pre-implantation genetic diagnosis could be suggested.

Conflict of interest

No conflict of interest was declared by the authors.

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Prenatal diagnosis of caudal regression syndrome without maternal diabetes mellitus

Maternal diabetes mellitusun eşlik etmediği kaudal regresyon sendromunun prenatal tanısı

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Abstract

Caudal regression syndrome is a rare congenital malformation with varying degrees of early gestational developmental failure. It is also known as sacral agenesis or caudal dysplasia. The cause of this malformation is thought to be defects in neuralization around the 28th day of the gestational period. Although maternal uncontrolled diabetes, genetic predisposition and vascular hypoperfusion are the possible risk factors, actual pathogenesis is unclear. CRS is generally diagnosed at prenatal assessment, but also a varying number of newborns with some degree of anomaly may be presented. In our case, we diagnosed a caudal regression syndrome fetus early in the second trimester. Determination of the pathology early in the gestational age gives parents a chance for termination of pregnancy. Although diabetes mellitus is the major risk factor for CRS, as in our case, sporadic presentations may occur. So clinicians should consider CRS when CRL is shorter than expected and incomplete vertebral ossification is observed both in gray scala and 3D imaging ultrasonography.

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Key words: Caudal regression syndrome, sirenomelia, diabetes mellitus, vertebral anomaly,3D ultrasonography
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Özet

Kaudal regresyon sendromu (KRS) çeşitli derecelerde erken gestasyonel gelişim bozukluğunun gözlendiği nadir bir konjenital anomalidir. Bu durum sakral agensesis ya da kaudal displazi olarak da bilinmektedir. Gebeliğin 28. günü civarında oluşabilecek nöralizasyon defekti bu malformasyonun nedeni olarak düşünülmektedir. Maternal kontrolsüz diabet, genetik yatkınlık ve vasküler hipoperfüzyon olası risk faktörleri olmasına rağmen, gerçek patogenezis belirsizdir. KRS tanısı genellikle prenatal olarak koyulur, ancak yine de değişen sayıda yeni doğan çeşitli derecelerde anomalilerle tanı alabilirler. Sunulan kaudal regresyon sendromlu fetüs olgusuna erken ikinci trimesterde tanı koyduk. Patolojinin erken gebelik haftalarında belirlenmesi ile aile gebelik terminasyonu şansına sahip olabilir. Diabetes mellitus, KRS için majör faktörü olmasına karşın, bizim olgumuzda olduğu gibi spoaradik durumlar da sözkonusu olabilir. Böylece, klinisyenler beklenenden kısa CRL ile karşılaştıklarında ve hem gri skala hem de 3D ultrasonografide tamamlanmamış vertebral ossifikasyon görünümü saptadıklarında kaudal regresyon sendromunu düşünmelidir.

(J Turkish-German Gynecol Assoc 2011; 12: 186-8)
Anahtar kelimeler: Kaudal regresyon sendromu, sirenomelia, diabetes mellitus, vertebral anomali, 3D ultrasonografi
Geliş Tarihi: 18 Nisan 2010 **Kabul Tarihi:** 03 Eylül 2010

Introduction

Caudal regression syndrome is a congenital malformation with varying degrees of early gestational developmental failure. It is also known as sacral agenesis or caudal dysplasia. These defects may include thoracic, lumbar, coccygeal vertebrae and lower extremities. The cause of this malformation is thought to be defects in neuralization around the 28th day of the gestational period (1). This results in motor and sensory deficits of the lower extremities. Cardiac diseases, gastrointestinal disorders, neutral tube defects, and genitourinary malformations may accompany CRS (2). Although uncontrolled maternal diabetes, genetic predisposition and vascular hypoperfusion are the possible risk factors, the actual pathogenesis is unclear (3).CRS is generally diagnosed during prenatal assessment, but also a varying number of newborns with some degree of anomaly may be presented.

Although the number of pregnancies complicated with caudal regression syndrome is 0,1 to 0,25 per 10000 pregnancies, this ratio increases to 1% of pregnancies when complicated with diabetes mellitus (DM) (4). DM is a major risk factor for CRS. The fetuses of insulin dependent diabetic mothers have 200 to 400 times more risk for CRS compared to those of non diabetic mothers (3-6).

Case

A twenty-five year old patient of 16 weeks gravida I, parity was 0 referred to our clinic for vertebral anomaly. In the parental history of the fetus, the couple were infertile for 5 years and pregnancy was achieved by intracytoplasmic sperm injection. Also, the couple had rh incompatibility, the mother's blood being B Rh(-) and the father's 0 Rh(+). Except for these issues, the patient's history was unremarkable. Biochemical and hematological parameters were all within the normal range.

