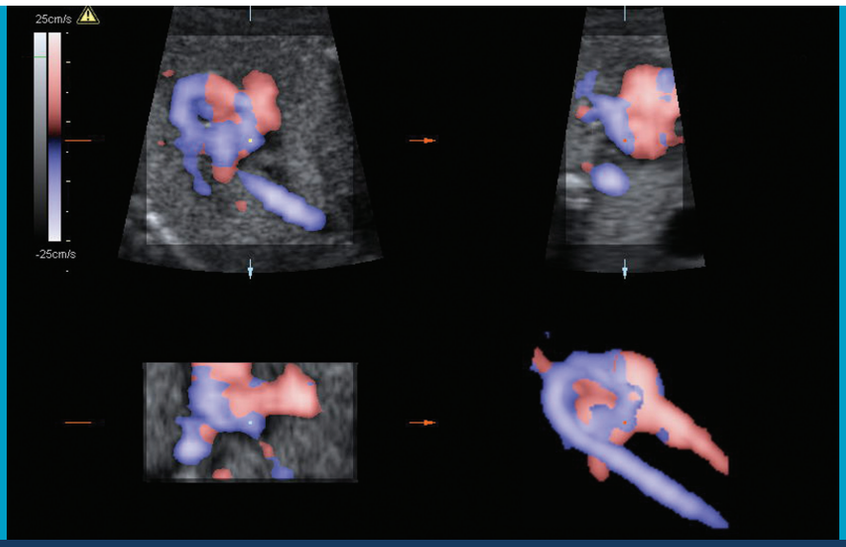
ISSN 1309-0399

TURKISH-GERMAN GYNECOLOGICAL EDUCATION and RESEARCH FOUNDATION

Journal of the Turkish-German Gynecological Association



Volume 12
Issue 4
December
2011

Original Investigations

Official Journal of the
Turkish-German Gynecological Education and Research Foundation
www.tajev.org

Turkish-German Gynecological Association
www.dtgg.de

Turkish Society of Reproductive Medicine
www.tsrm.org.tr

www.jtgga.org

Journal of the Turkish-German Gynecological Association Vol.: 12 Issue: 4 December 2011 Page: 199-285

Journal of the
Turkish-German Gynecological Association

This journal is a member of and subscribes to the principles of the Committee on Publication Ethics (COPE)

Official Journal of the
Turkish-German Gynecological Education and Research Foundation
www.tajev.org

Turkish-German Gynecological Association
www.dtgg.de

Turkish Society of Reproductive Medicine
www.tsrm.org.tr

Editorial Office
Address: Abdi İpekçi cad. 2/7 34367 Nişantaşı, İstanbul-Turkey
Phone: +90 212 241 45 45 Fax: +90 212 241 44 08
E-mail: tajev@tajev.org

Cover Picture:
4D view of the heart using STICK mode.
(Cemil Yaman, MD, Linz, Austria)

Publisher
Address: Kızılelma cad. 5/3 34096 Fındıkzade-İstanbul
Phone: +90 212 589 00 53 Fax: +90 212 589 00 94
E-mail: info@avesyayincilik.com
İmtiyaz Sahibi ve Sorumlu Yazı İşleri Müdürü: M. Cihat Ünlü
Basım Yeri: ADA Ofset Matbaacılık Tic. Ltd. Şti. - +90 212 567 12 42
Yayın Türü: Yerel Süreli Yayın Basım Tarihi: Ekim 2011

Journal of the
Turkish-German
Gynecological Association

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.

Papers written in English language are particularly supported and encouraged.

Journal of the Turkish-German Gynecological Association is indexed in EMBASE, Scopus, CINAHL, Gale/Cengage Learning, EBSCO, DOAJ, ProQuest, Index Copernicus, Tübitak/Ulakbim Turkish Medical Database and Turkiye Citation Index.

Subscription Information
Journal of the Turkish-German Gynecological Association is delivered free of charge to all physicians, specialists in gynecology field. For subscription please contact Turkish-German Gynecological Education and Research Foundation at www.jtgga.org. The access to tables of contents, abstracts and full texts of all articles published since 2000 are free to all readers. Visit the journal's home pages for details of the aims and scope and instruction to authors.

Permission
Permission requests to reproduce copies of articles for non-commercial use may be obtained from the Editorial Office:

Editor: Prof. Dr. Cihat Ünlü
Address: Abdi İpekçi cad. 2/7 34367 Nişantaşı-İstanbul-Turkey
Phone: +90 212 241 45 45
Fax: +90 212 241 44 08
E-mail: tajev@tajev.org

Advertising
Enquiries concerning advertisements should be addressed to Editorial Office:

Editor: Prof. Dr. Cihat Ünlü
Address: Abdi İpekçi cad. 2/7 34367 Nişantaşı-İstanbul-Turkey
Phone: +90 212 241 45 45
Fax: +90 212 241 44 08
E-mail: tajev@tajev.org

Instructions for Authors
Instructions for authors are available in the journal content and at www.jtgga.org.

Disclaimer
The statements and opinions contained in the articles of the Journal of the Turkish-German Gynecological Association are solely those of the individual authors and contributors not of the Turkish-German Gynecological Education and Research Foundation, Turkish-German Gynecological Association, Turkish Society of Reproductive Medicine, Editorial Board or Aves Yayincilik Co.

The journal is printed on acid-free paper.

Journal of the
Turkish-German
Gynecological Association

Instructions for Authors

The "Journal of the Turkish German Gynecological Association" (ISSN 1309-0399; Abbreviated as "J Turkish German Gynecol Assoc") is the official journal of the Turkish-German Gynecological Association and the Turkish Society of Reproductive Medicine. Formerly named "ARTEMIS" is printed quarterly (March-June, September, December) and publishes original peer-reviewed articles, reviews, case reports, brief reports and commentaries in the fields of Gynecology, Gynecologic Oncology, Endocrinology & Reproductive Medicine and Obstetrics in English (Mainly) and occasionally in Turkish and German languages. The title, abstract, and key words (according to medical subject headings) are provided in English at the beginning of each article. Reviews will be considered for publication only if they are written by authors who have at least three published manuscripts in the international peer reviewed journals and these studies should be cited in the review. Otherwise only invited reviews will be considered for peer review from qualified experts in the area.

The "Journal of the Turkish German Gynecological Association" is a peer reviewed journal and adheres to the highest ethical and editorial standards. The Editorial Board of the journal endorses the editorial policy statements approved by the WAME Board of Directors. The journal is in compliance with the uniform requirements for manuscripts submitted to biomedical journals published by the International Committee of Medical Journal Editors (NEJM 1997; 336: 309-315, updated 2001).

Submission of manuscripts
All manuscripts must be submitted via the online submission system after logging on to the web site www.jtgga.org. Authors who have any queries can contact the following addresses:

Prof. Dr. Cihat Ünlü
Editor in Chief (Turkey)
Abdi Ipekci Caddesi 2/7
Nisantasi, Istanbul / Türkiye

Prof. Dr. Peter Mallmann
Editor in Chief (Germany)
Universitäts-Frauenklinik KölnKerpener Str.
3450691 Köln/Germany

The manuscript, figures and tables, prepared under "Microsoft Office Word program", double spaced on one side of A4 sized page, with margins of at least 25 mm should be submitted. Original articles should not exceed 15 pages including the tables and figures. Brief reports should not exceed 5 pages including one figure and/or maximum two tables. As the journals policy only online submissions of manuscripts are accepted after May 2005.

Online Submissions
Only online submissions are accepted for quick peer-review and to prevent delay in publication. Manuscripts should be prepared as word document (*.doc) or rich text format (*.rtf) . After logging on to the web site www.jtgga.org double click the "submit an article" icon. All corresponding authors should be provided a password and an username after providing the information needed. After logging on the article submision system with your own password and username please read carefully the directions of the system to provide all needed information in order not to delay the processing of the manuscript. Attach the manuscript, all figures, tables and additional documents. Please also attach the cover letter with "Assignment of Copyright and Financial Disclosure" forms, check-list of below mentioned guidelines according to the type of the manuscript.

Editorial Policies
All manuscripts will be evaluated by the scientific board for their scientific contribution, originality and content. Authors are responsible for the accuracy of the data. The journal retains the right to make appropriate changes on the grammar and language of the manuscript. When suitable the manuscript will be send to the corresponding author for revision. The manuscript, when published, will become the property of the journal and copyright will be taken out in the name of the journal. Articles previously published in any language will not be considered for publication in the journal. Authors can not submit the manuscript for publication in another jounal. All changes in the manuscript will be made after obtaining written permission of the author and the publisher. Full text of all articles can be downloaded at the web site of the journal www.jtgga.org

Preparation of Manuscripts
The "Journal of the Turkish German Gynecological Association" follows the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" (International Commitee of Medical Journal Editors: Br Med J 1988; 296: 401-5). Upon submission of the manuscript, authors are to indicate the type of trial/research and provide the checklist of the following guidelines when appropriate: Consort statement for randomized controlled trials (Moher D, Schultz KF, Altman D, for the CONSORT Group. The CONSORT statement revised recommendations for improving the quality of reports of parallel group randomized trials. JAMA 2001; 285: 1987-91), the QUOROM statement for meta-analysis and systemic reviews of randomized controlled trials (Moher D, Cook DJ, Eastwood S, Olkin I, Rennie D, Stroup DF. Improving the quality of reports of meta-analyses of randomized controlled trials: the QUOROM statement. Quality of Reporting of Meta-Analyses. Lancet 1999: 354: 1896-900) and the MOOSE guidelines for meta-analysis and systemic reviews of observational studies (Stroup DF, Berlin JA, Morton SC, et al. Meta-analyis of observational studies in epidemiology: a proposal for reporting Meta-analysis of observational Studies in Epidemiology (MOOSE) group. JAMA 2000; 283: 2008-12).

Human and Animal Studies
Manuscripts submitted for publication must contain a statement to the effect that all human studies have been reviewed by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in an appropriate version of the 1975 Declaration of Helsinki. It should also be stated clearly in the text that all persons gave their informed consent prior to their inclusion in the study. Details that might disclose the identity of the subjects under study should be omitted.
Reports of animal experiments must state that the "Principles of laboratory animal care" (NIH publication No. 86-23, revised 1985) were followed, as well as specific national laws where applicable.
The editors reserve the right to reject manuscripts that do not comply with the abovementioned requirements. The author will be held responsible for false statements or for failure to fulfill the abovementioned requirements.
In a cover letter the authors should state if any of the material in the manuscript is submitted or planned for publication elsewhere in any form including electronic media. The cover letter must contain address, telephone, fax and the e-mail address of the corresponding author.

Conflict of Interest
Authors must indicate whether or not they have a financial relationship with the organization that sponsored the research. They should also state that they have had full control of all

primary data and that they agree to allow the Journal to review their data if requested. Therefore manuscripts should be accompanied by the "Conflict of Interest Disclosure Form." The form can be obtained from the journal webpage (www.jtgga.org).

Copyright
The author(s) transfer(s) the copyright to his/their article to the Journal of the Turkish German Gynecological Association effective if and when the article is accepted for publication. The copyright covers the exclusive and unlimited rights to reproduce and distribute the article in any form of reproduction (printing, electronic media or any other form); it also covers translation rights for all languages and countries. For U.S. authors the copyright is transferred to the extent transferable.

Manuscripts must be accompanied by the "Copyright Transfer Statement".

Manuscript Specifications

Title Page
The first page should include the title of the article, name(s), affiliations and major degree(s) of the author(s) and source(s) of the work or study. The name, address, telephone and fax numbers and e-mail address of the corresponding author should be listed on the title page.

Abstract
All manuscripts in Turkish and German should be accompanied by a structured abstract in English and in the language of the manuscript. Only English abstract suffice for manuscripts written in English. The structured abstract(s) should present the study objective, material and method, results and conclusions. Word limitation is 250 words for original articles and 150 words for brief reports and case reports.

Key Words
Below the abstract provide up to 5 key words or short phrases. Do not use abbreviations as key words. For key words in English, Medical Subject Headings (MeSH) terms (www.nlm.nih.gov/mesh/MBrowser.html) and for key words in Turkish, Turkish Science Terms (Türkiye Bilim Terimleri) (www.bilimterimleri.com) should be used.

Introduction
State concisely the purpose and rationale for the study and cite only the most pertinent references as background.

Material and Methods
Describe the plan, the patients, experimental animals, material and controls, the methods and procedures utilized, and the statistical method(s) employed. Address "Institutional Review Board" issues as stated above. State the generic names of the drugs with the name and country of the manufactures.

Results
Present the detailed findings supported with statistical methods. Figures and tables should supplement, not duplicate the text; presentation of data in either one or the other will suffice. Emphasize only your important observations; do not compare your observations with those of others. Such comparisons and comments are reserved for the discussion section.

Discussion
State the importance and significance of your findings but do not repeat the details given in the Results section. Limit your opinions to those strictly indicated by the facts in your report. Compare your finding with those of others. No new data are to be presented in this section.

References
Number references in Arabic numerals consecutively in the order in which they are mentioned in the text starting with number "1". Use the form of the "Uniform Requirements for Manuscript Submitted to Biomedical Journals" (http://www.amaassn.org/public/peer/wame/uniform.htm). If number of authors exceeds seven, list first 6 authors followed by et al. Journal titles should conform to the abbreviations used in "Cumulated Index Medicus".

Examples:
Journals;
Harrington K, Cooper D, Lees C, Hecher K, Campbell S. Doppler ultrasound of the uterine arteries: the importance of bilateral notching in the prediction of pre-eclampsia, placental abruption or delivery of a small-for-gestational-age baby. Ultrasound Obstet Gynecol 1996; 7: 182-8.
Book chapter;
Ertan AK, Tanriverdi HA, Schmidt W. Doppler Sonography in Obstetrics. In: Kurjak A, Chervenak FA, editors. Ian Donald School Textbook of Ultrasound in Obstetrics and Gynecology. New Delhi, India: Jaypee Brothers; 2003. p. 395-421.
Book;
Kohler G; Egelkraut H. In Kohler G and Egelkraut H (edts).Munchener Funktionelle Entwicklungsdiagnostik im zweitem und drittem Lebensjahr. Handanweisung. Munchen: Uni Munchen, Institut fur Soziale Paediatrie und Jugendmedizin; 1984.
Tables and Figures
Tables and figures should work under "Windows". Color figures or gray-scale images must be at least 300 dpi. Figures using "*.tiff", "*.jpg" or "*.pdf" should be saved separate from the text. All tables and figures should be prepared on separate pages. They should be numbered in Arabic numerals. Each table must have a title indicating the purpose or content of each table. Each figure must have an accompanying legend defining abbreviations or symbols found in the figure.

Revisions
Revisions will be sent to the corresponding author. Revisions must be returned as quick as possible in order not to delay publication. Deadline for the return of revisions is 30 days. The editoral board retains the right to decline manuscripts from review if authors' response delay beyond 30 days. All reviewers' comments should be addressed and revisions made should be started with page and line of the text. Send a highlighted copy indicating the revisions made and a clear copy of the revised manuscript. Authors are responsible for the truth of presented data and references. The Editors have the right to withdraw or retract the paper from the scientific literature in case of proven allegations of misconduct.

Journal and Society Web sites:
www.dtgg.de (Deutsch-Türkische Gynäkologengeselleschaft)
www.tajd.org (Türk-Alman Jinekoloji Derneği)
www.jtgga.org (Journal of the Turkish German Gynecological Association)

- Citation of published manuscripts in J Turkish German Gynecol Assoc should be as follows:
Tews G, Ebner T, Sommergruber M, Marianne M, Omar S. Ectopic Pregnancy in the Assisted Reproduction, J Turkish German Gynecol Assoc. 2004;5(1):59-62.
- The Journal name should be abbreviated as "J Turkish German Gynecol Assoc"

Journal of the
Turkish-German
Gynecological Association

Contents

Journal of the
Turkish-German
Gynecological Association

Editorial

Dear Colleagues,

We are pleased to introduce you the last issue of this year of JTGGA right on time. Having published for twelve years regularly and followed by an increasing number of international readers, the JTGGA is 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.

I would like to share my observation with you regarding the congress industry. The participation volume for the traditional Ob&Gyn congresses held for several years regularly in autumn was quite less than the expected this year. I believe that the most important reason of this situation is the consistent decrease in the support and sponsorship of the pharmaceutical industry to the congresses. In that case, our mission shall be combining our strengths and decreasing the total number of national congresses.

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 in the planning period of our forth 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 are determined to prepare a greater project in another region.

In one of the following pages of our journal, you will find the announcement of the ORReady project. It 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. 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 first issue of the next year.

Best regards,

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

A retrospective analysis of acute poisoning during pregnancy

Gebelikteki akut zehirlenmelerin geriye dönük incelenmesi

Sevdegül Karadaş[1], Ayşe Güler[2], İrfan Aydın[1]

[1]Department of Emergency Medicine, Faculty of Medicine, Yüzüncü Yıl University, Van, Turkey

[2]Department of Obstetrics and Gynecology, Faculty of Medicine, Yüzüncü Yıl University, Van, Turkey

Abstract

Objective: The aim of this study is to investigate and analyse pregnant women who were admitted to our emergency service due to acute poisoning.

Material and Methods: All cases were retrospectively collected from our computer records and emergency record book between 01.01.2006 and 01.01.2010; the registration data on age, gravidity, gestational week, whether the poisoning was deliberate or accidental, causative agent, admission time, treatment results and mortality outcome were collected and analyzed.