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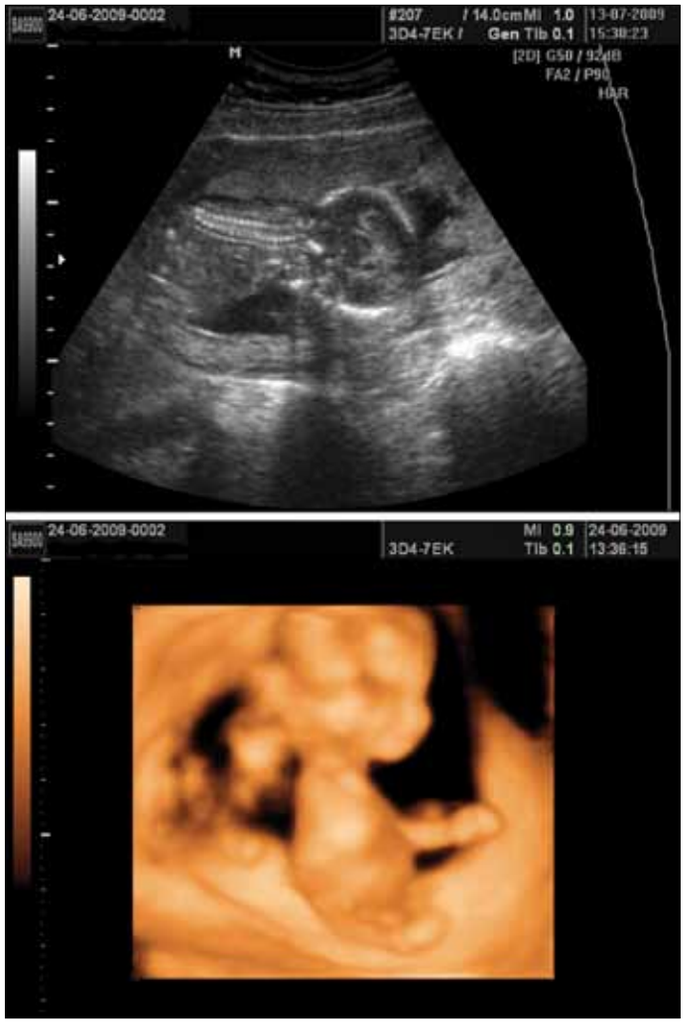

Figure 1 A-B. Vertebral progression was normal until lower thoracic level but after this level neither vertebra nor pelvic bone osssification was observed.

Figure 2 A-B. Talipes equinovarus was seen in both foot

There was no family or patient history of diabetes mellitus. The patient was refered for full ultrasonographic examination with General Electric Voluson 730 Expert and Medison SA 9900 ultrasounds. Ultrasound examinations were carried out both by gray scala and 3D imaging and revealed that the cranium and upper extremities were normal. Vertebral progression was normal until the lower thoracic level, but after this level neither vertebra nor pelvic bone osssification was observed (Figure 1 A-B). Also, the lower extremities were shorter than expected according to the last menstural period and hips were flexed. Other organ system examinations were unremarkable. The amniotic fluid and placenta seemed normal for gestational age. The couple were informed about the fetus' sonographic findings and probable complications after birth. The couple decided to terminate the pregnancy. After provoked abortus, physical examination of the fetus revealed that the upper portion of the body was normal but the pelvis was atrophic and narrow, abdomen was distended, lower extremities were shorter and legs fully flexed like frog legs. Talipes equinovarus was seen in both feet (Figure 2 A-B).

Discussion

CRS is a syndrome characterized by a series of lower vertebral anomalies that should be associated with pelvic bone deformity. Other than this, some anomalies of the lower limbs, NTD, gastrointestinal tract, genitourinary tract and cardiac organs may accompany the condition (7). In our case, the fetus was seen to have both vertebral, pelvic and lower limb deformities on physical examination.

Although the exact mechanism for this syndrome has not been established, it was thought that there is a defect in the induction of the caudal elements of the embryo before the 28th day of gestational period. The embryologic attack occurs at the midposterior axis of the mesoderm which causes absence of the progression of the mesoblastic caudal bud. Advances in the understanding of axial mesoderm patterning at early gestation reveal that one or more processes of primitive streak migration, primary or secondary neuralization, or differentiation are compromised (7, 8). The relationship and interdependence of developing caudal nervous, spinal, hindgut, and mesonephric elements involved in the closure of the neural tube result in the

development of neural, distal vertebral, anorectal, renal, and genital abnormalities that produce CRS. However, structures that are developmentally distant from these caudal elements, such as the brain, proximal spine, and spinal cord, are not generally involved in CRS (2).

Caudal regression syndrome is a rare disease and the true pathogenesis is unclear. Only 0.1 to 0.25 per 10,000 pregnancies have been complicated with CRS (4). Caudal regression syndrome is thought to occur in up to 1% of pregnancies in diabetic women. Up to 22% cases of CRS are associated with type 1 or type 2 diabetes mellitus in the mother. Insulin-dependent diabetic women are 200 to 400 times more likely to have a child with CRS than women without diabetes (3-6). For this reason, CRS is the most characteristic fetal abnormality of diabetic embryopathy (3). However, CRS has also occurred in nondiabetic women with confounding etiologic factors. Genetic factors have been proposed, but the lack of repetition of identical malformations in subsequent pregnancies does not support a genetic hypothesis (9). Also, no apparent enhancement is seen in chromosomal abnormalities.

Padmanabhan's experimental study showed the role of retinoic acid in producing CRS in the mouse fetus (10). Retinoic acid, when given in different dosages to TO mouse fetuses, resulted in CRS in most of the survivors. In another study, exposure of pregnant mice to all-trans retinoic acid, at a time when the metanephros has yet to form, causes failure of kidney development along with caudal regression (11). Drug-related etiology for extreme caudal agenesis in a human fetus has been suggested (12). The mother had used minoxidil solution for hair loss 4 years prior to and during gestation. She also received trimethoprim-sulfamethoxazole during the first trimester for an upper respiratory problem. There was no history of maternal diabetes or familial genetic diseases. All of these studies and cases strongly suggest that various chemicas may play a teratogenic role in the genesis of CRS. In our case, neither diabetes mellitus nor various chemicals were detected. Blood glucose level was under the normal range. The only treatment used before conception was ovulation induction with gonadotrophins because of primary infertility. This suggests that sporadic cases must be kept in mind.

Previously, it was thought that sirenomelia is the severe form of CRS but 1993 Twicker et al. (13) reported the differences of either clinical presentation or etiologic factors for both syndromes. Vascular steal mechanism is suspected for sirenomelia which results in severe ischemia of the caudal spinal segment. Apart from CRS, sirenomelia exhibits more severe caudal dysgenesis, fused lower limbs and renal agenesis that causes fatal progression with oligohydramnios and pulmonary hypoplasia. Our case is distinct from sirenomelia due to normal amniotic fluid volume, separate legs, normal number of lower extremity bones, and presence of normal appearing kidneys.

In conclusion, we described a case of early second trimester fetus with caudal regression syndrome without any known risk factor. Clinicians must keep in mind that CRS may be seen without any risk factors and a small CRL size or incomplete vertebral ossification may be the first signs of the syndrome. Early determination gives the opportunity for termination of pregnancy until we can prevent formation of the new cases by introducing the exact pathogenesis.

Conflict of interest

No conflict of interest was declared by the authors.

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Unicornuate uteri associated with contralateral renal agenesis and ovarian anomalies

Kontralateral renal agenezis ve over anomalileri ile birlikte tek boynuzlu uterus

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Abstract

Our findings regarding two cases of unicornuate uterus validate that conventional transvaginal ultrasound is helpful in diagnosing uterine anomalies. Moreover, anomalies of the urinary system and the contralateral ovary should always be considered.

(J Turkish-German Gynecol Assoc 2011; 12: 189-91)

Key words: Unicornuate uterus, renal agenesis, ovarian anomalies

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Özet

Tek boynuzlu uterusu olan iki olguyla ilgili bulgularımız, konvansiyonel transvajinal ultrasonun uterin anomalilerin tanısında yardımcı olduğunu doğrulamıştır. Bunun yanı sıra, üriner sistemin ve kontralateral overin anomalileri daima göz önünde bulundurulmalıdır.

(J Turkish-German Gynecol Assoc 2011; 12: 189-91)

Anahtar kelimeler: Tek boynuzlu uterus, renal agenezis, over anomalileri

Geliş Tarihi: 13 Ağustos 2010 **Kabul Tarihi:** 19 Eylül 2010

Introduction

The female reproductive system originates with the Müllerian ducts which, during embryogenesis, fuse to create the uterine tubes, uterus, and the upper two-thirds of the vagina. If one of the ducts does not develop, only one Müllerian duct contributes to uterine development. This so-called hemi-uterus has a single horn linked to the ipsilateral uterine tube facing its ovary. A unicornuate uterus has a single cervix and vagina (uterus unicornus unicollis, vagina simplex). On the opposite side, the contralateral Müllerian duct may undergo some development. In such a case, a rudimentary horn is present, which may or may not be connected to the hemi-uterus or to the ispilateral tube. Furthermore, the rudimentary horn may or may not have an endometrial cavity. When a functioning endometrium is present, haematometra and haematosalpinx with dysmenorrhoea soon after menarche can be observed. A blastocyst may also implant in a communicating or a noncommunicating cavitary rudimentary horn; the latter is only possible via transperitoneal migration of sperm. Since uterine rupture can occur, surgical resection of the rudimentary horn is indicated to avoid this life-threatening condition (1). Associated defects may involve the renal system (kidneys, urethra) due to their close embryological interactions, and, less commonly, the skeleton. Unicornuate uterus is much less common than other uterine malformations, with an estimated occurrence of about 1/4,000 (2). In the latest ASRM (American Society for Reproductive Medicine) (3) system for classification of uterine anomalies, formalized in 1988 and currently in use, the unicornuate uterus is classified as class II, and is subdivided into:

II a: with communicating rudimentary horn

II b: with noncommunicating rudimentary horn

II c: rudimentary horn without cavity

II d: without any rudimentary horn - also called true unicornuate uterus.

Regarding associated renal anomalies, the literature reports that 70-89% of patients with unilateral renal agenesis may have associated genital anomalies (4). On the other hand, many authors have investigated the presence of urinary anomalies each time a unicornuate uterus is diagnosed. In a study by Fedele et al. (5), 40.5% of the patients with a unicornuate uterus presented with urinary tract anomalies such as renal agenesis, ectopic kidney, horseshoe kidney, double renal pelvis, and/or unilateral medullary sponge kidney. Rolen et al. (6) found 67% of patients with unicornuate uterus had ipsilateral renal agenesis, 13% of them having a pelvic kidney. Furthermore, upon detection of a unicornuate uterus, anomalies of the contralateral ovary must be looked for. The entirely separate origin of the ovaries from the gonadal ridges explains the infrequent association of uterovaginal anomalies with ovarian anomalies (7). However, reports of ovarian anomalies in cases of unicornuate uteri are not rare. The ovary may be absent (8-10) or undescended,

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with attachment of the upper pole to an area above the level of the common iliac vessels (11). Ectopic ovaries are characterized by their attachment to an area above the level of the common iliac vessels. Dabirashrafi et al. (12) found that the incidence of ovarian malposition is higher in patients with congenital uterine anomalies than in a control group. This is especially true when the uterus is absent or only partially present. The incidence is reported to be as high as 42% in cases of unicornuate uterus (13). Despite the well-known association of ectopic ovaries with a unicornuate uterus, ectopic ovaries are reported only sporadically, suggesting the possibility that many cases go unrecognized. The contralateral ovary may also have some other pathology such as endometriosis, perhaps due to retrograde menstrual blood in case of the presence of a cavitated rudimentary horn (14). We present two cases of unicornuate uteri, one of which was associated with agenesis of the contralateral ovary and kidney and the other associated with a contralateral undescended ovary and renal agenesis.