Results: Eighty eight women admitted with acute poisoning were known to be pregnant and the poisoning was accidental in 23% of the cases, while 77% were suicidal intoxications. 74% of patients were in the 21-34 age group. Accidental intoxications were due to carbon monoxide, foods and cleaning products. 75.4% of the suicidal poisonings were caused by medical drugs, with analgesics, multiple drugs and psychiatric drugs being the top culprits and accounted for 53%, 31% and 16% of drug poisonings respectively. In terms of gestational week, 47.4% of suicidal poisonings were within the first trimester and the relationship between suicidal attempt and gestational week was found to be statistically significant ($p<0.015$). However, the relationship between gravidity and the rate of suicidal attempts was not statistically significant ($p=0.214$). All patients were followed up and treated in the emergency service and no mortality was observed in the study.

Conclusion: Most cases of acute poisonings during pregnancy were suicidal. Pregnant women attempted suicide mostly within the first trimester of gestation. The most common agents used for suicidal attempt were medical drugs.

(J Turkish-German Gynecol Assoc 2011; 12: 199-203)

Key words: Poisoning, pregnancy, emergency service

Received: 17 August, 2011 **Accepted:** 11 September, 2011

Özet

Amaç: Zehirlenme nedeniyle acil servise kabul edilen gebe kadınları araştırmak ve analiz etmektir.

Gereç ve Yöntemler: 01.01.2006 ve 01.01.2010 tarihleri arasında acil servise zehirlenme ile başvuran tüm gebe olgular bilgisayar kayıtları ve acil kayıt defteri kullanılarak geriye dönük toplandı; yaş, gravida, gebelik haftası, zehirlenme etkeni, zehirlenmenin özkıyım veya kazara oluşu, başvuru süresi, uygulanan tedaviler ve mortalite sonuçları analiz edildi.

Bulgular: Acil servise akut zehirlenme ile başvuran 88 gebe olgunun %77'si özkıyım amaçlı, %23'ü kazara oluşan zehirlenmeler idi. Olguların %74'ü 21-34 yaş grubundaydı. Kazara olan zehirlenmeler karbon monoksit, besin ve temizlik ürünlerine bağlıydı. Özkıyım amaçlı zehirlenmelerin %75.4'ünde etken medikal ilaçlar olup sırasıyla %53 analjezikler, %31 çoklu ilaçlar ve %16 oranında da psikiyatrik ilaçlar saptandı. Gebelik haftasına göre, özkıyım amaçlı zehirlenmelerin %47.4'ü 1. trimesterda olup gebelik haftası ile özkıyım girişimi arasındaki ilişki istatistiksel olarak anlamlı bulundu ($p<0.015$). Ancak, gravida ile özkıyıma teşebbüs etme arasındaki ilişki istatistiksel olarak anlamlı bulunmadı ($p=0.214$). Olguların tamamının takip ve tedavisi acil serviste yapıldı, çalışmada ölüm vakasına rastlanmadı.

Sonuç: Gebelikteki akut zehirlenme vakalarının çoğu özkıyım amaçlıdır. Gebeler özkıyıma en fazla gebeliğin ilk üç ayında teşebbüs etmektedir. İntihar amacıyla en sık kullanılan madde ise tıbbi ilaçlardır.

(J Turkish-German Gynecol Assoc 2011; 12: 199-203)

Anahtar kelimeler: Zehirlenme, gebelik, acil servis

Geliş Tarihi: 17 Ağustos 2011 **Kabul Tarihi:** 11 Eylül 2011

Introduction

Acute intoxications constitute a considerable number of admissions to the emergency services of all hospitals. Intoxications may occur intentionally or after accidental intake. Although there are a large number of studies on acute intoxications in the literature, studies performed on pregnant women are limited. Many physical, psychological and physiological changes occur during pregnancy. So, timing and planning of a gestation jointly by both spouses is very important. What happens if a woman gets pregnant unplanned or unwillingly? Most probably, it will be a source of stress for the mother and as a result the woman will seek an escape from this situation. Efforts to get rid of an unplanned gestation may

Address for Correspondence: *Sevdegül Karadaş, Department of Emergency Medicine, Faculty of Medicine, Yüzüncü Yıl University, 65000 Van, Turkey*
Phone: +90 534 987 03 31 e.mail: sevdegulkaradas@yahoo.com

doi:10.5152/jtgga.2011.51

vary from simple curettage to suicidal attempts. Poisonings resulting from suicidal attempts are not common during pregnancy. Suicidal attempts most frequently occur by oral intake of drugs or other substances. The approach to acute poisonings during pregnancy is different because of the physiology of pregnancy and the potential risks to two lives. The mother's life should always be the first concern. Unless the mother is in danger, any intervention that may harm the fetus should be avoided if possible.

In this study we aimed to define the demographic, etiological and clinical characteristics of pregnant women with acute poisoning who were admitted to the Emergency Department of Yüzüncü Yıl University in Van/Turkey.

Material and Methods

Cases of acute poisoning during pregnancy who were admitted to our emergency service between 01.01.2006 and 01.01.2010 were screened retrospectively by using the registration data from computer records and emergency record book. Cases of food poisoning and stings by animals were not included.

Eighty seven cases were enrolled to the study. Data on age, month of admission, gravidity, gestational week, toxic agent, route of poisoning, whether the poisoning was suicidal or accidental and outcome of treatment were recorded in the specifically designed forms. In addition, data about gestation obtained from the obstetrics and gynecology consultation note that was routinely asked every pregnant were filled in the forms. Data extraction and analyses were all done by two researchers of the study.

Statistical analysis

Descriptive statistics were expressed as means and standard deviations for continuous data and count and percent for categorical data. Chi square test was used to determine the association between categorical variables and Z test was used to compare proportions. In addition, Pearson correlation analysis was used to determine the linear relationships among the continuous variables. The SPSS (ver. 13) statistical program was used for all statistical computations and 5% level was considered as statistically significant.

Results

A total of 142,456 patients were admitted to the emergency service of Yüzüncü Yıl University between 01.01.2006-01.01.2010. Of these, 1623 (11/1000) cases were diagnosed as acute intoxication and 1163 (71%) were females. Eighty eight (7.5%) female patients were pregnant. While 1 of these 88 patients did not accept any intervention, 13 of them accepted only emergency intervention but refused any obstetrical and gynecological examination.

Sixty seven (77%) of the 87 cases of acute intoxication were suicidal poisonings. Intoxication in the remaining 20 (23%) was due to accidental intake.

The mean age of the cases was 25.25±5.85 years, and age range was 17-45 years. Average age was 24.78±5.38 years in the suicidal intoxication group and 26.35±6.80 in accidental ones (Table 1). Concerning the age, cases were divided into three groups as ≤20 (group 1), 21-34 (group 2) and ≥35 years (grup 3). There were 16 (18%), 64 (74%) and 7 (8%) patients in the first, second and third groups, respectively. In terms of age groups, no significant difference was found between intentionally and accidentally poisoned pregnants (p=0.795) (Figure 1).

When cases were analysed in terms of the causative agents, carbon monoxide (CO) intoxication, food poisonings (mushroom+fish, mushroom..etc) and cleaning products inhalation (bleach, thinner, hydrochloric acid) were determined in 7, 6 and 7 cases of accidental poisonings (n=20) respectively. No causative agents could be determined in 10 cases of suicidal poisonings (n=67). Forty three (75.4%) of the remaining cases attempted suicide by taking medical drugs. Bleach and rat poison were determined in the second and third order, respectively. The drugs taken in suicidal poisonings were analgesic drugs in 53% of cases, multiple drugs in 31% and psychiatric drugs in 16% (Table 2).

When cases were analysed in terms of gestational weeks, 27 (47.4%) of 57 suicidal cases were in the first trimester, 17 (29.8%) were in the second and 13 (22.8%) were in the third trimester. The relationship between gestational week and suicide attempt was found to be statistically significant (p<0.015) (Figure 2).

Concerning the month of admission, the highest record was in May with the rate of 14.8 %. When suicidal intoxications were separately analysed, it was found that the most frequent appli-

Table 1. Age distribution of study groups

	Minimum (years)	Maximum (years)	Average (years)
Accidental intoxication	18	45	26.35±6.80
Suicidal intoxication	17	42	24.78±5.38
Total	17	45	25.25±5.85

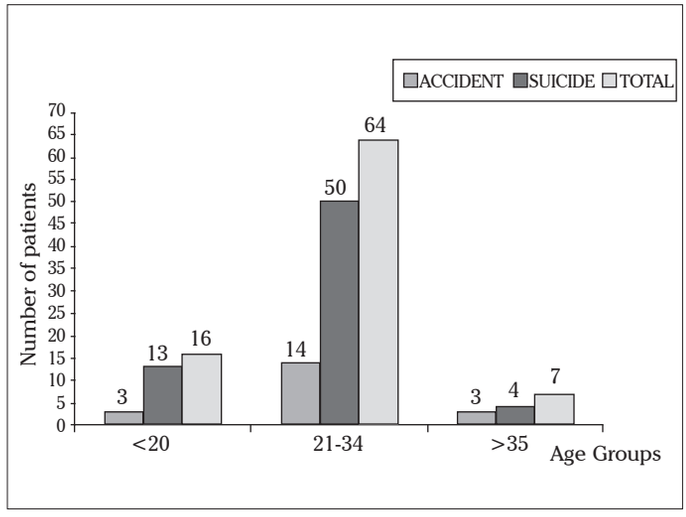


Figure 1. Dispersion of cases according to age groups

Table 2. Dispersion of poisonings according to causative agent

Causative Agent	Number (n)	Ratio (%)
Food	6	6.9
Carbon monoxide (CO)	7	8.1
Cleansing substance inhalation	7	8.1
Drugs	43	49.4
Analgesics (53%)		
Multiple drugs (31%)		
Psychiatric drugs (16%)		
Corrosive substance intake	6	6.9
Ratsbane	8	9.1
Others	10	11.5
Total	87	100

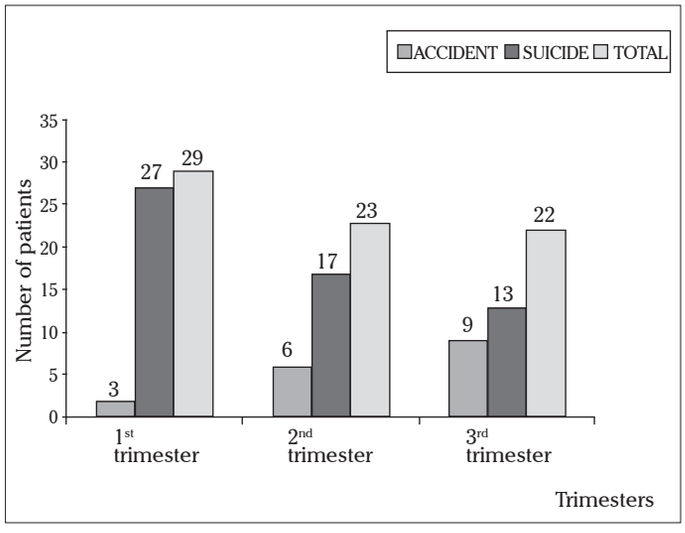


Figure 2. Dispersion of patients according to gestational week

Table 3. Distribution of cases according to gravidity

Gravidity	≤3	>3	Total
	n (%)	n (%)	n (%)
Accidental intoxication	18 (20.7)	2 (2.3)	20 (23.0)
Suicidal intoxication	49 (56.3)	18 (20.7)	67 (77.0)
Total	67 (77.1)	20 (22.9)	87 (100.0)

cation was observed in August with the rate of 12.3%. There was no statistically significant relationship between the rate of suicide attempts and month of admission ($p>0.05$).

Concerning the gravidity (G), cases were grouped as G≤3 (group 1) and G>3 (group 2). Rates of groups were found as 77% (n=67) and 23% (n=20) in the first and second groups, respectively. When the suicidal poisonings were reviewed separately, rates were determined as 73.1% and 26.9% in the first and second groups, respectively. The relationship between gravidity and the rate of suicidal attempt was not found to be statistically significant ($p=0.214$).

All of the patients were followed up and treated in the emergency service and were discharged from there. In this study, no case of mortality was observed.

Discussion

To our knowledge, this is the first study from Turkey that analyzes acute poisoning cases which occurred during pregnancy. Poisonings may vary according to country, region, traditions and degree of development. The American Association of Poison Control Centers reported 2,380,000 cases of poisoning in 2002 and a 4.9% increase was observed in poisonings comparing to that in 2001 (1). The incidence was found as 5.4% in a study from Iran (2). Rates of 1.8/1000 and 6.9/1000 were reported by Hassens et al. (3) and Bajo Bajo et al. (4) respectively. According to the results of quite a few epidemiologic studies conducted in our country, the annual poisoning incidence was determined as 0.8-5% (5-8). So the rate of 1.1% that we found in this study performed at a university hospital in the province of Van, located in the most eastern part of Turkey, is in accordance with the literature findings. We think that the difference among the rates reported might be due to the difference among the developmental status, economical and sociocultural levels of the countries.

According to studies conducted in different parts of Turkey (6, 9, 10), it was reported that the majority of acute poisoning cases were women. In our study, too, the ratio of women was 71% and this was 2.5 times higher than men. Pregnant cases constituted 7.5% of all women admitted with acute intoxication. Suicidal poisonings are reported in the literature to be more than accidental ones (11). In a study on acute poisonings from our country, it was reported that 48.2% were accidental, 51.8% were suicidal (5), however in another study, 76.7% were accidental and 23.4% were suicidal (12). Ahmadi et al. reported in their study conducted in Iran that 85% of poisonings were suicidal (13). According to the study conducted by K. Candace et al. on women of reproductive age and during pregnancy with acute poisoning; 69.6% of cases occurred after suicidal intake and 21.5% were accidental (14). In our study, 77% of pregnant cases were suicidal and 23% were accidental. This result can be due to conditions in our region such as life style and suppression of the society on women, women's lower authority in the family and society, their lack of economic independence and lack of knowledge about family planning. Further studies are needed to determine the effects of all those factors.

Watson et al. reported that 32% of poisonings occurred in the first trimester, 37.6% in the second trimester and 30.5% in the third trimester (15). The rates of acute poisonings in our study were 39.2%, 31.1% and 29.7% in the first, second and third trimesters, respectively. If suicidal poisoning was taken out for analysis, the rates were 49%. 24.5% and 26.5% in the first, second and third trimesters respectively. It can be appreciated that the rate of suicidal attempts was significantly reduced with advanc-

ing gestational age. This observation was supported by the study by Czeiel et al. conducted in Budapest in 1985-1993 (16). This decrease may be attributed to the increasing perception of the gestation by the women as their gestation advances.

In our study, the most common causative agents were found to be drugs, bleach and rat poison. Similar to other reported case series (17-19), poisonings with medical drugs were the most frequent and the rate was 75.4%. Acute poisonings during pregnancy constituted a small part of maternal demise in antenatal and postnatal periods. The most common method of suicidal attempt is oral intake of drugs or other toxic substances. Overuse of analgesics, vitamins, iron tablets, antibiotics and psychotropic drugs makes up 50-79% of those attempts (20, 21). It was reported that 50% of adult poisonings were with 2-3 agents by Mc Mahon et al. (22).

Fifty three percent of poisonings with drugs were with analgesics, 31% with multiple drugs (vitamin, antihistamines, antibiotics, digestive system drug, antitussive) and 16% with psychiatric drugs (tricyclic antidepressants, SSRIs). Similarly, poisonings occurred most commonly with analgesics according to the results of the study by K. Candace et al. (14). Analgesics are the most common agents leading to poisonings in our country because they can be easily bought over the counter.

All acute intoxication cases in our study were followed up and treated in the emergency service from where they were discharged. While death has been reported from some centers in our country (19, 23-25), no death occurred in our study. Mortality rates among cases admitted with acute poisoning were found as 0.03% in Serinken et al.'s (19) study and 2.8% in the study performed in Gaziantep by Göksu et al. (24). We postulate that aero mortality observed in our study is due to intake of drugs with low toxicity and to rapid application of an effective treatment protocol to potentially lethal poisonings. It is notable in our study that the majority of pregnant women who attempted suicide were in the first trimester and suicidal attempts decreased as gestational weeks increased. It is postulated that women with unplanned pregnancy commit suicide as a way to seek attention and to get rid of the pregnancy; which is known as a secondary gain. To reduce unplanned pregnancies, spouses in Van region should be better informed about family planning methods. Moreover, these patients should be evaluated psychiatrically before being discharged. We believe that such cases will decrease with increasing educational level, improving socioeconomical status and especially with increasing family planning services.

Pregnancy is a special period during which physical, psychological and physiological changes will occur in women. Untimely and unplanned pregnancy can be a source of additional stress for women. This is the first study from our country that analyses the epidemiology of acute intoxications during pregnancy. Nevertheless, our study is limited by being retrospective and including cases from only one center. In order to obtain more generalizable results; prospective, multicentered studies, including follow up of fetal outcomes as well, should be performed.

Conflict of interest

No conflict of interest was declared by the authors.