Case Reports

Patient 1

A 19-year-old woman (G0P0) was asked to return for a transvaginal examination because "the left ovary was not visualized" during a routine transabdominal scan.

Her past medical history included a corrected lateral sacral congenital lipomyelomeningocele. The patient had a neurogenic bladder. Furthermore, left renal agenesis was reported.

During the 2D transvaginal examination, the uterus was found to be markedly deviated to the right; a perfect transverse section at the fundal level could not be obtained because of inability to follow the interstitial portion of the left uterine tube at the uterine cornu. Uterine dimensions were approximately those of a normal nulliparous woman, that is, longitudinal diameter 81 mm, AP diameter 28 mm, and transverse diameter 32 mm, slightly narrow. Endometrial thickness and echostructure were concordant with the menstrual phase.

The right ovary was visualized cranial to the fundus; an anechoic structure was present consistent with a simple follicle corresponding to the menstrual phase. The left ovary was not visualized even on searching transabdominally, attempting to verify an extremely high adnexal position. No adnexal mass attributable to a rudimentary horn was visualized.

The patient was referred to Radiology where an MRI was performed, confirming the diagnosis of right type D unicornuate uterus and agenesis of the left ovary and left kidney.

Patient 2

A 34-year-old nulliparous woman, G1P0, was referred to the ultrasound service for a routine examination. Her past medical history included excision of the ocular bulbus performed at age 9 for left ocular retinoblastoma (the patient wore glasses with an opaque left lens). A previous spontaneous abortion, with no subsequent uterine cavity revision, was also reported.

During 2D transvaginal examination, the uterus was found to be deviated to the right but less markedly than in case 1. Once more, a perfect transverse section at the fundal level could not be obtained. Uterine dimensions were approximately those of a normal nulliparous woman, that is, longitudinal diameter 78 mm, AP diameter 34 mm, and transverse diameter at 40 mm. The right ovary was visualized and no adnexal mass attributable to a rudimentary horn was visualized.

Then, using a transabdominal approach, the left ovary containing two small follicular cysts was visualized, positioned very cranially in the left abdomen.

We searched for renal anomalies and found no visualization of the left kidney. The right kidney appeared normal, with a longitudinal diameter longer than normal (138 mm), denoting compensatory hypertrophy.

The patient was referred to Radiology. Hysterosalpingography and MRI were performed, confirming the diagnosis of right type D unicornuate uterus, undescended left ovary, and left renal agenesis.

Discussion

Women with this condition may be asymptomatic and unaware of having a unicornuate uterus; normal pregnancy may occur. In a review of the literature, Reichman et al. analyzed the data on pregnancy outcomes of 290 women with a unicornuate uterus. 175 women conceived, for a total of 468 pregnancies. They found that about 50% of patients delivered a live neonate. The incidence of ectopic pregnancy was 2.7%; of miscarriage 34%; and of preterm delivery 20%; while the incidence of intrauterine demise was 10% (2). Thus, patients with a unicornuate uterus are at elevated risk for pregnancy loss and obstetrical complications.

Transvaginal ultrasonography is an important diagnostic tool for the study of uterine anomalies (15). The transvaginal probe allows detailed study of the uterus through combined transverse, coronal and longitudinal planes and an accurate study of the adnexa. It is an important instrument in the diagnosis of unicornuate uteri as well. In these cases, transabdominal and transvaginal scanning complementing each other aids in the diagnosis of associated renal and ovarian anomalies. In fact, in cases of unicornuate uterus, renal contralateral agenesis is common, as are contralateral ovarian anomalies such as agenesis or undescended ovary. It seems that a right unicornuate uterus is more frequent than a left. Most cases in the literature describe a right unicornuate uterus with left renal agenesis, left ovarian agenesis, or left undescended ovary. The findings of our report also confirm the association between urinary and genital anomalies, suggesting that each time renal agenesis is diagnosed, it is important to look for genital anomalies, and equally, each time a uterine anomaly is suspected, the physician should look for renal anomalies.

Our findings prove that conventional transvaginal sonography is helpful in diagnosing uterine anomalies, but suspicion should be confirmed by other techniques. Furthermore, anomalies of the urinary system and contralateral ovary should be always sought.

Conflict of interest

No conflict of interest was declared by the authors.

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A sexually transmitted disease: History of AIDS through philately

Cinsel yolla geçen bir hastalık: AIDS'in filatelik tarihi

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Abstract

AIDS has become the new plague; a disease that is not only physically and psychologically debilitating, but culturally and socially devastating as well. Like the plague, AIDS has caused fear, prejudice and even panic in society. Although there are remarkable improvements in the diagnosis and treatment of the disease, AIDS continues its grim passage around the globe. After a slight downturn in the early 1990's, it then returned with a vengeance. By the end of the 20th century, AIDS was reliably estimated to have caused over 20 million deaths throughout the world. At the same time, 40 million people were estimated to be HIV positive. This paper provides an overview of the history of AIDS, including the discovery and its progress in the world through philately. Philately is the study of stamps and postal history and other related items. Philately involves more than just stamp collecting, it contains the study of the design and educational impact of a philatelic material. We have presented AIDS stamps produced world-wide to emphasize the history of AIDS. (J Turkish-German Gynecol Assoc 2011; 12: 192-6)

Key words: AIDS, HIV, infection, immunity, history, philately

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Özet

Geçtiğimiz yüzyıl ortaya çıkan yeni hastalıklardan birisi AIDS'tir. Kısa sürede çağımızın en tehlikeli hastalığı haline gelen AIDS, ilk kez 1981'de ABD'de kaposi sarkoma (kemik, kıkırdak, deri ve lifli dokularda tutunan bir kanser türü) adlı bir hastalığın olağandışı artışı sonucunda tespit edildi. "Edinilmiş bağışıklık yetersizliği sendromu" sözcüklerinin İngilizce baş harflerinden oluşan AIDS, birkaç yıl içinde dünyanın dört bir köşesine yayılarak ölümcül bir salgın boyutuna ulaştı. BM'nin geçen yıl yayınladığı rapora göre, dünyada 40 milyona yakın insan AIDS'in pençesinde boğuşuyor. Bu yeni hastalığın farkına varılmasından dört yıl sonra, hastalığa sebep olan ve cinsel ilişkiyle, kan yoluyla ve anneden bebeğe geçerek insandan insana bulaşan HIV virüsü tanımlandı. Bu yazıda, tematik filateli yoluyla HIV virüsünün keşfinin öyküsü anlatılamaktadır. (J Turkish-German Gynecol Assoc 2011; 12: 192-6)

Anahtar kelimeler: AIDS, HIV, enfeksiyon, bağışıklık, tarih, filateli

Geliş Tarihi: 28 Temmuz 2011 **Kabul Tarihi:** 19 Temmuz 2011

Introduction

By the 1960's informed medical opinion was firmly convinced that the age of pandemics and plagues was over. Indeed, in 1969 no less a figure than the US Surgeon General solemnly announced that "the book of infectious disease is now closed." However, towards the end of the twentieth century, the world suddenly found itself faced with what appeared to be potentially the worst pandemic in human history-namely, AIDS (acquired immunity deficiency syndrome). This is caused by a virus known as HIV (human immunodeficiency virus) which, over a period, attacks a variety of white blood cells essential to the body's immune system. When this system finally breaks down, the body is powerless to defend itself against attack by opportunistic diseases, including cancer, until it is overwhelmed and the victim dies (1). This disease attracted so much attention all over the world that many philatelic materials have been published from different countries to draw public attention to the importance and severity of the disease. The collection and study of philatelic materials like stamps, FDC's, entries and cancellations related to medicine is termed medical philately and the concept of AIDS has been presented frquently in medical philately (Figure 1).

The historical origins of AIDS remain something of a mystery. It appears likely that chimpanzees in the jungles of the Congo basin had long been carriers of a virus genetically similar to HIV, but remained unaffected by it. Sometime around 1970, this virus evolved into HIV, which was transferred to humans who hunted and ate these chimpanzees (Retrospective examination of Central African blood samples reveal no evidence of HIV before 1971). From there it spread, at first largely unnoticed (1). However, there is some curious evidence that appears to contradict this hypothesis. Retrospective tests indicate that a British seaman died of AIDS in Manchester as early as 1959. Another case has been traced to a young adolescent from Missouri who died in 1968. Also, there were outbreaks of diseases now known to be AIDS-related in Central Africa during the 1950's. All this indicates that HIV may initially have crossed into humans much earlier than 1970, and then either died out or lain dormant (Figure 2).

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Figure 1. Chimpanzees had long been carriers of a virus

Figure 2. This stamp was issued to Kaposi stamp from Hungary

In 1981 doctors in New York and San Francisco began reporting cases of young, active homosexual men dying from Kaposi's Sarcoma, a rare type of skin cancer which usually occurs after a breakdown in the immune system. Other types of death resulting from immunodeficiency then began occurring with alarming frequency, and the medical profession realized that it was faced with a new and highly dangerous infectious disease, which it named AIDS. As panic spread,

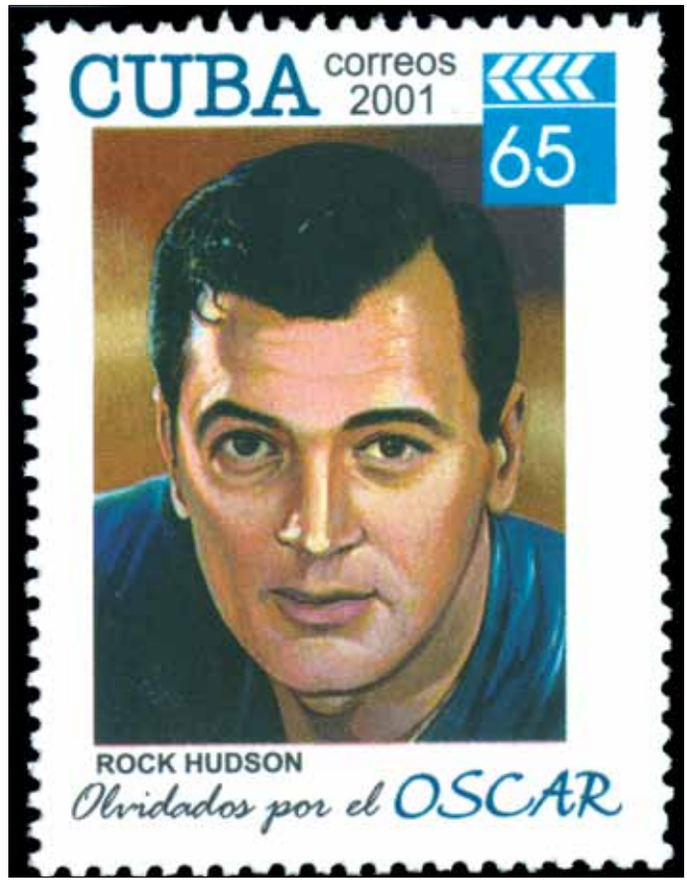

Figure 3. The famous American actor Rock Hudson, who died from AIDS in 1985 on a Cuban stamp