References

1. Watson WA, Litovitz TL, Rodgers GC Jr, Klein-Schwartz W, Youniss J, Rose SR, et al. 2002 Annual Report of the American Association of Poison Control Centers Toxic Exposure Surveillance System. Am J Emerg Med 2003; 21: 353-411. [CrossRef]
2. Islambulchilar M, Islambulchilar Z, Kargar-Maher MH. Acute adult poisoning cases admitted to a university hospital in Tabriz, Iran. Hum Exp Toxicol 2009; 28: 185-90. [CrossRef]
3. Hanssens Y, Deleu D, Taqi A. Etiologic and demographic characteristics of poisoning:a prospective hospital-based study in Oman. J Toxicol Clin Toxicol 2001; 39: 371-80. [CrossRef]
4. Bajo Bajo A, Santos Perez ME, Sanz Ortega F, Zapico Alvarez N, Okatsu KT, Garcia Perez A. An epidemiological study of acute intoxications and provision of medical-cabinet antidotes. An Med Interna 1999; 16: 285-9.
5. Mert E, Bilgin NG. Demographical, etiological and clinical characteristics of poisonings in Mersin, Turkey. Hum Exp Toxicol 2006; 25: 217-23. [CrossRef]
6. Ozkose Z, Ayoglu F. Etiological and demographical characteristics of acute adult poisoning in Ankara, Turkey. Hum Exp Toxicol 1999; 18: 614-8. [CrossRef]
7. Pınar A, Fowler J, Bond GR. Acute poisoning in Izmir, Turkey- a pilot epidemiologic study. J Toxicol Clin Toxicol 1993; 31: 593-601. [CrossRef]
8. Karakaya A, Vural N. Acute poisoning admissions in one of the hospitals in Ankara. Hum Toxicol 1985; 4: 323-6. [CrossRef]
9. Pekdemir M, Kavalci C, Durukan P, Yildiz M. Evaluation of poisoning cases presented to our emergency department. Turkish Journal of Emergency Medicine 2002; 2: 36-40.
10. Kekec Z, Sozuer EM, Duymaz H, Okkan S. Evaluatian of the patients applied to the emergency department due to multiple drug poisoning: analysis of 7 years. Turkish Journal of Emergency Medicine 2005; 5: 69-72.
11. Lapatto-Reiniluoto O, Kivisto KT, Pohjola-Sintonen S, Luomanmaki K, Neuvonen PJ. A prospective study of acute poisonings in Finnish hospital patients. Hum Exp Toxicol 1998; 17: 307-11. [CrossRef]
12. Deniz T, Kandiş H, Saygun M, Büyükkoçak Ü, Ülger H, Karakuş A. Evaluation of intoxication cases applied to emergency department of Kirikkale university hospital. Düzce Medical Journal 2009; 11: 15-20.
13. Ahmadi A, Pakravan N, Ghazizadeh Z. Pattern of acute food, drug and chemical poisoning in Sari city, Northern Iran. Hum Exp Toxicol 2010; 29: 731-8. [CrossRef]
14. McClure CK, Katz KD, Patrick TE, Kelsey SF, Weiss HB. The epidemiology of acute poisonings in women of reproductive age and during pregnancy, California, 2000-2004. Matern Child Health J 2011; 15: 964-73. [CrossRef]
15. Watson WA, Litovitz TL, Rodgers GC Jr, Klein-Schwartz W, Reid N, Youniss J, et al. 2004 annual report of the American association of poison control centers toxic exposure surveillance system. Am J Emerg Med 2005; 23: 589-666. [CrossRef],
16. Czeizel AE, Timar L, Susanszky E. Timing of suicide attempts by self-poisoning during pregnancy and pregnancy outcomes. Int J Gynaecol Obstet 1999; 65: 39-45. [CrossRef]
17. Gandhi SG, Gilbert WM, McElvy SS, El Kady D, Danielson B, Xing G, et al. Maternal and neonatal outcomes after attempted suicide. Obstet Gynecol 2006; 107: 984-90. [CrossRef]
18. Yavuz MS, Aydın S. Profile of poisoning cases. Journal of Toxicology 2003; 1: 47-52.
19. Serinken M, Yanturali S. A retrospective analysis of suicidal poisoning in the emergency department. The Turkish Journal of Toxicology 2003; 1: 15-9.

20. Perrone J, Hoffman RS. Toxic ingestions in pregnancy: Aborticifacient use in a case series of pregnant patient overdose. Acad Emer Med 1997; 4: 206-9. [CrossRef]
21. Raybum W, Anarow R, Delancey B, et al. Drug overdose during pregnancy: An overview from a metropolitan poison control center. Obstet Gynecol 1984; 64: 611-4. [CrossRef]
22. McMahon GT, McGarry K. Delibrate self-poisoning in an Irish country hospital. Ir J Med Sci 2001; 170: 94-7. [CrossRef]
23. Guloglu C, Kara IH. Acute poisoning cases admitted to a university hospital emergency department in Diyarbakir, Turkey. Hum Exp Toxicol 2005; 24: 49-54. [CrossRef]
24. Goksu S, Yildirim C, Kocoglu H, Tutak A, Oner U. Characteristics of acute adult poisoning in Gaziantep, Turkey. Toxicol Clin Toxicol 2002; 40: 833-7. [CrossRef]
25. Celiker H, Tezcan E, Gunal AI, Celebi H, Donder E. Demographic characteristics of suicidal poisonings in Elazig. Fırat University Medical Journal of Health Sciences 1996; 10: 33-7.

Sentinel lymph node biopsy in endometrial cancer: description of the technique and preliminary results

Endometrium kanserinde sentinel lenf nodu biopsisi: tekniğin tanımı ve ilk sonuçlar

Oğuzhan Kuru, Samet Topuz, Serhat Şen, Cem İyibozkurt, Sinan Berkman

Department of Obstetrics and Gynecology, İstanbul Faculty of Medicine, İstanbul University, İstanbul, Turkey

Abstract

Objective: To measure the feasibility of sentinel lymph node technique in endometrial cancer.

Material and Methods: The study was designed as a prospective non-randomized case-control trial. Between 2010-2011, in Istanbul University, Istanbul Medical Faculty, Gynecologic Oncology department, 26 patients who were preoperatively evaluated as endometrial cancer enrolled in the study. Patients' detailed informed consent and ethics committee approval were obtained. Sentinel lymph node (SLN) detection rate was determined as the primary outcome. Sensitivity, specificity, positive and negative predictive values and particularly false negative results were determined as secondary outcomes. As a technique of SLN, injection of methylene blue to the subserosal myometrium of the uterine fundus via 5 cc syringe following peritoneal aspiration cytology procedure was obtained. Surgery was made after injection for an average of 5 minutes due to the physiological spread of the blue dye. Then, the standard protocol of hysterectomy was performed and the retroperitoneum was opened to perform lymphadenectomy. The presence of lymph node regions, and presence of a sentinel node was recorded on the trial record form. Positive staining nodes were sent separately for pathological examination. In the course of the study due to insufficient rate of staining, the technique has been changed to cervical and multiple uterine injections.

Results: As the primary outcome, an SLN positivity rate of 23% in 6 patients with a total of 8 lymph nodes were found. The remarkable finding was that in the first technique, the rate was 1/16 (6%), while the second technique, 5/10 (50%), respectively. The difference is statistically significant (p=0.001). In endometrial cancer stage I and II, secondary outcomes for sensitivity, specificity, positive predictive value, negative predictive value were 23%, 0%, 100%, 43%, respectively. Because there were no metastatic lymph nodes found, false negative rate was 0%.

Conclusion: SLN approach is not valuable enough to eliminate the need for lymphadenectomy. On the other hand, it facilitates scanning micrometastases and ultrastaging, while its clinical value has not yet been established. However, according to the recent pilot studies, it provides a means for assessing micrometastases for the medium-risk group for local recurrence.

(J Turkish-German Gynecol Assoc 2011; 12: 204-8)

Key words: Sentinel lymph node, endometrium cancer, surgical staging

Received: 3 August, 2011 **Accepted:** 16 September, 2011

Özet

Amaç: Sentinel lenf nodu tekniğinin endometriyum kanserinde uygulanabilirliğini ölçmek.

Gereç ve Yöntemler: Prospektif non-randomize vaka kontrol çalışması olarak, 2010-2011 yılları arasında İstanbul Üniversitesi İstanbul Tıp Fakültesi, Kadın Hastalıkları ve Doğum Anabilim Dalı, Jinekolojik Onkoloji servisinde yapılan preoperatif değerlendirmesi endometriyum kanseri saptanan 26 olgu çalışmaya alındı. Çalışma için hastalardan aydınlatılmış onam formları ile etik kurul onayı alındı. Birincil sonuç olarak sentinel lenf nodu (SLN) saptanma oranı, sekonder sonuç olarak da yalancı negatiflik başta olmak üzere duyarlılık, özgüllük, pozitif ve negatif prediktif değerlerin saptanması belirlendi. SLN tekniği olarak periton yıkantı sitolojisini takiben uterus fundusta subserozal myometriyuma 5 cc injektör ile metilen mavisi verildi. Boya verilmesini takiben ortalama 5 dakika cerrahiye ara verilerek boya maddenin yayılması beklendi. Broad ligaman ve tubalar klempe edilerek tümör hücrelerinin mobilizasyonunun engellenmesi amaçlandı. Ardından standart histerektomi protokolü uygulandı. Retroperitoneum açılarak lenfadenektomi yapıldı. Çalışma formuna, çıkarılan lenf nodu bölgeleri ve varsa sentinel nodu varlığı kaydedildi. Pozitif boyanma gösteren lenf nodlarının patolojik incelemeye ayrıca gönderilmesi öngörüldü. Çalışmanın seyrinde boyanma oranı yetersiz görüldüğü için servikal ve multipl uterus injeksiyonu tekniğine geçildi.

Bulgular: Primer sonuç olan SLN pozitifliği 6 hastada toplam 8 lenf nodu ile %23 oranında bulunmuştur. İlk teknikte oran 1/16 (%6) iken ikinci teknikte 5/10 (%50) olarak bulunmuştur. Aradaki fark istatistiksel olarak anlamlıdır (p=0.001). Sekonder sonuçlar olarak evre I ve II endometriyum kanserinde SLN tekniğinin duyarlılığı %23, özgüllüğü %0, pozitif prediktif değeri %100, negatif prediktif değeri %43 olarak bulunmuştur. Yalancı negatiflik değeri de pozitif lenf nodu bulunmadığından %0'da kalmıştır.

Sonuç: SLN yaklaşımı lenfadenektomi ihtiyacını bertaraf edecek kadar değerli olmamakla birlikte rutin pratikte tüm lenf nodlarına uygulamanın mümkün görünmediği ve tedavi yönetiminde oturmuş bir yeri olmayan ancak pilot çalışmalarda elde edilen verilere göre nüks için orta risk grubu kabul edilen mikrometastaz incelemesine imkan vererek ultrastaging şansı yaratmaktadır.

(J Turkish-German Gynecol Assoc 2011; 12: 204-8)

Anahtar kelimeler: Sentinel lenf nodu, endometriyum kanseri, cerrahi evreleme

Geliş Tarihi: 03 Ağustos 2011 **Kabul Tarihi:** 16 Eylül 2011

Address for Correspondence: *Oğuzhan Kuru, Department of Obstetrics and Gynecology, İstanbul Faculty of Medicine, İstanbul University, İstanbul, Turkey*
Phone: +90 212 414 20 00 e.mail: drokuru@yahoo.com

doi:10.5152/jtgga.2011.52

Introduction

Endometrial cancer is the most common gynecologic cancer in developed countries and its incidence has been increasing (1, 2). Although the data has poor reliability, according to Turkish Health Statistics 2006 reports; the incidence of endometrium cancer is 0.0029, which is the second most common gyenocologic cancer. Surgical staging of endometrial cancer in the early stage covers peritoneal cytology, total hysterectomy, bilateral salphingoopherectomy (BSO) and lymph node sampling. Mean prognostic factors are based on histological grade, depth of myometrial invasion and lymph node status (3). Positive lymph node status is the major prognostic factor and restricts the duration of disease-free survival. Positive lymph nodes at stage 1 endometrial cancer reduces 5 year disease-free survival rate to 54%, while it was 90% if the nodes were negative (4). The chance of lymph node invasion depends on tumor size, proximity to the lymphatic space, the degree of tumor differentiation, and infiltration (5). Lymph node metastases ranged from 0% to 34% in early-stage endometrial cancer, and it is crucial to determine an accurate staging (6-8). Retrospective studies have shown the benefits of therapeutic lymphadenectomy (9, 10).

Hypothetically, if the presence of intraoperative lymphatic involvement is determined, the extent of surgery should be established. Burke and his colleagues published the first study on the use of sentinel lymph node in endometrial cancer 12 years ago (11). In this study, we aimed to measure the success of the feasibility and effectiveness of sentinel lymph node technique in early-stage endometrial cancer in our clinic.

Materials and Methods

Study design

This is a pilot study which was designed as a prospective non-randomised case-control research. The primary outcome obtained was sentinel lymph node (SLN) detection rate, with false negativity, sensitivity, specificity, negative predictive value as secondary outcomes. Between 2010-2011, in Gynecologic Oncology, Obstetrics and Gynecology, department of the Istanbul University, Istanbul Medical Faculty, 26 patients who were preoperatively evaluated as endometrial cancer were enrolled in the study. Patients' detailed informed consent and ethics committee approval were obtained. The patients' demographic data, histological diagnosis, grade, intraoperative and postoperative complications of the surgery, body mass index (BMI), and presence of diabetes were recorded.

Inclusion criteria

The presence of the histological diagnosis of primary endometrial cancer, signing of informed consent form, having no evidence of distant metastases or extra-uterine disease in preopative imaging procedures, non-recurrent disease, having no medical disease which contraindicated general anesthesia or conditions that makes it difficult to perform lymphadenectomy such as morbid obesity and congenital anomalies were obtained as inclusion criteria.

Surgical tehnique

The patients were operated with a Phannenstiel or midline incision according to their BMI and gynecological examination. Minimum surgical protocol involved abdominal washing cytology, abdominal hysterectomy, BSO, omental biopsy and external-internal-common iliac and obturator lymphadenectomy. Under the guidance of clinical datas and if surgical tehnique was feasible; total omentectomy, presacral and/or paraaortic lymphadenectomy were also performed.

As a technique of SLN, injection of methylene blue to the subserosal myometrium of uterine fundus via a 5 cc syringe following periton aspiration cytology procedure was performed. Surgery was carried out after injection for average of 5 minutes due to the physiological spread of the blue dye. The broad ligament and the uterine tubes were clamped to prevent mobilization of tumor cells. The standard protocol of hysterectomy was performed; the retroperitoneum was opened to perform lymphadenectomy. The presence of lymph node regions, and a sentinel node was recorded in the trial record form. Positive staining nodes were sent separately for pathological examination. During the course of the study, due to an inadequate rate of staining, the technique was switched to cervical and multiple uterine injections after 16 patients in the serial. The new technique was performed as bilateral paratubal, posterior and anterior isthmic uterine wall injections of 0.5 cc isosulphane blue dye in each of the areas. The period of time increased to 10 minutes before clamping the ligaments.

Histological evaluation

Pathologists evaluated the sentinel node and other nodes macroscopically. They were divided into those with gross metastatic nodes and normal-looking ones and were cut perpendicular to the axis. Each half was cut parallel at intervals of up to 5 mm across, and hematoxylin-eosin (HE) staining was performed. After the parallel sections of 3 mm were obtained, sentinel nodes were cut at 150 μm intervals and at least 4 primary sections for HE staining, followed by 4 secondary cross-section were taken for immunohistochemistry (IHC). For IHC staining, the anti-cytokeratin antibody (Cytokeratin AE1-AE3, Dako Corporation, Glostrup, Denmark) was used. The definition of micrometastasis in lymph node metastases between 0.2 and 2 mm in size were identified. The histological typing, grading and staging of the primary tumor were based on the FIGO system.

Statistical analysis

The results of 16 cases with myometrial subserosal methylene blue dye injection and 10 cases with both cervical and uterine isosulphane blue dye injection were compared by the Mann-Whitney test. Because of the absence of lymphatic metastasis, logistic regression analysis could not be performed to investigate the possibility of lymphatic metastasis.

Results

In demographic data; the mean age of the patients was 57.73±6.36 (42-79), the mean gravida was 3.34 (0-8), average parity was 2.07 (0-5) and except for 6 cases (23%), all cases

were at the postmenopausal stage. The average body mass index was 35.25±5.58 (20.3 to 45.7). Histological types were endometrioid adenocarcinoma (24 cases, 92%), serous papillary carcinoma (1 case, 4%), and clear cell carcinoma (1 case, 4%) (Table 1).

The second technique was applied in two cases of non-endometrioid type. In 5 cases (19.2%), over 50% of the depth of myometrial invasion was detected. Para-aortic lymphadenectomy was applied in only two cases (7.6%). On the other hand, lymph node metastases were not found in any of the patients (0%). The average tumor volume was 20.15 cc. Lymphovascular invasion (LVAI) was found in 6 (23%) cases. In fewer than expected only 5 cases, (19.2%) had diabetes mellitus while 14 patients (53.7%) had hypertension. It was found to be remarkable that only half of 4 cases which were shown to have greater than 50% myometrial invasion, correlate with the final pathology report. The primary outcome of SLN positivity was detected with 6 patients (23%) with a total of 8 lymph nodes. The detection rate of the first technique was 1/16 (6%), while the second technique 5 /10 (50%), which was found statistically significant (p=0.001).