others began calling it "the gay plague", while fundamentalist preachers declared that it was the wrath of God descending on the inhabitants of the licentious San Francisco and other sinks of homosexual iniquity. Soon, however, reports of AIDS began coming in from countries worldwide, and it became clear that this was not a disease limited to homosexuals. In sub-Saharan Africa, the disease had spread along the main highways running east and west, transmitted by male lorry drivers to the female prostitutes along their routes. By 1984, 50 percent of Kenya's prostitutes were HIV positive. By the following year, 10.000 people in the United States alone were infected, and the majority would die within two years of being diagnosed. The disease spread through all levels of society, taking a particularly heavy toll on the artistic and intellectual community. In France, the philosopher Michel Foucault would be the first well-known American figure to declare publicly that he had AIDS shortly before he died in November 1985. Also, there was a big scandal when a very famous American actor Rock Hudson died from AIDS in October 1985 (1) (Figure 3).

Transmission of the disease

By now considerable research had been carried out into AIDS. It had been discovered that the disease was spread through the transmission of sexual fluids or blood. It could enter by way

of cuts or abrasions, particularly those caused in the sensitive tissues of the sexual organs and the anus. Another prevalent way of passing on the disease was the shared use of syringes by drug abusers. Pregnant women could pass on HIV to their fetuses by way of their bloodstream, and even after birth HIV could be passed on through the mother's milk. It was also found to be passed on to people requiring blood transfusions, such as hemophiliacs-there was still no test which could detect the HIV virus in blood samples. However, despite all the hysteria, it was soon understood that the disease was not passed on by normal social contact, or even deep kissing; and "safe sex" (involving careful use of a condom) also prevented possible contamination (1) (Figure 4).

Structure of the Virus

HIV was found to be a retrovirus that affected the white blood cells known as Helper T cells, which were essential to the body's immune system. Alarmingly, after the initial infection there was a long period during which the patient showed no symptoms of illness. This asymptomatic period could last for months, or even years, with the infected person unaware that he was HIV positive, while at the same time passing on the virus. During this stage Helper T cells were attacked by the HIV virus and gradually reduced from their normal density of 1000 per micro-liter to the critical point around 200 per micro-liter, when the patient suffered from immune deficiency (AIDS). As a result he/she would be exposed to illnesses from tumors to diseases of the nervous system, and at the same time also be liable to suffer from such distressing symptoms as personality changes, memory blanks and dementia. On top of this, viruses which had long lain dormant in the body were no longer suppressed and became active. This latter aspect contributed to the rise in tuberculosis around this period (1) (Figure 5, 6).

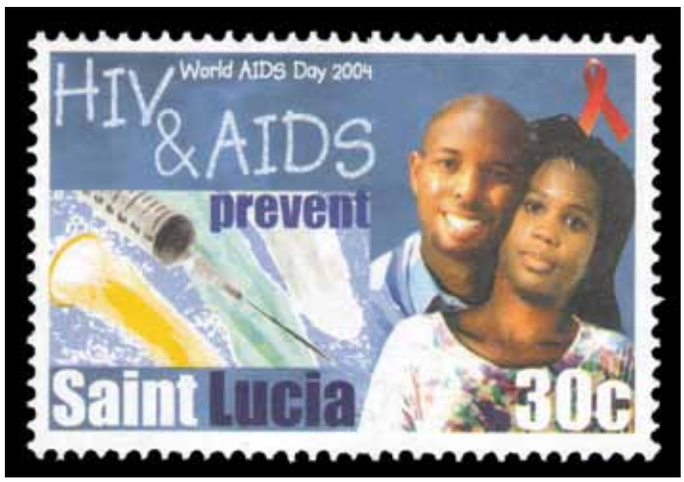

Figure 4. The propaganda of safe-sex is exhibited by a stamp from Saint Lucia

The quest for a vaccine to combat AIDS began early, but major difficulties soon became apparent. The HIV virus was found to be capable of mutating into new strains even more rapidly than the influenza virus. Normally, a vaccine would only be capable of combating a single strain. At the same time,, anyone who did come up with a successful vaccine would be assured of worldwide gratitude, to say nothing of the unimaginable fortune which the patent rights would bring.

Two front runners in the race to develop a vaccine soon emerged-the American Robert Gallo and his team at the National Institutes of Health in Maryland, and a French team at the Pasteur Institute under Luc Montagnier. One after another, these two researchers discovered the structure of the virus that causes AIDS what we now called HIV . In 2008, Francoise Barre-Snoussi and Luc Montagnier shared the Nobel Medicine Prize for their discovery of the HIV virus (2) (Figure 7).