None of the patients suffers from intraoperative or postoperative complications which prolongs the duration of hospital stay or requires re-hospitalization. As secondary outcomes, the sensitivity, specificity, positive predictive value, negative predictive value of SLN biopsy technique in stage I and II endometrial cancer was 23%, 0%, 100% and 43%, respectively. The negative predictive value was 0% because of the absence of positive lymph nodes. The most common region of 8 positive sentinel lymph nodes in 6 cases was detected in the obturator areas with 5 nodes (62.5%). There were only two high graded patients in the series of 26 cases, and SLN was found in both cases. On the other hand, other patients with SLN-positivity remained as stage 1 and grade 1 (Table 2).

Table 1. Demographic and clinical data of the patients

Age range years	
40-50	6 (23%)
50-60	9 (35%)
60-70	8 (31%)
70-80	3 (11%)
BMI kg/m^2	
<35	11 (42%)
>35	15 (58%)
Parity	
≤3	21 (80%)
>3	5 (20%)
Stage	
I	24 (92%)
II	2 (8%)
Histologic type	
Endometrioid	24 (92%)
Serous	1 (4%)
Clear cell	1 (4%)
Grade	
I	20 (77%)
II	6 (23%)

Discussion

In our study injecting the blue dye to the uterine fundus and SLN biopsy in the early stage endometrial cancer appeared to be a poor technique as in the data in the literature.

Sentinel lymph node biopsy and lymphatic mapping can be applied in many solid tumors. It was used as the standard procedure in cutaneous melanoma and breast cancer therapy. The technique allows evaluation of lymphatic involvement, and assists in reducing the frequency of unnecessary complete lymphadenectomy. However, although endometrial cancer is a solid tumor, due to the uncertain and complex field of lymphatic drainage, application is not ideal for lymphatic mapping. Potential regions of involvement are the obturator, internal-external-common iliac, tibia, parametrial and presacral areas around the aortic artery. In our study, the distribution of SLN were: obturator region in 62.5%, external iliac region in 25% and internal iliac region in 12.5%. Due to the insufficient number of cases, the clinical relevance of SLN detection rate failed to be tested.

The injection technique and sites are also crucial. In our study, switching the technique from subserosal- fundal myometrial injection to uterine-servical combined injection reduces the detection rate. Thus, it indicates that mimicking the natural path of lymphatic drainage of the uterus has to be the priority. Considering that the technique aims to represent lymphatic drainage of the tumor, simple contradiction is inevitable; non-hysteroscopic techniques represent uterine lymphatic flow, not the tumors. To obtain accurate results, the blue dye should be injected peritumorally. Because the study was designed in a pilot manner and the hospital conditions, we did not use that protocol.

Our major handicap was the insufficient number of the patients. The criteria obtained and time interval decided was the cause of this lack. On the other hand, when data over the last decade in our clinic as the minimum specified method of surgical staging similar to this study was analysed retrospectively; in the 378 patients, only 33 (8%) lymph node metastases were detected, and only two of them were at the early stage. In other words, only two of 313 patients (0.06%) who were surgically staged had lymph node metastases. In this context, since the number of cases was statistically inadequate, lymph node metastases in early-stage endometrial cancer are much less frequent, so this is one of the most important issues that limits our study.

From the perspective of the learning curve, because of the low incidence of cervical and vulvar cancer, the surgeon's experience seems to be difficult to improve. However, the high incidence of endometrial cancer provides better conditions for experience. In fact, the presence of 5 SLN of the last 9 cases in

Table 2. Summary of all cases

Case		Age	Grade	Histology	Depth of invasion (>%50)	Periton cytology	Tumor volume (cm^3)	Para-aortic	Lymph node Met.	LVAI	HT	DM	BMI	G*	P^n	Menapause	Stage
G	Z	59	1	Endomet.	+	-	60.0		-		+		31.1	8	2	+	2A
Z	T	56	2	Endomet.		-	22.5		-				40.4	1	0	+	1A
A	K	49	1	Endomet.		-	17.5	+	-				38.2	5	1		2A
N	Ö	57	1	Endomet.		-	5.3		-			+	37.5	4	3	+	1A
F	Y	63	1	Endomet.		-	0.0		-				30.0	4	2	+	1A
N	G	58	2	Endomet.		-	9.0		-	+			20.5	0	0	+	1A
N	Ö	67	1	Endomet.		-	8.4		-		+	+	36.0	3	2	+	1A
T[1]	**İ**	**61**	**1**	**Endomet.**	**+**	**-**	**87.5**		**-**	**+**	**+**		**37.7**	**3**	**3**	**+**	**1B**
H	D	62	1	Endomet.	+	-	1.4		-				34.2	0	0	+	1B
G	U	71	2	Endomet.		-	2.0		-	+	+	+1	38.1	0	0	+	1A
A	Ü	79	2	Endomet.		-	10.0		-				29.1	6	3	+	1A
F	A	46	2	Endomet.		-	16.2	+	-				26.1	3	2		1A
F	C	44	1	Endomet.		-	2.6		-				33.8	2	2	+	1A
R	U	73	1	Endomet.		-	8.4		-		+		28.4	4	4	+	1A
F	G	66	1	Endomet.		-	120.0		-		+		34.3	2	2	+	1A
A	K	47	1	Endomet.		-	1.4		-		+		32.1	5	5	+	1A
Ş	K	54	1	Endomet.		-	6.6		-				31.2	4	2	+	1A
G[1]	**F**	**58**	**1**	**Endomet.**	**+**	**-**	**12.0**		**-**		**+**		**40.1**	**0**	**0**	**+**	**1B**
Z[1]	**A**	**60**	**1**	**Endomet.**		**-**	**8.0**		**-**		**+**		**38.7**	**5**	**3**	**+**	**1A**
M	**B**	**58**	**3**	**Serous**		**-**	**0.4**		**-**	**+**	**+**		**39.5**	**1**	**1**	**+**	**1A**
N[1]	C	62	1	Endomet.		-	10.5		-		+		37.5	4	4		1A
B	T	47	1	Endomet.		-	1.9		-	+	+		37.5	6	2		1A
S[1]	**B**	**60**	**1**	**Endomet.**	**+**	**-**	**7.5**		**-**		**+**		**37.8**	**3**	**3**	**+**	**1B**
F	G	42	1	Endomet.		-	4.5		-				42.1	6	4		1A
G[1]	**Ö**	**52**	**3**	**Clear**		**-**	**107.8**		**-**	**+**	**+**		**45.7**	**0**	**0**	**+**	**1B**
A	S	50	1	Endomet.		-	2.0		-				39.0	8	4		1A

G*: gravidity, P^n: parity, Paraaortic column represents the patients which were performed paraaortic dissection. Second injection technique was performed to the cases marked in dark tone, [1]: SLN was found positive in cases which are shown in bold

the study, appears to be related to the surgeon's experience. Thus, the chance to detect SLN increases with the surgeon's experience.

Another possible area of SLN technique in endometrial cancer is to determine micrometastases. Detailed histopathological examination and immunohistochemical study of endometrial cancer is indispensable for evaluating lymph nodes. In various studies, when all the lymph nodes were examined with very thin sections and IHC staining, it revealed a greater number of metastatic nodes, but it does not affect the sensitivity and negative predictive value. Therefore, the concept of sentinel lymph node biopsy provides the opportunity to establish diagnosis of micrometastases by using less effort. In our study no micrometastases were found in SLN's.

In our study, we have questioned the waiting period after injection prior to surgery and assigning the correct priority for dissection. After the subserosal and cervical injection, the mean drainage time of the dye is 30 to 60 seconds. The error rates may increase if the time interval between blue injection exceeds 20 minutes (11). According to these observations in our study, the obtained time interval seems to be appropriate, but hysterectomy before retroperitoneal lymphatic dissection appears to be the wrong option.

As a result, the basic strategy of sentinel node biopsy includes four steps: to minimize the number of patients undergoing unnecessary surgical procedure, to minimize the number of patients given unnecessary adjuvant therapy, to perform complete lymphadenectomy in patients with significant risk

factors, and facilitating the appropriate amount of lymph node sampling by unexperienced surgeons in low risk patients. Although the SLN technique is not valuable enough to exclude lymphadenectomy, it appears to be feasible for scanning micrometastases and ultrastaging, while its clinical value has not yet been established. Although, according to the recent pilot studies, micrometastases may be adapted for the medium-risk group for local recurrence.

Conflict of interest

No conflict of interest was declared by the authors.

References

1. Silverberg E, Boring CC, Squires TS. Cancer statistics, 1990. CA Cancer J Clin 1990; 40: 9-26. [CrossRef]
2. Gusberg SB: Diagnosis and principles of treatment of cancer of the endometrium. In: Gusberg SB, Shingleton HM, Deppe G, eds. Female genital cancer. New York: Churchill 337, 1988.
3. Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics 2007. CA Cancer J Clin 2007; 57: 43-66. [CrossRef]
4. Lurain JR, Rice BL, Rademaker AW, Poggensee LE, Schink JC, Miller DS. Prognostic factors associated with recurrence in clinical stage I adenocarcinoma of the endometrium. Obstet Gynecol 1991; 78: 63-9.
5. Mariani A, Webb MJ, Keeney GL, Aletti G, Podratz KC. Predictors of lymphatic failure in endometrial cancer. Gynecol Oncol 2002; 84: 437-42. [CrossRef]
6. Mariani A, Webb M, Galli L, Podratz KC. Potential therapeutic role of paraaortic lymphadenectomy in node positive endometrial cancer. Gynecol Oncol 2000; 76: 348-56. [CrossRef]
7. Orr JW, Roland PY, Leichter D, Orr PF. Endometrial cancer: is surgical staging necessary? Curr Opin Oncol 2001; 13: 408-12. [CrossRef]
8. Holub Z, Jabor A, Kliment L. Comparison of two procedures for sentinel node detection in patients with endometrial cancer: a pilot study. Eur J Gynaecol Oncol 2002; 23: 53-7.
9. Kilgore LC, Partridge EE, Alvarez RD, Austin JM, Shingleton HM, Noojin III F, et al. Adenocarcinoma of the endometrium: survival comparison of patients with and without pelvic node sampling. Gynecol Oncol 1995; 56: 29-33. [CrossRef]
10. Cragun JM, Havrilesky LJ, Calingaert B, Synan I, Secord AA, Soper JT, et al. Retrospective analysis of selective lymphadenectomy in apparent early-stage endometrial cancer. J Clin Oncol 2005; 16: 3668-75. [CrossRef]
11. Echt ML, Finan MA, Hoffman MS, Kline RC, Roberts WS, Fiorica JV. Detection of sentinel lymph nodes with lymphazurin in cervical, uterine, and vulvar malignancies. South Med J 1999; 92: 204-8. [CrossRef]

Serum adenosine deaminase and its isoenzyme activities in pregnancy

Gebelerde serum adenozin deaminaz ve izoenzim aktivitesi

Göksel Bahadır[1], Yasemin Erdoğan Döventaş[1], Rana Turkal[1], Macit Koldaş[1], Filiz Basınoğlu[1], Banu Dane[2], Emine Altunkaynak[3]

[1]Clinic of Clinical Biochemistry, Haseki Education and Research Hospital, İstanbul, Turkey

[2]Clinic of Obstetrics and Gynecology, Haseki Education and Research Hospital, İstanbul, Turkey

[3]Clinic of Clinical Biochemistry, Yedikule Chest Diseases Education and Research Hospital, İstanbul, Turkey

Abstract

Objective: ADA is widely distributed in human tissues, which may contribute to the maturation of the immunological system, especially the proliferation and differentiation of lymphoid cells, and seems to be critical at different stages of the maturation process. The activity of ADA changes in diseases characterized by the alteration of cell-mediated immunity. In this study we examined changes in serum total ADA activity and the patterns of two ADA isoenzymes, ADA-1 and ADA-2, in healthy pregnant women, and evaluated the possible role of the alteration of cell-mediated immunity during pregnancy as causes of changes in ADA activity.

Materials and Methods: We measured serum activities of total ADA, ADA-1 and ADA-2 in healthy pregnant women (n=129) and age-matched healthy nonpregnant women (n=42). We divided the study group into three different subgroups: first trimester, second trimester and third trimester.

Results: Serum ADA, ADA-1 and ADA-2 activities in healthy pregnant women were significantly lower than in nonpregnant women ($p<0.001$, $p<0.001$ and $p<0.01$ respectively). ADA ($p<0.001$) and ADA-2 ($p<0.001$) activities in the first trimester were significantly lower than in the control group. However, there were no significant differences between the first trimester and control group according to their ADA-1 activities ($p=0.016$). ADA ($p<0.001$), ADA-1 ($p<0.001$) and ADA-2 ($p<0.008$) activities in the second trimester were significantly lower than in the control group. Combined trisomy 21 risk, biochemical trisomy 21 risk, age risk and trisomy 18 + Nuchal translucency (NT) risk were calculated using a first trimester screening test in 63 pregnant women. Furthermore, trisomy 21 risk, age risk and trisomy 18 risk were calculated by triple test in 52 pregnant women. ADA, ADA-1 and ADA-2 activities were not significantly correlated with risks in the first trimester screening test. ADA-1 activity was slightly significantly negative correlated with age risk ($r= -0.314$, $p<0.05$) and trisomy 18 risk ($p<0.05$) in the triple test. ADA ($p<0.05$) and ADA-2 ($p<0.05$) activities were slightly significantly correlated with gestational age, while there was no significant correlation between ADA-1 activity and gestational age.

Conclusion: Serum ADA activity may be useful for clinical diagnosis and observation of high-risk pregnancies in which cell-mediated immunity has been altered. (J Turkish-German Gynecol Assoc 2011; 12: 209-13)

Key words: Adenosine deaminase, immunity, pregnancy

Received: 13 May, 2011 **Accepted:** 16 September, 2011

Özet

Amaç: ADA insan dokularında yaygın bir dağılım göstermektedir. ADA immünolojik sistemin olgunlaşmasına, özellikle lenfoid hücrelerin çoğalma ve farklılaşmasına katkıda bulunur ve olgunlaşma işleminin farklı aşamalarında kritik bir rol oynar. ADA aktivitesi hücre-aracılı immünitenin değiştiği hastalıklarda değişmektedir. Bu çalışmada sağlıklı gebe kadınlarda serum total ADA ve ADA izoenzim (ADA-1 ve ADA-2) aktivitelerini inceledik ve gebelik boyunca ADA aktivitesinde değişikliklere neden olan hücre-aracılı immünitedeki değişikliklerin olası rollerini değerlendirdik.

Gereç ve Yöntemler: Sağlıklı gebe kadınlarda (n=129) ve yaşça uyumlu sağlıklı gebe olmayan kadınlarda (n=42) serum total ADA, ADA-1 ve ADA-2 aktivitelerini ölçtük. Çalışma grubunu 1. trimester, 2.trimester ve 3. trimester olmak üzere üç farklı gruba ayırdık.

Bulgular: Gebe grubunun ADA, ADA-1 ve ADA-2 değerleri, kontrol grubunun değerlerine göre anlamlı olarak daha düşük bulundu (ADA ve ADA-1 için $p<0.001$, ADA-2 için $p<0.01$). 1. trimester'e ait ADA ($p<0.001$) ve ADA-2 ($p<0.001$) değerleri, kontrol grubunun değerlerinden anlamlı olarak düşük bulundu. Fakat ADA-1 değerleri açısından kontrol grubu ile 1. trimester arasında anlamlı bir fark bulunamadı ($p=0.016$). 2. trimester'e ait ADA ($p<0.001$), ADA-1 ($p<0.001$) ve ADA-2 ($p<0.008$) değerleri, kontrol grubunun değerlerinden anlamlı olarak düşük bulundu. 63 gebe kadında, 2'li test kullanılarak kombine trizomi 21 riski, biyokimyasal trizomi 21 riski, yaş riski ve trizomi 18 + Nuchal translucency (NT) riski hesaplandı. Ayrıca, 52 gebe kadında 3'lü test kullanılarak trizomi 21 riski, yaş riski ve trizomi 18 riski hesaplandı. 2'li test riskleri ile ADA, ADA-1 ve ADA-2 arasında bir ilişki bulunamadı. 3'lü teste ait yaş riski ($r= -0.314$, $p<0.05$) ve trizomi 18 riski ($r=-0.314$, $p<0.05$) ile ADA-1 arasında negatif yönde zayıf bir ilişki tespit edildi. 3'lü teste ait diğer riskler ile ADA, ADA-1 ve ADA-2 arasında anlamlı bir ilişki bulunamadı. Gestasyonel yaş ile ADA ($r=0.201$, $p<0.05$) ve ADA-2 ($r=0.195$, $p<0.05$) arasında zayıf bir ilişki tespit edildi fakat gestasyonel yaş ile ADA-1 arasında anlamlı bir ilişki tespit edilemedi.

Sonuç: Serum ADA aktivitesi hücre-aracılı immünitenin değiştiği yüksek riskli gebeliklerin teşhis ve incelenmesinde faydalı olabilir.

(J Turkish-German Gynecol Assoc 2011; 12: 209-13)

Anahtar kelimeler: Adenozin deaminaz, immünite, gebelik

Geliş Tarihi: 13 Mayıs 2011 **Kabul Tarihi:** 16 Eylül 2011

Address for Correspondence: *Yasemin Erdoğan Döventaş, Clinic of Clinical Biochemistry, Haseki Education and Research Hospital, 34080 İstanbul, Turkey*
Phone: +90 532 548 59 51 e.mail: yasemined@hotmail.com

doi:10.5152/jtgga.2011.53

Introduction

Adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4.; ADA) is an enzyme in the purine salvage pathway which is primarily responsible for the intracellular disposition of transported adenosine (1). ADA catalyzes the irreversible hydrolytic deamination of adenosine and 2-deoxyadenosine to inosine and 2-deoxyinosine, respectively (2). ADA is an important enzyme that participates in the degradative pathways of adenosine monophosphate (AMP), a purine nucleotide (3). Patients who inherently lack this enzyme have defects in both humoral and cellular immunity that are manifested as severe combined immunodeficiency disease (1). When ADA fails to catalyze the deamination of adenosine, those compounds that accumulate are readily converted into their respective nucleotides. Deoxyadenosine triphosphate (dATP) is one of the nucleotides generated in this way. In ADA deficiency, deoxyadenosine accumulates intracellularly as dATP. dATP has been recognized as the toxic metabolite in the immunodeficiency disease associated with ADA deficiency. This compound is a potent inhibitor of DNA replication, because it prevents the synthesis of deoxyribonucleotides from ribonucleotides by interference with ribonucleotide reductase (3).

ADA is widely distributed in human tissues, and it is detected in high levels particularly in lymphoid tissues (4). Furthermore, ADA may contribute to the maturation of the immunological system, especially the proliferation and differentiation of lymphoid cells, and seems to be critical at different stages of the maturation process (1). Thus it has been considered as an indicator of a nonspecific marker of T-cell activation (5). ADA has been used for monitoring several diseases in which immunity has been altered. As an indicator of cellular immunity, the serum activity of this enzyme has been suggested to be altered in diseases that cause a cell-mediated immune response such as rheumatoid arthritis, systemic lupus erythematosus and tuberculosis (5). On the other hand, it has been shown that serum ADA activity was increased in several diseases where cellular immunity is stimulated. Lymphocytes or the monocyte-macrophage cell system have been assumed to contribute to changes in serum ADA activity, however, the exact mechanisms by which serum ADA activity is altered has not been elucidated (2, 5).

Human ADA exists in at least three molecular isoforms, ADA-1, ADA-2 and ADA-1 and ADA-complexing protein. Although ADA-1 is present in almost all human tissues and cells, ADA-2 is the predominant isoenzyme in the serum of normal subjects. The majority of ADA activity is derived from ADA-1. Most human cells contain very small amounts of ADA-2 and its tissue sources may be lymphocytes or the monocyte-macrophage cell system (2). It is assumed that ADA-1 arises mainly from injured tissues or cells, while ADA-2 primarily derives from stimulated T-cells (4). Adenosine and 2-deoxyadenosine are molecules with many effects on human cells. Thus, the homeostasis of these substances and the activity of the isoenzymes, ADA-1 and ADA-2, in human cells are of extreme importance (6).

Pregnancy is an immunological balancing act in which the maternal immune system has to remain tolerant of paternal human leucocyte antigens (HLA) expressed by the fetus and yet maintain normal immune competence for defense against microorganisms (7, 8). Immunologically, the human fetus has always been considered as an allograft to the pregnant mother (8). Despite being biological allografts, fetuses are not normally rejected by the matenal immune system. One likely explanation for this phenomenon is that the feto-placental unit is an immunologically privileged site that creates a mechanical barrier which reduces interactions between fetal tissues and maternal lymphocytes, and/or functionally impairs the maternal immune response (9). Cytokine-secreting T cells play a central role in the immune response and have been classified into subsets based on their type of cytokine production. T helper 1 cells synthesize mainly interleukin-2 (IL-2) and interferon gamma, which induce cellular immunity. T helper 2 cells produce predominantly IL-4, -5, -6 and -10, which promote humoral immunity. The shift of Th1/Th2 balance to Th2 predominance occurs in normal pregnancy and appears to protect the fetus and placenta from being rejected and to aid in the maintenance of normal pregnancy (10). The deviation of the immune response from Th1 to Th2 may leave the mother more open to infection whose control is Th1-dependent, but increased production of Th1 cytokines has been linked to spontaneous abortion and small-for-date babies (7). Not only T cells, but also B cells, decrease during pregnancy, and serum levels of IgG, IgM and IgA also decrease during pregnancy (11). In this study, we analyzed changes in serum total ADA activity and the patterns of two ADA isoenzymes, ADA-1 and ADA-2, in healthy pregnant women and evaluated the possible role of the alteration of cell-mediated immunity during pregnancy as causes of changes in ADA activity. We also performed prenatal screening tests in pregnant women and calculated prenatal risks by the Prisca package program, and studied a possible correlation between prenatal risks and serum ADA activity and its isoenzyme pattern in pregnant women.

Materials and Methods

One hundred and twenty-nine healthy pregnant women from the department of gynecology and obstetric of Haseki Education and Research Hospital, were analyzed. Among the 129 healthy pregnant women, 38 were in the first trimester, 79 were in the second trimester, and 12 were in the third trimester. The inclusion criteria for eligibility were as follows; a singleton fetus, normal fetal anatomy, well-established gestational age corroborated by ultrasonography, nonsmoker, no evidence of recent infection, no prescribed medication and no maternal medical complications. In this study, 42 age and sex-matched healthy nonpregnant women with no known history of any disease were taken as controls. The informed written consents were obtained from each patient and healthy controls.

A blood sample was taken from the antecubital vein, which was then centrifuged at 3000xg for 10 min. within half an hour and stored at -20°C until assay. Stability of the ADA enzyme in the serum lasts 24 hours at 25°C, 7 days at 4°C and 3 months at -20°C. ADA, ADA-1 and ADA-2 activities of all serum samples were measured. Furthermore, pregnancy associated plasma

Table 1. Demographic characteristics of subjects

	All pregnant (n=129)	1. Trimester (n=38)	2. Trimester (n=79)	3. Trimester (n=12)	Control (n=42)
Age (year)	26.5±5.44	25.7±4.50	26.9±5.96	26.5±4.54	28.1±6.99
Weight (kg)	63.6±11.95	59.8±9.71	64.2±11.66	71.3±16.21	62.5±11.59

Table 2. Serum total ADA, ADA-1 and ADA-2 activities in healthy pregnant women and nonpregnant women (controls). Data are presented as mean±SEM

		All Pregnant (n=129)	1. Trimester (n=38)	2. Trimester (n=79)	3. Trimester (n=12)	Control (n=42)
ADA	Mean±SEM	33.93±0.47	32.97 ±0.76	34.10 ±0.64	35.91±1.45	39±0.99
IU/L	Median (min-max)	34 (23-51)	33 (25-42)	34 (23-51)	35 (26-45)	39 (28-55)
ADA -1	Mean±SEM	4.14±0.18	4.42 ±0.36	4±0.21	4.25±0.91	5.78±0.32
IU/L	Median (min-max)	4 (1-12)	4.5 (1-9)	4 (1-11)	4 (1-12)	5.5 (1-11)
ADA -2	Mean±SEM	29.79±0.44	28.55±0.70	30.10±0.58	31.66±1.65	33.21±0.92
IU/L	Median (min-max)	29 (20-44)	29 (20-37)	29 (21-44)	31 (25-43)	31.5 (22-45)

protein-A (PAPP-A) and free β-human chorionic gonadotropin (free β-hCG) used for the first trimester prenatal screening test were measured in the first trimester pregnant women. Alpha-fetoprotein (AFP), free estriol (FE3) and β-hCG used for triple test were measured in the second trimester pregnant women.

Concentrations of PAPP-A, free β-hCG, AFP, FE3 and β-hCG were assayed using an automatic analyzer (Immulite 2500) with a commercial kit according to the instructions of the manufacturer (Siemens Medical Solutions Diagnostics Limited, Llanberis, Gwynedd. LL55 4EL United Kingdom). Measurement of these parameters were performed by a chemiluminescent immunometric assay which is based on the antibody sandwich complex method. Prenatal screening tests were evaluated using the Prisca package program (version 4.0.20.4) obtained from Siemens.

The manual kinetic ADA activity assay was optimized for the automated analyser (Konelab 60 I). For the determination of ADA activity, the ammonia produced by the enzymatic activity was coupled to 2-oxoglutarate by glutamate dehydrogenase. 2-oxoglutarate was activated by adenosine diphosphate (ADP). In this reaction, NADH was used as indicator and the reaction was followed by the decrease of absorbance at 340 nm. This method was developed by Ellis (12). To distinguish ADA-1 from ADA-2, the activity was measured using the same techniques with Erythro-9 (2-hydroxy-3-nonyl) adenine (EHNA) which is a potent inhibitor of only ADA-1 isoenzyme, showing the ADA-2 activity. The activity of ADA-1 was calculated by subtracting the ADA-2 activity from total ADA activity. All chemicals for ADA assay were obtained from Sigma.

Statistical analysis

Statistical analyses were carried out using the SPSS 17.0 (SPSS Inc., Chicago, IL, USA) software. Results were expressed as the mean±standard error of the mean (SEM) and as the median and range. Distributions of the groups were analyzed with the Kolmogorov-Smirnov test. Because all groups did not show normal distribution, nonparametric statistical methods were used to analyse the data. The Kruskal-Wallis test was used for skewed data, and data obtained from the study groups were compared by the nonparametric Mann-Whitney U test. Bonferroni's test was used to evaluate the repeated measurements. Spearman correlation analysis was performed to evalaute relations between variables.

Table 3. Serum total ADA, ADA -1 and ADA -2 activities in pregnant and control group

	All Pregnant (n=129)	Control (n=42)	p
ADA	33.93±0.47	39±0.99	<0.001
ADA -1	4.14±0.18	5.78±0.32	<0.001
ADA -2	29.79±0.44	33.21±0.92	<0.01

Results

Demographic data are given in Table 1. Serum total ADA, ADA-1 and ADA-2 activities are shown in Table 2. Serum ADA, ADA-1 and ADA-2 activities in healthy pregnant women were significantly lower than those of nonpregnant women ($p<0.001$, $p<0.001$ and $p<0.01$, respectively). Table 3 summarizes the ADA activities in both pregnant and nonpregnant groups. ADA ($p<0.001$) and ADA-2 ($p<0.001$) activities in the first trimester were significantly lower than those in the control group. However, there was no significant difference between the first trimester and control group according to their ADA-1 activities ($p=0.016$). ADA ($p<0.001$), ADA-1 ($p<0.001$) and ADA-2 ($p<0.008$) activities in the second trimester were significantly lower than those of the control group. There was no significant difference between the third trimester and control group, and between each trimester of pregnancy with respect to their ADA, ADA-1 and ADA-2 activities. These comparison results are given in Table 4.

Combined trisomy 21 risk, biochemical trisomy 21 risk, age risk and trisomy 18 + Nuchal translucency (NT) risk were calculated

Table 4. Comparison of results between each trimester of pregnancy and control group, and among each trimester of pregnancy according to their ADA, ADA -1, and ADA -2 activities

	ADA	ADA -1	ADA -2
Control - 1. Trimester	<0.001	0.016	<0.001
Control - 2. Trimester	<0.001	<0.001	<0.008
Control - 3. Trimester	0.166	0.040	0.478
1. Tri - 2. Tri	0.417	0.291	0.196
1. Tri - 3. Tri	0.086	0.542	0.118
2. Tri - 3. Tri	0.230	0.877	0.356
Differences were considered significant a p<0.008 according to Bonferroni's correction			

using the first trimester screening test in 63 pregnant women. Furthermore, trisomy 21 risk, age risk and trisomy 18 risk were measured by the triple test in 52 pregnant women. ADA, ADA-1 and ADA-2 activities were not significantly correlated with risks in the first trimester screening test. ADA-1 activity was slightly significantly negative correlated with age risk (r= -0.314, p<0.05) and trisomy 18 risk (r=-0.314, p<0.05) in the triple test. ADA, ADA-1 and ADA-2 were not significantly correlated with other risks in the triple test. Results in the correlation analysis are shown in Table 5 and Table 6. ADA (r=0.201, p<0.05) and ADA-2 (r=0.195, p<0.05) activities were slightly significantly correlated with gestational age, while there was no significant correlation between ADA-1 activity and gestational age.

Discussion

ADA is an essential enzyme for the differentiation of lymphoid cells, so changes in ADA activity reflect alterations in immunity (2). This enzyme is widely distributed in human tissues, especially in the lymphoid tissues (5). Normal pregnancy is characterized by depressed cell-mediated immunity in conjunction with enhanced humoral immunity, so serum ADA activity may be altered (10). In this study, we examined changes in serum total ADA activity and the patterns of two ADA isoenzymes, ADA-1 and ADA-2, in healthy pregnant women and they were compared with those of age-matched healthy nonpregnant women. We divided the study group into three different subgroups: first trimester, second trimester and third trimester. Serum ADA, ADA-1 and ADA-2 activities in healthy pregnant women were significantly lower than those of nonpregnant women (p<0.001, p<0.001 and p<0.01 respectively). ADA (p<0.001) and ADA-2 (p<0.001) activities in the first trimester were significantly lower than those in the control group. However, there was no significant difference between the first trimester and control groups according to their ADA-1 activities (p=0.016). ADA (p<0.001), ADA-1 (p<0.001) and ADA-2 (p<0.008) activities in the second trimester were significantly lower than that in the control group. There was no significant difference between the third trimester and control groups and between each trimester of pregnancy according to their ADA, ADA-1 and ADA-2 activities. Jaqueti et al. found similar results (13). Our results were thus compatible with the literature.

Table 5. Correlation between risk in the first trimester screening test and ADA, ADA -1, and ADA -2

	r	p
ADA - Combined Trisomy 21 Risk	-0.045	0.72
ADA - Biochemical Tri. 21 Risk	-0.044	0.724
ADA - Age Risk	0.076	0.546
ADA - Trisomy 18 + NT Risk	-0.159	0.204
ADA 1- Combined Trisomy 21 Risk	-0.012	0.921
ADA 1-Biochemical Tri. 21 Risk	0.097	0.438
ADA 1- Age Risk	-0.043	0.734
ADA 1– Trisomy 18 + NT Risk	-0.161	0.197
ADA 2 – Combined Trisomy 21 Risk	-0.022	0.863
ADA 2 – Biochemical Tri. 21 Risk	-0.09	0.471
ADA 2– Age Risk	0.136	0.276
ADA 2– Trisomy 18 + NT Risk	-0.094	0.451

Table 6. Correlation between risk in the triple test and ADA, ADA -1, and ADA -2

	r	p
ADA - Trisomy 21 Risk	0.01	0.942
ADA - Age Risk	-0.028	0.844
ADA - Trisomy 18 Risk	-0.028	0.844
ADA 1- Trisomy 21 Risk	-0.228	0.101
ADA 1- Age Risk	-0.314	0.022
ADA 1- Trisomy 18 Risk	-0.314	0.022
ADA 2- Trisomy 21 Risk	-0.07	0.618
ADA 2- Age Risk	0.058	0.68
ADA 2- Trisomy 18 Risk	0.058	0.68

Yoneyama et al. (2) measured serum activities of total ADA, ADA-1 and ADA-2 in normal pregnant women in the third trimester and age-matched healthy nonpregnant women. The authors found that, in normal pregnant women, serum total ADA and ADA-2 activities were lower than those of the nonpregnant women, while there was no difference in ADA-1 activity. In the light of these results, they concluded that reduced serum total ADA activity might be reflected by decreased ADA-2 activity, which may be in part associated with depressed cell-mediated immunity during normal pregnancy (2). In our study, there was also no difference between third trimester and control groups according to their ADA-1 activity. Because no significant difference was found in ADA and ADA-2 activities between third trimester and control groups, our results differed from the findings of Yoneyama et al. (2). Therefore, we believe that this current study contributes to the literature. In a different study performed by Yoneyama et al. (10), they found results that were similar to ours.

Serum ADA activity in normal pregnant women was significantly lower than that in nonpregnant women. In addition, Oladipo

et al. (14) demonstrated that the mean serum ADA level in nonpregnant women was higher than that of normal pregnant women. Lee et al. (5) measured the catalytic values of serum ADA from normal pregnant women, who were divided into four groups according to the gestational age in weeks (Gwks) (Group I: 5-9 Gwks; Group II: 15-20 Gwks; Group III: 24-30 Gwks; Group IV: 30-39 Gwks). The serum ADA activity of group III was significantly higher than the other groups. The significant increase in ADA activity which was detected in Lee et al.'s study (5) during 24 to 30 gestational weeks may be associated with the fact that the increase in maternal cardiac output reaches its peak during the late second trimester to early third trimester and decreases in late pregnancy. In contrast, in the present study there was no significant difference among each trimester of pregnancy according to their ADA, ADA-1 and ADA-2 activities. Jaqueti et al. found results that were similar to ours (13). Our study were slightly different from Lee et al.'s study (5). However, the study population differed from that of our study, which may account for the discrepancy in the results. Lee et al. did not find significant correlation between the serum ADA activity and gestational age in normal pregnant women (5). On the contrary, in our study ADA and ADA-2 activities were mildly sifnificantly correlated with gestational age, but there was no significant correlation between ADA-1 activity and gestational age.

Similar results for ADA activity have been reported in an animal study carried out by Chaudhry et al. (15). In this study, total serum ADA and ADA-2 activities were lower in pregnant than in nonpregnant buffaloes. However, ADA-1 activity did not differ between the pregnant and nonpregnant buffaloes. Similarly, the activities of total ADA, ADA-1 and ADA-2 did not differ among pregnant buffaloes of three trimesters. Our study is supported by these results.