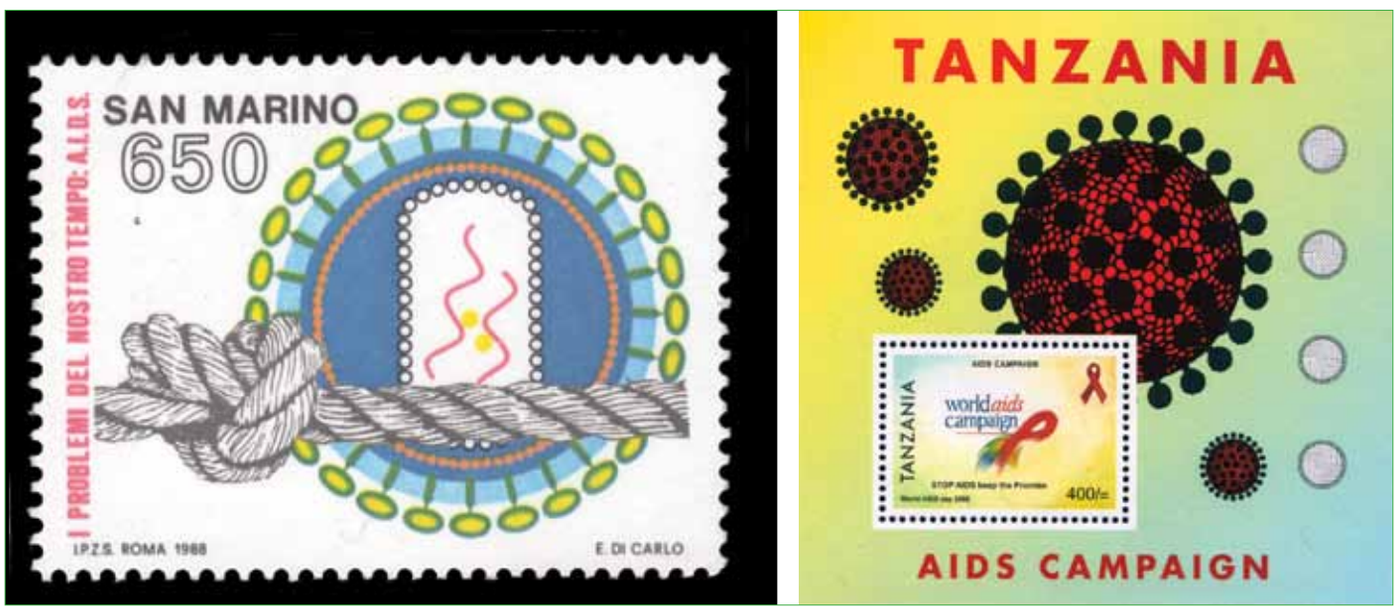

Figure 5, 6. HIV virus represented on San Marino and Tanzania stamps

Figure 7. A stamp from Botswana emphasizing stigma and discrimination in AIDS

Some Ethical Considerations

Despite all the scientific researches developing new drugs and producing really effective pharmaceutical agents, the main ethical issues related to HIV/AIDS testing including confidentiality, informed consent, end of life, research design, conflict of interest, vulnerable populations, and vaccine research are still continuing (3).

According to the contemporary approach, all medical information is generally considered confidential and protected under the law. Because of the sensitivity of HIV-related information, many countries have adopted laws that provide additional protection to HIV-related medical records. For example, in many countries, HIV information may not be disclosed based on a general release of medical information-specific authorization for release of HIV-related information must be obtained (4) (Figure 8).

Vulnerability is particularly important in the context of HIV-related research. Those infected with HIV may be medically vulnerable because of their infection. In addition, homosexuals, injection drug users, minorities, and women, who, for various reasons, may be at higher risk of HIV infection, are more likely to be socially and economically vulnerable because of historical

Figure 8. World AIDS day on the stamp from United Nations Organization

Figure 9. Stamp is showing the importance of fighting against AIDS

attitudes and discrimination. Accordingly, investigators conducting HIV-related research must pay particular attention to vulnerability and take steps to protect potentially vulnerable research participants (5) (Figure 9).

Conclusion

Most agree that the epidemic is gradually being brought under control in many advanced Western countries, whereas it is undeniably out of control in Africa and many Third World

Table 1. Philatelic table of the history of AIDS through Philately

Name	Year	Country	Scott Nr
Year of monkeys	2004	Antigua & Barbuda	2752e
14th Int. Cancer Congr.	1986	Hungary	2997
Rock Hudson	2001	Cuba	4192
HIV & AIDS	2004	Saint Lucia	1198
AIDS	1988	San Marino	1163
AIDS	2006	Tanzania	2483
AIDS	2002	Botswana	759
AIDS	2002	United Nations, Vienna	325
AIDS	2007	Serbia	371

countries. How long this bipartite state of affairs can continue is uncertain. However, one thing is certain: similar diseases will continue to appear at more or less lengthy intervals. In the blind process of natural selection, human beings are far from being alone in their struggle for survival on this planet. In order to survive, viruses too will evolve and discover their methods of adapting to circumstances. Yet, in discovering how to overcome AIDS, and finding a vaccine against such an elusive virus as HIV, humanity may well discover the means to win these battles in the future to. Above all, the total list of the philatelic material used in this article is shown in Table 1.

Conflict of interest

No conflict of interest was declared by the authors.

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What is your diagnosis?

A 78-year-old woman living in a nursing home was admitted to our clinic with chronic left-lower quadrant pain. She had to stay in her wheelchair and was not able to walk because of paralysis. She was diagnosed as having Alzheimer's disease. She had no one with her except the nursing home staff. They brought her to the general surgeon because of suspicion of acute appendicitis. After non-conclusive blood tests, a whole abdominal computerized tomography (CT) was performed. The CT revealed a cystic mass with a small notch on the mid pelvis (Figure 1). They referred the patient to our clinic for further evaluation.

She had some debilitating conditions such as hypertension and diabetes as well as Alzheimer. She refused the gynecological examination. On abdominal examination, there was no significant finding but around the patient there was a malodorous smell. The abdominal ultrasound examination showed the same cyst that was seen on CT. The patient was persuaded to undergo a gynecologic exam but, in the supine not the lithotomic position. Inspection of external genitalia were normal. There was bad smelling discharge.