Uslu et al. measured serum ADA activity in the second trimester pregnant women who were of advanced age and Down syndrome risk as compared with nonpregnant women (16). In this study, serum ADA activity was lower in pregnant women with low Down syndrome risk, in all pregnant women and in pregnant women with high age risk and higher in pregnant women with high Down syndrome risk when compared to the control group. Since there were a few pregnant women with high Down syndrome risk in our study, we could not perform such comparison. However, in the present study, ADA, ADA-1 and ADA-2 activities were compared to the prenatal risks. ADA, ADA-1 and ADA-2 activities were not significantly correlated with risks in the first trimester screening test. ADA-1 activity was slightly significantly negatively correlated with age risk and trisomy 18 risk in the triple test. ADA, ADA-1 and ADA-2 were not significantly correlated with other risks in the triple test.

In conclusion, normal pregnancy is characterized by depressed cell-mediated immunity, so serum ADA activity and its isoenzyme pattern alter in normal pregnancy. That is, changes in serum ADA activity reflect changes in the immune system during pregnancy. Moreover, the clinical significance of changes in ADA activity and the regulatory mechanisms that alter the activity of serum ADA have not been elucidated. Serum ADA activity may be useful for clinical diagnosis and observation of high-risk pregnancies in which cell-mediated immunity has been altered. Further studies are needed to evaluate the usage of ADA activity in pregnancy for prenatal screening test and to determine the relationship between ADA activities and preeclampsia.

Conflict of interest

No conflict of interest was declared by the authors.

References

1. Yagawa K, Okamura J. Role of adenosine deaminase in activation of macrophages. Infect Immun 1981; 32: 394-7.
2. Yoneyama Y, Suzuki S, Sawa R, Otsubo Y, Miura A, Kuwabara Y, et al. Serum adenosine deaminase activity and its isoenzyme pattern in women with normal pregnancies. Arch Gynecol Obstet 2003; 267: 205-7.
3. Kutlar I, Aksoy F, Koyluoglu O, Ugur MG, Balat O, Tarakcioglu M. Adenosine deaminase activity in serum and placenta of patients with anembryonic pregnancies and missed abortions. Arch Gynecol Obstet 2005; 272: 124-6. [CrossRef]
4. Kurata N. Adenosine deaminase. Nippon Rinsho 1995; 53: 1178-83.
5. Lee SJ, Hwang HS, Kim BN, Kim MA, Lee JW, Park YW, et al. Changes in serum adenosine deaminase activity during normal pregnancy. J Korean Med Sci 2007; 22: 718-21. [CrossRef]
6. Gakis C. Adenosine deaminase (ADA) isoenzymes ADA-1 and ADA-2: diagnostic and biological role. Eur Respir J 1996; 9: 632-3. [CrossRef]
7. Weetman AP. The immunology of pregnancy. Thyroid 1999; 9: 643-6. [CrossRef]
8. Kumar V, Medhi B. Emerging role of uterine natural killer cells in establishing pregnancy. Iran J Immunol 2008; 5: 71-81.
9. Marzi M, Vigano A, Trabattoni D, Villa ML, Salvaggio A, Clerici E, et al. Characterization of type 1 and type 2 cytokine production profile in physiologic and pathologic human pregnancy. Clin Exp Immunol 1996; 106: 127-33. [CrossRef]
10. Yoneyama Y, Sawa R, Suzuki S, Yoneyama K, Doi D, Araki T. Relationship between adenosine deaminase activity and cytokine-secreting T cells in normal pregnancy. Obstet Gynecol 2002; 100: 754. [CrossRef]
11. Shimaoka Y, Hidaka Y, Tada H, Nakamura T, Mitsuda N, Morimoto Y, et al. Changes in cytokine production during and after normal pregnancy. Am J Reprod Immunol 2000; 44: 143-7. [CrossRef]
12. Ellis G, Goldberg DM. A reduced nicotinamide adenine dinucleotide linked kinetic assay for adenosine deaminase activity. J Lab Clin Med 1970; 76: 507-17.
13. Jaqueti J, Martinez-Hernández D, Hernández-Garcia R, Navarro-Gallar F, Arenas-Barbero J. Adenosine deaminase in pregnancy serum. Clin Chem 1990; 36: 2144.
14. Oladipo O, Afolabi B, Okorodudu A. Adenosine deaminase activity in subjects with normal pregnancy, pregnancy induced hypertension and pre-eclampsia. West Afr J Med 2009; 28: 161-4.
15. Chaudhry SM, Naseer Z, Rabbani S and Alrokayan SA. Activity of adenosine deaminase and its isoenzymes in serum of pregnant buffaloes. Pakistan Vet J 2007; 27: 152.
16. Uslu S, Sütken E, Güçlüer Z, Çolak Ö, Alataş Ö. Yüksek yaş ve Down Sendromu riski taşıyan gebelerde, seruloplazmin ve adenozin deaminaz aktiviteleri. Türk Klinik Biyokimya Dergisi 2004; 2; 121-6.

Determination of serum CRP, VEGF, Leptin, CK-MB, CA-15-3 and IL-6 levels for malignancy prediction in adnexal masses

Adneksiyel kitlelerde malignite prediksiyonunda tümör belirteci olarak serumda CRP, VEGF, Leptin, CK-MB, CA-15-3, IL-6 bakılması

Serhat Şen[1], Oğuzhan Kuru[1], Özgür Akbayır[2], Hilal Oğuz[3], Vildan Yasasever[3], Sinan Berkman[1]

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

[2]Department of Obstetrics and Gynecology, Kanuni Sultan Süleyman Research and Training Hospital, İstanbul, Turkey

[3]Department of Basic Oncology, Institute of Oncology, İstanbul University, İstanbul, Turkey

Abstract

Objective: Investigation of serum markers which could be used in the malignancy prediction of adnexal masses.

Material and Methods: Vascular endothelial growth factor (VEGF), interleukin 6 (IL-6), leptin, C-reactive protein (CRP), creatine-kinase-MB (CK-MB) and cancer antigen 15-3 (CA 15-3) levels were determined prospectively in serum samples that were obtained from patients who underwent surgery for an adnexal mass and who were referred to Istanbul University, Faculty of Medicine, Department of Obstetrics and Gynecology, between 2009 and 2011, and then were compared with the serum samples of completely healthy outpatient patients as a control group. Based onto the ovarian cancer status, cases were divided into four groups: 13 patients were included in the early-stage malignant group, 12 patients were included in the advanced-stage malignant group, 25 in the benign group and 19 in the healthy control group. Patients with only epithelial ovarian cancer were included into the cancer group. Ethics Commitee approval was obtained for this study. The budget was supported by the Istanbul University Scientific Research Projects Unit.

Results: Results related with sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and odds ratio (OR), respectively, and the following values were calculated: 48%, 95%, 92%, 59% and +OR 9.6 -OR 0.5 for CA; 15-3; 52%, 75%, 72%, 55%, +OR 2.08 -OR 0.64 for leptin; 72%, 70%, 75%, 66% 2.4-0.5 for IL-6; 24%, 80%, 60%, 45%, 1.2-0.92 for VEGF; 68%, 30%, 55%, 43%, 0.97-1.06 for CRP; and 8%, 70%, 25%, 38%, 026-1.31 for CK-MB.

Conclusion: CA 15-3, IL-6, Leptin, VEGF and CRP were effective in the prediction of benign and malignant masses; however they may be more suitable in selected cases as they have a limited use because of their inadequate potential regarding sensitivity and specificity.

(J Turkish-German Gynecol Assoc 2011; 12: 214-9)

Key words: Adnexal masses, malignancy prediction, epithelial ovarian cancer, serum biomarkers

Received: 12 July, 2011 **Accepted:** 6 September, 2011

Özet

Amaç: Adneksiyel kitlelerde malignite ayrımında kullanılabilecek serum markerlarının araştırılması.

Gereç ve Yöntemler: İstanbul Üniversitesi İstanbul Tıp Fakültesi Kadın Hastalıkları ve Doğum Anabilim Dalı'nda 2009-2011 yılları arasında prospektif olarak, adneksiyel kitle endikasyonu ile opere olan hastalardan alınan serum örneklerinde vascular endotelial growth factor (VEGF), interlökin 6 (IL-6), leptin, C-reaktif protein (CRP), kreatin kinaz-MB (CK-MB) ile kanser antijeni 15-3 (CA 15-3) düzeyleri belirlendi ve kontrol grubu olan tamamen sağlıklı poliklinik hastalarının serum örnekleriyle karşılaştırıldı. Olgular over kanseri durumuna göre 4 grupta toplandı: 13 olgu erken evre malign, 12 olgu ileri evre malign, 25 olgu benign, 19 olgu sağlıklı kontrol grubunda yer aldı. Over kanseri grubunda sadece epitelyal over kanseri olan olgular çalışmaya dahil edildi. Çalışma için etik kurul onayı alındı ve bütçe ise İstanbul Üniversitesi Bilimsel Araştırma Projeleri Birimi tarafından desteklendi.

Bulgular: Belirteçlerin duyarlılık, özgüllük, pozitif prediktif değer (PPD), negatif prediktif değer (NPD) ve odds ratio (OR) sonuçlarına bakıldığında CA 15-3 için sırasıyla %48, %95, %92, %59 ve +OR 9.6 -OR 0.5; leptin için sırasıyla %52, %75, %72, %55, +OR 2.08 -OR 0.64; IL-6 için %72, %70, %75, %66 2.4-0.5; VEGF için %24, %80, %60, %45, 1.2-0.92; CRP için %68, %30, %55, %43, 0.97-1.06; CK-MB için %8, %70, %25, %38, 026-1.31 değerleri hesaplandı.

Sonuç: CA 15-3, IL-6, Leptin, VEGF ve CRP benign-malign over tümörü ayrımında etkin bulundu ancak duyarlılık ve özgüllükleri yeteri kadar kuvvetli olmadığı için rutinde kullanımı kısıtlı olup seçilmiş vakalarda kullanımı uygun olabilir.

(J Turkish-German Gynecol Assoc 2011; 12: 214-9)

Anahtar kelimeler: Adneksiyel kitleler, malignite prediksiyonu, epitelyal over kanseri, serum biomarkerları

Geliş Tarihi: 12 Temmuz 2010 **Kabul Tarihi:** 06 Eylül 2011

Address for Correspondence: *Serhat Şen, Department of Obstetrics and Gynecology, İstanbul Faculty of Medicine, İstanbul University, İstanbul, Turkey*
Phone: +90 212 414 20 00 e.mail: cerrahserhat@yahoo.com

doi:10.5152/jtgga.2011.54

Introduction

Ovarian cancer is the second most common and lethal gynecological cancer seen in women (1). The condition ranks in 5th place in cancers among women. Ovarian cancers are listed in 4th place for death due to cancer in women. In the U.S.A, more than 15.000 women die every year because of ovarian cancer (2). At the early stage, life expectancy can be as high as 90%, but unfortunately only 19% of the patients can be diagnosed during the early stage of the disease (3). Most of the patients are diagnosed at the advanced stage when consequently their life expectancy falls below 30%. Early ovarian cancer may not cause obvious symptoms. Ovarian cancer is generally diagnosed when considerable abdominal fluid is observed (at this stage the tumor can be at an advanced stage) or when a mass in the pelvic region is found by the imaging methods (ultrasonography, magnetic resonance imaging, computerized tomography). However, these imaging methods may not be capable of having a high specificity to differentiate benign and malignant tumors. Furthermore, additional surgical interventions may be required when an abnormal image is determined. Consequently, imaging methods may not be reliable in detecting ovarian cancers at the early stage and can be variable (4).

However, there are no biomarkers that are known to diagnose ovarian cancer. Cancer antigen 125 (CA-125) was frequently employed to help the diagnosis of ovarian cancer, but it was basically approved to determine the recurrent disease and monitor response to the therapy (5-7). The sensitivity of CA-125 in detecting an early stage ovarian cancer varies between 29% - 75%. Additionally, CA-125 level may also increase during certain circumstances such as endometriosis, pregnancy, menstruation, cardiac failure and cirrhosis, which are considered as normal or benign conditions. In a recent study, it was observed that an abnormal level of CA-125 failed to indicate the presence of a cancer in 86% of the cases, whereas the mentioned level returned to normal within 3-6 months (8). Today, we still require good biomarkers which can be used in the diagnosis of ovarian cancer.

Materials and Methods

Blood samples were collected from patients who were operated on due to an adnexal mass and from outpatient patients who were completely healthy as a control group, prospectively between 2009-2011 at the Istanbul University, Istanbul Medical Faculty Department of Obstetrics and Gynecology. Blood samples were kept 30 minutes in room temperature and then were centrifuged for 10 minutes at 4000 rpm. Separated serums were stored at -80°C until the number of tests were completed.

Serum C-reactive protein (CRP) levels of patients and control groups were determined by a Toshiba Accute auto-analyzer and immuno-turbidimetric method, while creatine-kinase-MB (CK-MB) and cancer antigen 15-3 (CA 15-3) levels were determined with an Abbott Axsym device and micro-particle enzyme immune-assay (MEIA) method. Vascular endothelial growth factor (VEGF), interleukin 6 (IL-6) and leptin levels were determined by a dual antibody sandwich enzyme immune-assay (ELISA) method.

All pathological samples were examined and diagnosed by 3 specialists from Istanbul Faculty of Medicine, Department of Gynecopathology. The FIGO surgical staging system was used in the staging of ovarian cancer. Histopathological classification was carried out according to the World Health Organization. Serum samples obtained from patients were examined at the Istanbul University, Institute of Oncology, Department of Cancer Biochemistry.

Patients were classified in four groups: Early Stage (Stage 1 and 2), Advanced Stage (Stage 3 and 4), benign ovarian tumors and healthy control groups. Only patients with epithelial ovarian cancer were included into the cancer groups. Patients were distributed into appropriate groups according to their postoperative diagnosis and surgical stage. Accordingly, 13 patients were included in the early stage malignant group, 12 patients in the advanced stage malignant group, 25 patients in the benign group and 19 patients in the healthy (control) group. The control group was used to determine the cut-off values of markers which did not have a cut-off value. The upper limit value was estimated by an average +2 standard deviation formula at the 95% confidence interval. Demographic similarities of patients' data were compared by the student's t-test. Statistical power analysis was also made. Because of time limitation and inadequate cases, only 70% statistical power was reached with 25 ovarian cancer cases.

A comparison was made by the student's t-test and Mann-Whitney U-test to determine if there was a difference between the mean value of each tumor marker in the groups. The Wilcoxon method was used to calculate the value of markers regarding the difference between benign and malignant tumors. However, the number of patients with a malignant tumor in the study group was low and therefore ANOVA testing was not applied. Instead, the Kruskal-Wallis test was used to determine the relationship of the stage of the tumor of each parameter. The correlation between markers in malignant tumors was determined by a Pearson correlation analysis. A linear regression study was carried out on markers which showed significant correlation. Cut-off values were obtained as the following:

CA 15-3: 0-30 U/mL

CRP: 5 mg/dl

Cut-off values were obtained from the healthy control group for CK-MB, IL-6, VEGF and Leptin. The following values were estimated: 9.65 pg/mL for IL-6, 201 pg/mL for VEGF, 1.225 pg/mL for Leptin and 0.9 ng/mL for CK-MB. Ethics Commitee approval was obtained for this study. The budget was supported by the Istanbul University Scientific Research Projects Unit.

Results

Clinical characteristics of patients showed that the most frequent histological diagnosis was serous carcinoma; 20% of the patients were at the early stage malignant, 18.75% at the advanced stage malignant, 31% at the benign ovarian tumor and 29.6% were the healthy control group. The distribution of these ratios are displayed in Table 1. Average age and parity were found statistically significant at higher levels in the

advanced stage cancer group, respectively 49.6 and 3.4 when compared to other groups ($p<0.05$) (Table 2).
Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and odds ratio (OR) for CA 15-3, respectively, were 48%, 95%, 92%, 59% and +OR 9.6 -OR 0.5.
Sensitivity, specificity, PPV, NPV and OR for IL-6, respectively, were 72%, 70%, 75%, 66%, 2.4-0.5.

Table 1. Clinical characteristics of patients

	Number of Patients (%)
Ovarian Cancer Groups	
Early (I and II)	13 (20.3%)
Advanced (III and IV)	12 (18.75%)
Benign Ovarian Tumor	25 (31.25%)
Healthy (control) group	19 (29.6%)
Histology	
Serous carcinoma	12 (17.39%)
Endometrioid carcinoma	5 (7.24%)
Mucinous carcinoma	6 (8.69%)
Mixed carcinoma	2 (2.89%)
Endometrioma	9 (13.04%)
Serous cystadenoma	7 (10.14%)
Mucinous cystadenoma	4 (5.79%)
Teratoma	3 (4.3%)
Fibrotectoma	2 (2.8%)

Table 2. Demographic data of the groups

Group		Year	Gravida	Parity
Early stage	N	13	10	10
	Mean	44.6	3.8	2.1
	Standard deviation	4.45	1.1	0.6
	Minimum	25	0	0
	Maximum	62	6	4
Advanced Stage	N	12	10	10
	Mean	49.6	4.2	3.4
	Standard deviation	6.4	1.4	0.8
	Minimum	36	0	0
	Maximum	66	10	6
Benign	N	25	19	19
	Mean	41.6	3.5	2.1
	Standard deviation	10.3	1.3	1.0
	Minimum	23	0	0
	Maximum	68	10	7
Control	N	19	17	16
	Mean	40.1	3.4	2.3
	Standard deviation	8.9	1.4	1.1
	Minimum	27	0	0
	Maximum	57	13	12

Sensitivity, specificity, PPV, NPV and OR for leptin, respectively, were 52%, 75%, 72%, 55%, +OR 2.08 -OR 0.64.
Sensitivity, specificity, PPV, NPV and OR for VEGF, respectively, were 24%, 80%, 60%, 45%, 1.2-0.92.
Sensitivity, specificity, PPV, NPV and OR for CRP, respectively, were 68%, 30%, 55%, 43%, 0.97-1.06.
Sensitivity, specificity, PPV, NPV and OR for CK-MB, respectively, were 8%, 70%, 25%, 38%, 0.26-1.31.
Accordingly, it was significant that CA 15-3 was the most specific and IL-6 the most sensitive markers (Table 3). Pearson correlation analysis displayed a correlation coefficient value of 0.193 among these two values, while p value was 0.013 which was considered statistically significant. On the other hand, leptin with relatively high positive and negative predictive ratios, showed a significant correlation with CA 15-3 (correlation coefficient: 0.185, $p=0.020$) (Table 4).
The logistic regression analysis of CA 15-3 and IL-6 was found more sensitive in the malignancy prediction of ovarian tumors when used concomitantly, but failed to reach a level which can be considered as significant.
Among tumor markers, CA 15-3, IL-6 and CRP showed a significant increase in the malignant tumor group, while VEGF and Leptin displayed a significant increase both in the malignant and benign tumor groups when compared with the control group (Table 5). Also, a statistical difference was found in CA 15-3, IL-6 and CRP levels between the early stage and the advanced stage groups. However, these markers were not capable of making a prediction related with stage differentiation (Table 6).
We have analysed the success of these markers by the Wilcoxon rank test to understand what parameter they were successful in detecting an early stage ovarian tumor, and after separate measurements, it was concluded that CA 15-3, IL-6, Leptin and VEGF were suitable to be used in the early stage differentiation of ovarian tumors ($p<0.05$) (Table 7). Also, a ROC curve (Figure 1) was determined. It was also concluded that CA 15-3, IL-6 and CRP were useful in differentiating ovarian cancers from benign tumors (Table 8). The sensitivity, specificity, PPV and NPV of these markers must be considered.

Conclusion

There is no ideal model for scanning of an ovarian cancer nor is there an approved clinical test for diagnosis at the early stage. Currently, early stage sensitivity of the many biomarkers which were used to clinically diagnose ovarian cancer, is low (9). At present CA 125 is the most reliable serum marker in ovarian cancer but its role in scanning the disease is challengeable. High CA 125 levels can be encountered in 30% during Stage I and 90% during the advanced stage (10). Additionally, mucinous ovarian tumors may secrete a lesser degree of CA 125 when compared to non-mucinous type ovarian cancers (11).
Many researchers agree that a minimum 10% positive predictive value must be achieved in the scanning process of an ovarian cancer. To attain this value, it may be necessary to reach a minimum ratio of 99.6% specificity. Nevertheless, specificity of CA 125 is considerably lower than this value (12-14). Therefore,

Table 3. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) of 6 biomarkers

Marker	Cut-off value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
CA 15-3	30 U/mL	48	95	92	59
IL-6	9.65 pg/mL	72	70	75	66
Leptin	1225 pg/mL	52	75	72	55
VEGF	201 pg/mL	24	80	60	45
CRP	5 mg/dl	68	30	55	43
CK-MB	0.9 ng/mL	8	70	25	38

Table 4. Pearson's correlation coefficients of 6 biomarkers

	CA 15-3	IL-6	Leptin	VEGF	CRP	CK-MB
CA 15-3						
CC	1	0.193*	-0.185¥	0.131	0.095	0.024
p value		0.013	0.020	0.087	0.120	0.95
IL-6						
CC	0.193	1	-0.108	0.101	0.126	0.031
p value	0.013		0.091	0.130	0.130	0.87
Leptin						
CC	-0.185	-0.108	1	-0.113	-0.098	0.28
p value	0.020	0.091		0.081	0.24	0.98
VEGF						
CC	0.131	0.101	-0.113	1	0.361	0.020
p value	0.087	0.112	0.081		0.361	0.91
CRP						
CC	0.095	0.126	-0.098	0.071	1	0.076
p value	0.120	0.130	0.24	0.361		0.210
CK-MB						
CC	0.024	0.031	0.28	0.020	0.076	1
p value	0.85	0.87	0.88	0.91	0.210	

CC: correlation coefficient, *: significant positive correlation $p<0.05$, ¥: significant negative correlation $p<0.05$

to achieve this purpose; various biomarkers must be used synchronically (15, 16).

Because of the heterogenic structure of the ovarian cancer, a single biomarker may not be capable of covering the entire types and stages. Use of multiple biomarkers may be necessary to determine ovarian cancer at an early stage (17). Gorelik et al. were successful in differentiating 44 early stage ovarian cancers in the serum samples of 45 healthy women by using a combination of IL-6, IL-8, VEGF, EGF and CA 125 biomarkers, where a ratio of 84% for sensitivity and 95% for specificity were obtained (18). In a study performed by Zhang et al. early stage ovarian cancer was diagnosed with 72% sensitivity after CA 125, CA 72-4, CA 15-3 and M-CSF biomarkers were used in combination according to an artificial estimation system (19). Similarly, Skates et al. determined early stage ovarian cancer with a 98% specificity and 75% sensitivity by using a CA 125, CA 15-3, CA 72-4 and M-CSF combination (20). Additionally, different biochemical and ultrasonographic parameters can be used to carry out a differentiated diagnosis of adnexal masses based on logistic regression models (21). In a study performed by Woolas et al.

Table 5. Comparison of levels of tumor markers among the groups (mean±standard deviation)

Tumor marker	Ovarian Cancer n=25	Benign ovarian tumor n=25	Control n=19
CA 15-3	61.3±14*	13.86±7.1	15.2±5.1
IL-6	70±11.0*	26±7.7	26.5±8.4
Leptin	745.5±39*	862.11±41.9¥	941±28.4
VEGF	162.2±10.7*	142.2±12¥	96.4±9.2
CRP	16±1.5*	11.8±1.3	11.6±1.1
CK-MB	0.63±0.01	0.74±0.04	0.58±0.02

* Statistical difference was determined in the cancer group compared with the control group, $p<0.05$. (Negative correlation was also observed in the leptin levels), ¥ Statistical difference was determined in the benign ovarian tumor group compared with the control group, $p<0.05$

effectiveness was enhanced by combining 8 different tumor markers (MCSF, OVX1, LASA, CA 15-3, CA 72-4, CA 19-9, CA 54 and CA 61) in 492 patients, where 192 of these patients had ovarian cancer. The single sensitivity and specificity of CA 125

was found as 78.1% and 76.8% respectively. Different combinations were used in logistic regression analysis where sensitivity achieved a ratio of 90.6% and a specificity of 93.2% where this improvement can be considered as satisfactory (22).

Table 6. Deviation of significant tumor markers among the stages (mean±standard deviation)

Tumor Marker	Stage I and II n=13	Stage III and IV n=12
CA 15-3	23.21±2.6	102.7±19.7*
IL-6	31.1±4.4	113.3±14.3*
Leptin	731±38.9	761.2±39.3
VEGF	178.9±13.6	144.9±6.6
CRP	7.5±0.6	25.5±1.7*

*statistical difference was encountered between the early stage and the advanced stage groups $p<0.05$

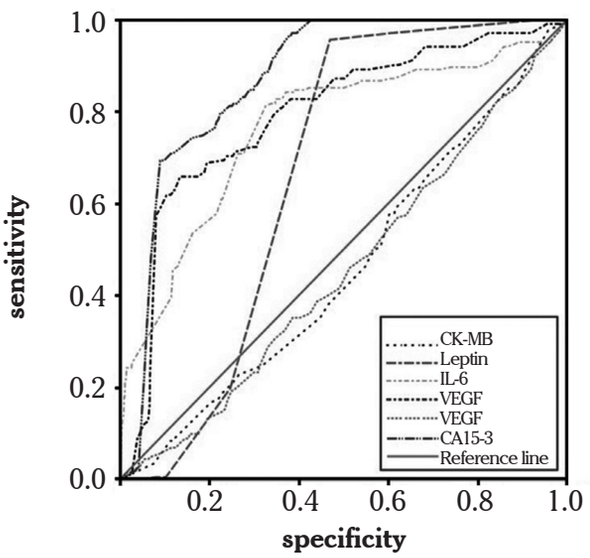


Figure 1. ROC Curve

In our study, CA 15-3 was found to be the most specific and IL-6 the most sensitive marker. Pearson correlation analysis demonstrated that concomitant use of CA 15-3 and IL-6 in logistic regression analysis, as these markers display a positive correlation, could be more sensitive in the differentiation of benign-malignant ovarian tumors, but these findings were unable to reach a significant level. In a similar study, CA 15-3 showed a lower sensitivity ratio, while higher specificity in the malignancy prediction of adnexal masses was found (23).

Gil Mor et al. found that 4 biomarkers (leptin, prolactin, osteopontin, insulin-like growth factor-II) may not be useful alone in the prediction of malignancy, but the combination of these four markers may be effective in the prediction of an early stage ovarian cancer as they possess a 95% ratio of sensitivity, 95% ratio of specificity, 95% ratio of PPV and 94% ratio of NPV (15). In our study, leptin alone was also found effective in the prediction of early stage ovarian cancer. However its 52% ratio of sensitivity and 75% ratio of specificity may cause a handicap for clinical significance.

The most comprehensive study in the literature was carried out by Bertenshaw et al. where a total number of 204 molecules were investigated. Among these molecules, 104 antibodies, 44 autoimmune markers, and 56 infection molecules were significant. Also, levels of 77 biomarkers were found different and the most significant molecules were CA 125, CRP, EGFR, IL-10, IL-8, CTGF, haptoglobulin, and TIMP-1. Excluding CA 125, no specificity higher than 80% was determined in any of the mentioned markers. The 8 markers herein mostly consist of inflammation markers and acute phase reactants. CRP level was found significantly increased and was accepted as the second most determinative biomarker. Increased IL-6 level was stated to be responsible for the high correlation of CRP level. The inflammatory response and the active state of the tumor, may prepare a suitable environment for angiogenesis and metastasis, as there is also some information that it may contribute to the progression of the cancer (24, 25). In our study CRP and IL-6 levels were also found significant in the scanning of ovarian cancer (Table 8). Angiogenesis plays a significant role in the tumorigenesis. Bertenshaw et al. (25) showed a significant increase in the VEGF level, which is a

Table 7. p values of the markers for the detection of early-stage ovarian cancer

	CA 15-3	IL-6	Leptin	VEGF	CRP	CK-MB
p value	0.044	0.038	0.001	0.0003	0.213	0.762

Table 8. Analysis of the ROC Curve

Marker	Area Under the Curve (AUC)	Standard Deviation	p value	95% confidence
CA 15-3	0.861*	0.009	0.000	0.810- 0.823
IL-6	0.785*	0.016	0.000	0.751- 0.814
Leptin	0.063	0.012	0.000	0.638- 0.681
VEGF	0.458	0.022	0.040	0.404- 0.462
CRP	0.761*	0.021	0.000	0.718- 0.796
CK-MB	0.451	0.023	0.051	0.398- 0.458

*CA 15-3, IL-6 and CRP were useful in differentiating ovarian cancers from benign tumors

potent angiogenetic factor. In our study, a similar increase was also detected in the VEGF levels of the ovarian cancer group.

We found higher ratios of mean age and higher parity values in patients included in the advanced stage ovarian cancer group when compared with other groups ($p<0.05$). Even though this finding is compatible with the information in the literature related with the increased risk of ovarian cancer due to higher age, it is contrary to the information that ovarian cancer risk may decrease when parity is increased (26-28).

Although a ROC curve was created in this study, the number of early stage ovarian cancer cases in the sample group was fewer than expected and could cause a bias for our study. Information was obtained from a limited number of patients and therefore clinical significance is readily decreased. However, according to the current data, CA 15-3, IL-6 , leptin, VEGF and CRP were found effective in the differentiation process of benign-malignant ovarian tumors. Parameters such as sensitivity and specificity alone were not as effective as estimated, and may not be convenient for routine applications, but differential diagnosis can be used in solving problematic cases.

There is a strong need for large-scale serial studies which may confirm and strengthen the findings of this study. Additionally, this study could be an important indicator that it may not be suitable to use CK-MB as a biomarker in future studies.

Conflict of interest

The budget was supported by the Istanbul University, Scientific Research Projects Unit.

References

1. American Cancer Society. Cancer facts and figures 2007. Atlanta: American Cancer Society; 2007.
2. Cannistra SA. Cancer of the ovary. N Engl J Med 1993; 329: 1550-9. [CrossRef]
3. Res LAG, Melbert D, Krapcho M, et al. SEER cancer statistics review, 1975-2004. Bethesda (MD): National Cncer Institute; 2007. Based on Novermber 2006 SEER data submission, posted to the SEER web site.
4. Teneriello MG, Park RC. Early detection of ovarian cancer. CA Cancer J Clin 1995; 45: 71-87. [CrossRef]
5. Klug TL, Bast RC Jr, Niloff JM, Knapp RC, Zurawski VR Jr. Monoclonal antibody immunoradiometric assay for an antigenic determinant (CA-125) associated with human epithelial ovarian carcinomas. Cancer Res 1984; 44: 1048-53.
6. Rose PG, Fusco N, Fluellen L, Rodriguez M. Second-line therapy with paclitaxel and carboplatin for recurrent disease following first-line therapy with paclitaxel and platinum in ovarian or peritoneal carcinoma. J Clin Oncol 1998; 16: 1494-7.
7. Schilthuis MS, Aalders JG, Bouma J, Kooi H, Fleuren GJ, Willemse PH, et al. Serum CA-125 levels in epithelial ovarian cancer: relation with findings at second-look operations and their role in the detection of tumour recurrence. Br J Obstet Gynaecol 1987; 94: 202-7. [CrossRef]
8. Oei, AL, Massuger LF, Bulten J, et al. Surveillance of women at high risk for hereditary ovarian cancer is inefficient. Br J Cancer 2006; 94: 814-9. [CrossRef]
9. Jacobs U, Menon U. Progress and challenges in screening for early detection of ovarian cancer. Mol Cell Proteomics 2004; 3: 355-66. [CrossRef]
10. Berek JS, Bark JR RC. The CA 125 tumor associated antigen: a review of the literature. Hum Repr 1989; 4: 1-12.
11. Gadducci A, Ferdeghini M, Prontera C, Moretti L, Mariani G, Bianchi R, et al. The concomitant determination of different tumor markers in patients with epithelial ovarian cancer and benign ovarian masses: relevance for differential diagnosis. Gynecol Oncol 1992; 44: 147-54. [CrossRef]
12. Gezginç K, Çelik Ç, Bala A, Acar A, Çiçek N, Akyürek C. Comparison of ultrasonography and Ca-125 in differential diagnosis of malign adnexial masses. J Turkish-German Gynecol Assoc 2003; 4: 42-5.
13. Tanrıverdi HA, Sade H, Akbulut V, Barut A, Bayar Ü. Clinical and ultrasonographic evaluation of pelvic masses. J Turkish-German Gynecol Assoc 2007; 8: 67-70.
14. Jacobs IJ, Oram DH, Bast RC Jr. Strategies for improving the specificity of screening for ovarian cancer with tumor-associated antigens CA 125, CA 15-3, and TAG 72.3. Obstet Gynecol 1992; 80: 396-9.
15. Mor G, Visintin I, Lai Y, Zhao H, Schwartz P, Rutherford T, et al. Serum protein markers for early detection of ovarian cancer. Proc Nati Acad Sci USA 2005; 102: 7677-82. [CrossRef]
16. Zhang Z, Bast RC Jr, Yu Y, Li J, Sokoll LJ, Rai AJ, et al. Three biomarkers identified from serum proteomic analysis for the detection of early stage ovarian cancer. Cancer Res 2004; 64: 5882-90. [CrossRef]
17. Kingsmore SF. Multiplexed protein measurement: technologies and applications of protein and antibody arrays. Nat Rev Drug Discov 2006; 5: 310-20. [CrossRef]
18. Gorelik E, Landsittel DP, Marrangoni AM, Modugno F, Velikokhatnaya L, Winans MT, et al. Multi-plexed immunobead-based cytokine profiling for early detection of ovarian cancer. Cancer Epidemiol Biomarkers Prev 2005; 14: 981-7. [CrossRef]
19. Zhang Z, Yu Y, Xu F, Berchuck A, van Haaften-Day C, Havrilesky LJ, et al. Combining multiple serum tumor markers improves detection of stage I epithelial ovarian cancer. Gynecol Oncol 2007; 107: 526-31. [CrossRef]
20. Skates SJ, Horick N, Yu Y, Xu FJ, Berchuck A, Havrilesky LJ, et al. Pre-operative sensitivity and specifity for early stage ovarian cancer when combining CA 125, CA 15-3, CA72-4 and M-CSF using mixtures of multivariate normal distributions. J Clin Oncol 2004; 22: 4059-66. [CrossRef]
21. Timmerman D, Bourne TH, Tailor A, Collins WP, Verrelst H, Vandenberghe K, et al. A comparison of methods for preoperative discrimination between malignant and benign adnexal masses: the development of a new logistic regression model. Am J Obstet Gynecol 1999; 181: 57-65. [CrossRef]
22. Woolas RP, Conaway MR, Xu F, Jacobs IJ, Yu Y, Daly L, et al. Combination of multiple serum markers are superior to individual assays for discriminating malignant from benign pelvic masses. Gynecol Oncol 1995; 59: 111-6. [CrossRef]
23. Makar AP, Kristensen GB, Kaern J, Børmer OP, Abeler VM, Tropé CG. Prognostic value of pre- and post-operative serum CA 125 levels in ovarian cancer: new aspects and multivariate analysis. Obstet Gynecol 1992; 79: 1002-10.
24. Marx J. Cancer biology. All in the stroma: Cancer's Cosa Nostra. Science 2008; 320: 38-41.
25. Bertenshaw GP, Yip P, Seshaiah P, Zhao J, Chen TH, Wiggins WS, et al. Multianalyte profiling of serum antigens and autoimmune and infectious disease molecules to identify biomarkers dysregulated in epithelial ovarian cancer. Cancer Epidemiol Biomarkers Prev 2008; 17: 2872-81. [CrossRef]
26. Runnebaum IB, Stickeler E. Epidemiological and molecular aspects of ovarian cancer risk. J Cancer Res Clin Oncol 2001; 127: 73-9. [CrossRef]
27. Yancik R, Ries LG, Yates JW. Ovarian cancer in the elderly: an analysis of Surveillance, Epidemiology and End Results Program data. Am J Obstet Gynecol 1986; 154: 639-47.
28. Purdie DM, Bain CJ, Siskind V, Webb PM, Green AC. Ovulation and risk of epithelial ovarian cancer. Int J Cancer 2003; 104: 228-32. [CrossRef]