Laboratory parameters including erythrocyte sedimentation rate, complete blood count

and blood biochemistry were normal as were tumor markers including CA 125, CA 19-9, carcino embryonic antigen, and α-fetoprotein.

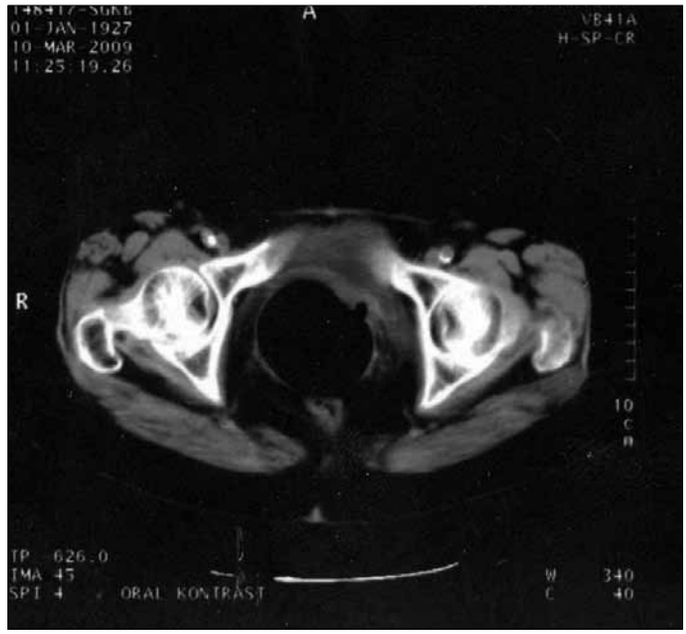

Figure 1. Computerized tomography showed a uniform cyst with a notch on its wall

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Answer

Answer Adnexal masses are common among peri- and post-menopausal women. Although ovarian cancer is a significant cause of mortality in menopausal women, large population-based studies demonstrate that the majority of adnexal masses are benign (1).
Despite this, the appearance of an adnexal mass is a concern for the patient and an insight exercise for physicians. In most cases, an adnexal enlargement is an incidental finding, generally corresponding to a benign cyst and easily diagnosed by conventional ultrasound. Exceptionally, an ovarian tumour may be malignant and should be treated as early as possible. When conventional ultrasound renders complex morphology, other diagnostic tools must be used such as: colour Doppler and functional tumour vessel properties, serum CA 125 levels, nuclear magnetic resonance imaging and in some cases laparoscopy. Several new tumour markers are being studied for clinical application, although there are presently no clear recommendations. The postmenopausal ovary continues to produce cysts; the prevalence in an ovarian cancer screening population approaches 18%. Yet 60% to 70% of unilocular cysts resolve spontaneously (2). Optimal management of an asymptomatic adnexal mass allows surveillance of women at low malignancy risk while triaging intermediate/high-risk women to surgery.
In our case, after convincing the patient to undergo the genital exam in the supine position, digital palpation revealed a balloon in the vagina. The balloon was deflated and removed. It was an inflatable pessary (Figure 2). Probably she forgot about it. The nursing home staff reported that she had spent her last years in America. We thought that somehow in USA a pessary had been inserted for her pelvic organ prolapsus and after time it was totally forgotten. With this case, the importance of the physical examination and history was revealed once more.

Figure 2. The truth is this cyst is an inflatable pessary

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CONGRESS CALENDAR

8-11 September 2011	**The International Congress on Controversies in Stem Cell Transplantation and Cellular Therapies (COSTEM) Andel's Hotel** *Berlin, Germany* www.comtecmed.com/costem/2011/
8-11 September 2011	**26th IUSTI-Europe Congress LATVIA (Diseases - Sexually Transmitted Infections & Disea) ACOG (21st)** *Riga, Latvia* www.iusti-europe2011.org/
11-17 September 2011	**ESGO-Oncology (17th)** *Milan, Italy* www2.kenes.com/esgo17
11-17 September 2011	**ACOG (21th)** *Chicago, USA* www.agosonline.org
14-17 September 2011	**SLS (20th)** *Los Angeles (Hyatt Regency Century Plaza), CA USA* www.sls.org
18-22 September 2011	**ISUOG (21th)** *Los Angeles, USA* http://www.isuog.org
28 September - 2 October	**1st Annual congress on stem cell research** *Sapanca-Turkey* *www.stemcell2011.org*
5-9 October 2011	**UTD (3rd)** *Antalya, Turkey* http://www.utd.org.tr
15-19 October 2011	**ASRM (67th)** *Orlando, Florida, USA* http://www.asrm.org
3-6 November 2011	**The Oocyte: from Basic Research to Clinical Practice,** *Princesa Sofia Hotel, Barcelona, Spain,* www.comtecmed.com/OC/2011/
17-20 November 2011	**Controversial issues and clinical debates in Obstetrics, Gynecology and Infertility (COGI)** *Paris-France* www.congressmed.com/cogi
24-27 November 2011	**Asia Pacific COGI Congress on Building Consensus: Gynecology, Infertility & Perinatology** *Bangkok,THAILAND* www.bcgip.com/2011/
1-2 December 2011	**3rd ANNUAL SEMINAR LAPAROSCOPIC & ROBOTIC Hysterectomy, and Intensive Hands-on Laparoscopic Suturing&Knot Tying** *New York, USA* www.nywomenshealth.com
1-4 July 2012	**28th Annual Meeting of ESHRE** *Istanbul, Turkey* http://www.eshre.eu/home