Second trimester serum alpha-fetoprotein level is a significant positive predictor for intrauterine growth restriction in pregnant women with hyperemesis gravidarum

İkinci trimester serum Alfa-Fetoprotein düzeyi hiperemezis gravidarumu olan gebe kadınlarda intrauterin gelişme geriliği için pozitif bir prediktördür

Enis Özkaya[1], Evrim Çakır[2], Mehmet Çınar[3], Metin Altay[3], Orhan Gelişen[3], Fadıl Kara[1]

[1]Department of Obstetrics and Gynecology, Dr. Sami Ulus Maternity and Women's Health Teaching and Research Hospital, Ankara, Turkey

[2]Department of Endocrinology and Metabolism, Dışkapı Yıldırım Beyazıd Teaching and Research Hospital, Ankara, Turkey

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

Abstract

Objective: The aim of this study was to determine the association between three parameters of second trimester serum secreening and preterm labor and intrauterine growth restriction (IUGR) in patients with hyperemesis gravidarum (HG).

Material and Methods: A prospective study on 429 pregnancies with HG was designed to determine the association between alpha-fetoprotein (AFP), unconjugated estriol (uE3), human chorionic gonadotropin (HCG) and pregnancy prognosis in terms of preterm labor, IUGR and birth weight.

Results: In our study group the mean age of patients was 25.4±3.8 years. Mean birth weight was 3180±555 g. Mean AFP, uE3, hCG levels in the study group were 1.44±0.65 MoM, 0.91±0.38 MoM, 1.09±0.64 MoM, respectively. Twenty nine (6.8%) patients delivered before 37 weeks of gestation and 52 (12.1%) patients developed IUGR. Mean MoM values of AFP among patients with preterm labor, IUGR and normal delivery were 1.35±0.45, 1.97±0.81, 1.34±0.58 MoM, respectively (p<0.001). Mean MoM values of hCG among patients with preterm labor, IUGR and normal delivery were 1.46±0.90, 1.35±0.89, 1±0.5 MoM respectively (p<0.001). Mean MoM values of uE3 among patients with preterm labor, IUGR and normal delivery were 0.75±0.25, 0.80±0.30, 0.95±0.40 MoM, respectively (p=0.003). Odds ratio of AFP>1.55 was 3.73 (95% CI, 1.99-6.98, p<0.001) for IUGR after adjustment for HCG.

Conclusion: Our study suggests that AFP levels of the second trimester screening test higher than 1.55 MoM is significantly associated with IUGR in hyperemesis gravidarum. The second trimester screening test can predict poor outcome in HG.

(J Turkish-German Gynecol Assoc 2011; 12: 220-4)

Key words: Second trimester screening, alpha-fetoprotein, intrauterine growth restriction, preterm labor, hyperemesis gravidarum

Received: 28 July, 2011 **Accepted:** 7 September, 2011

Özet

Amaç: Bu çalışmanın amacı hiperemezis gravidarumu olan hastalarda ikinci trimester taramasında kullanılan üç parametre ile preterm eylem, intrauterin gelişme geriliği arasındaki ilişkiyi belirlemek.

Gereç ve Yöntemler: Bu prospektif çalışma hiperemezis gravidarumu olan 429 gebe üzerinde alfafetoprotein (AFP), unkonjuge estriol (uE3), human koryonik gonadotropin (HCG) ile preterm eylem, intrauterin gelişme geriliği arasındaki ilişkiyi belirlemek amacı ile dizayn edildi.

Bulgular: Çalışma grubunda ortalama yaş 25.4±3.8 idi. Ortalama doğum ağırlığı 3180±555 g olarak hesaplandı. Ortalama AFP, uE3, HCG düzeyleri sırası ile ortalama 1.44±0.65 MoM, 0.91±0.38 MoM, 1.09±0.64 MoM idi. Yirmidokuz (%6.8) hasta 37 hafta öncesinde doğurdu ve 52 (%12.1) hastada gelişme geriliği mevcuttu. Preterm eylem, IUGR ve normal doğum yapan grupta ortalama AFP değerleri sırası ile 1.35±0.45, 1.97±0.81, 1.34±0.58 MoM idi (p<0.001). Preterm eylem, IUGR ve normal doğum yapan grupta ortalama HCG değerleri sırası ile 1.46±0.90, 1.35±0.89, 1±0.5 MoM (p<0.001). Preterm eylem, IUGR ve normal doğum yapan grupta ortalama HCG değerleri sırası ile 0.75±0.25, 0.80±0.30, 0.95±0.40 MoM (p=0.003). IUGR için HCG ye göre düzeltilmiş AFP>1.55 için odds oranı 3.73 (%95 CI, 1.99-6.98, p<0.001) olarak saptandı.

Sonuç: Çalışmamıza göre 1.55 in üstündeki AFP düzeyleri hiperemezis gravidarum hastalarında IUGR için risk faktörüdür. İkinci trimester serum taraması HG de kötü prognozu öngörebilir.

(J Turkish-German Gynecol Assoc 2011; 12: 220-4)

Anahtar kelimeler: İkinci trimester tarama, alfa feto protein, intrauterin gelişme geriliği, preterm doğum, hyperemezis gravidarum

Geliş Tarihi: 28 Temmuz 2011 **Kabul Tarihi:** 07 Eylül 2011

Address for Correspondence: *Enis Özkaya, Demetevler 10. Sok. 63/15 Yenimahalle, Ankara, Turkey*
Phone: +90 505 474 24 59 e.mail: enozkaya1979@gmail.com

doi:10.5152/jtgga.2011.55

Content

Hyperemesis gravidarum is associated with high hCG levels. This study investigated the association between parameters of the second trimester screening test and pregnancy outcome in selected group of pregnancies. A high hCG level was not found to be a predictor for poor outcome of pregnancy, while the AFP level was associated with intrauterine growth restriction, with a cut off value lower than the reported value in normal pregnancies.

Introduction

A small percentage of women experience a severe form of nausea and vomiting known as hyperemesis gravidarum. Estimates of the incidence of hyperemesis vary from 0.3-1.5% of all live births. Diagnosis is subjective, but the condition is usually described as intractable vomiting leading to fluid, electrolyte and acid base imbalance, nutritional deficiency and weight loss (1). Hyperemesis gravidarum (HG) is the most common cause of hospitalization in the first half of pregnancy and the second most common cause of antenatal hospitalization during pregnancy. Extreme weight loss is common among women with HG, suggesting that HG is a form of prolonged starvation in pregnancy and that the long-term effects of this condition on women and their offspring warrant further investigation (2). Measurement of maternal serum alpha fetoprotein (AFP), human chorionic gonadotropin (HCG), and unconjugated estriol (uE3) at the beginning of the second trimester of pregnancy is a well established screening test for trisomies. Association between second trimester screening test parameters and several pregnancy complications have been studied in unselected study populations. Previous studies have described the association of abnormal levels of maternal serum AFP and hCG with a variety of problems and complications of pregnancy, such as preterm delivery, fetal growth retardation, fetal death and severe hypertensive disorders in pregnancy (3-5).

Serum human chorionic gonadotropin (hCG) and estradiol levels are higher in women with hyperemesis gravidarum (HG) than in unaffected pregnant controls (6). Concentrations of hCG that are above or below the normal range are associated with poor pregnancy outcomes. It seems that HG and hCG are related and that nutrient restriction during critical periods of embryonic development may ensure production of hCG at optimal levels, protect placental development, and optimize nutrient partitioning between maternal and fetal tissues (7).

The aim of this study was to determine the association between three parameters of second trimester serum screening and preterm labor (PL) and intrauterine growth restriction (IUGR) in patients with HG.

Material and Method

Four hundred twenty nine patients with first to second trimester diagnosis of hyperemesis gravidarum who had been followed for their pregnancies in Etlik Zübeyde Hanım Maternity and Women's Health Teaching and Research Hospital between January 2005 to December 2009 were included in the study. The study was approved by the local ethics committee. Informed consents were obtained from all the participants. Diagnosis of hyperemesis gravidarum was based on intractable vomiting leading to fluid, electrolyte and acid base imbalance, nutritional deficiency and weight loss. All patients had repeat hospitalizations during pregnancy follow up for hyperemesis.

Patients with a history of pregnancy complications (preeclampsia, eclampsia, preterm labor, intrauterine growth restriction etc.) and patients with genetic, structural abnormality, preeclampsia, gestational diabetes, elective cesarean, placental ablation in the present pregnancy were excluded from the study in order to select patients without a risk factor for investigated outcomes. None of the patients had systemic disorders (stomach disease, cholelithiasis, gastroenteritis, chronic gastric disease, multiple pregnancy, etc.). Thyroid function tests were all in normal limits. At the beginning of the second trimester, screening test was performed for each patient between 15th to 19th weeks of gestation. After blood serum AFP levels and hCG levels were calculated using the ELISA method; unconjugated estriol was calculated using RIA method for each patients. Patients with increased risk of trisomy and open spina were not excluded from the study. Patients were followed until delivery at monthly intervals. Complications during pregnancies were recorded. Preterm labor was accepted as spontaneous deliveries before 37 weeks of gestation and IUGR was diagnosed in patients with a neonate birth weight smaller than the 10th percentile of that gestational age. IUGR was also evaluated according to 3rd and 5th percentiles. Preterm infants were evaluated for their birth percentiles in order not to miss any IUGR among them.

Statistical analysis

Collected data was entered into SPSS version 11. For group comparisons, analysis of variance and posthoc Tukey test was used. P value smaller than 0.05 was accepted as statistically significant. Binary logistic regression was used to calculate odds ratio. Correlation analysis was used to calculate the degree of associations and ROC analysis was performed for cut off calculation. Regression analysis was used to determine associations.

Result

Means of AFP, hCG, uE3 among groups of complicated and uncomplicated pregnancies and groups divided according to birth weight percentiles:

In our study group mean age of patients was 25.4±3.8 years. Mean birth weight was 3180±555 g Mean AFP, uE3, hCG levels in the study group were 1.44±0.65 MoM, 0.91±0.38 MoM, 1.09±0.64 MoM respectively. Twenty nine (6.8%) patients delivered before 37 weeks of gestation and fifty two (12.1%) patients developed IUGR. Mean MoM values of AFP level among patients with preterm labor, IUGR and normal deliveries were 1.35±0.45, 1.97±0.81, 1.34±0.58 MoM respectively ($p<0.001$).

Mean MoM values of hCG level among patients with preterm labor, IUGR and normal deliveries were 1.46±0.90, 1.35±0.89, 1±0.5 MoM respectively ($p<0.001$).

Table 1. Relationship between AFP-MoM, uE3-MoM, hCG-MoM levels in hyperemesis gravidarum and pregnancy outcomes

	IUGR		p value	Preterm Labor		p value
	No	Yes		No	Yes	
	n=377	n=52		n=400	n=29	
AFP-MoM	1.34±0.50	1.97±0.81	0.00	1.45±0.67	1.35±0.45	0.00
uE3-MoM	0.93±0.39	0.80±0.29	0.02	0.92±0.38	0.75±0.25	0.02
hCG-MoM	1.05±0.57	1.35±0.88	0.02	1.06±0.59	1.46±0.90	0.00

Mean MoM values of uE3 level among patients with preterm labor, IUGR and normal deliveries were 0.75±0.25, 0.80±0.30, 0.95±0.40 MoM, respectively (p=0.003). Mean ages were similar among groups (p=0.189). Mean values between pregnancy complications were shown in Table 1.

Mean MoM values of AFP levels among patients with birth weights below 3rd (n=5, 1.2%), 3- 5th (n=12, 2.8%) and 5-10th (n=35, 8.1%) percentiles of that gestational age were 2.66±0.25, 1.98±0.23, 1.83±0.91 MoM, respectively (p<0.001).

Mean MoM values of hCG levels among patients with birthweights below 3rd, 3-5th and 5-10th percentiles of that gestational age were 3, 0.92±0.84, 1.20±0.61 MoM, respectively (p<0.001).

Mean MoM values of uE3 among patients with birth weights below 3rd, 3-5th and 5-10th percentiles of that gestational age were 0.5, 0.86±0.22, 0.82±0.32 MoM, respectively (p=0.023). Highest AFP (p<0.001, p=0.012) and hCG (p<0.001) levels were seen in patients with newborn birth weights below the 3rd percentile. Mean ages were similar among different birth weight percentile groups (p=0.141).

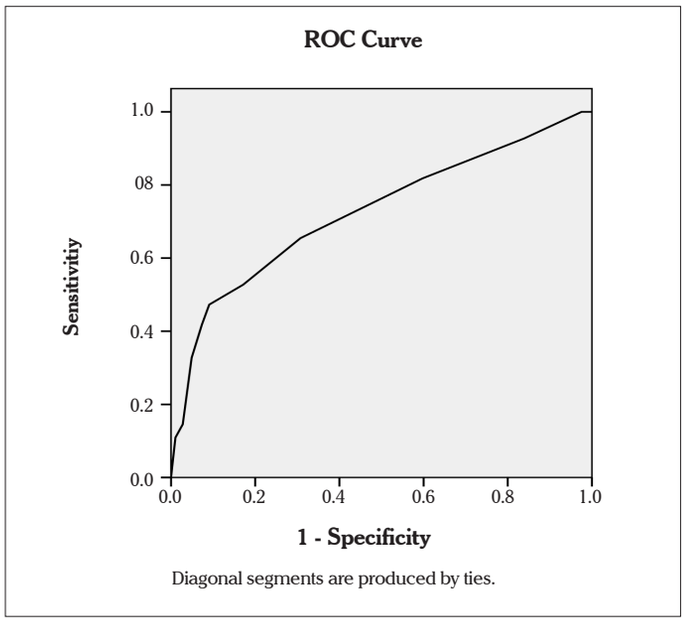


Figure 1. ROC analysis revealed that AFP levels is a significant predictor for IUGR

Correlations

AFP level was positively correlated with IUGR (r=0.351, p<0.001), negatively correlated with birth weight (r=-0.222, p<0.001). uE3 level was positively correlated with birth weight (r=0.245, p<0.001) and negatively correlated with IUGR (r=-0.123, p=0.023) and PL (r=-0.122, p=0.024). hCG level was negatively correlated with birth weight (r=-0.199, p<0.001) and positively correlated with IUGR (r=0.167, p=0.002) and PL (r=0.175, p=0.001).

Associations

Regression analysis revealed that uE3, hCG adjusted AFP; AFP, hCG adjusted uE3; uE3, AFP adjusted hCG were significantly associated with birth weight and IUGR (p<0.001, p<0.001, p<0.05, respectively, Figure 1). uE3, AFP adjusted hCG was significantly associated with PL (p<0.05).

Hyperemesis gravidarum was first diagnosed at different gestational ages in the study group: at 9th week (n=22, 5%), 10th week (n=43:10%), 11th week (n=51, 12%), 12th week (n=61, 14%) and 13th week (n:252.59%).

Gestational age of hyperemesis gravidarum diagnosis was positively correlated with birth weight (r=0.288, p=0.001), negatively correlated with PL (r=-0.189, p=0.001) and IUGR (r=-0.181, p=0.001).

ROC analysis

ROC analysis revealed that the AFP level is a significant predictor for IUGR (Area under curve 0.716, p<0.001, Figure 2). Cut off value was 1.55 MoM for the AFP level to predict IUGR with 66% sensitivity and 69% specificity. uE3 and hCG levels were not found to be significant predictors for either preterm labor or IUGR in ROC analysis (p>0.005).

Odds ratio of AFP>1.55 was 3.73 (95% CI, 1.99-6.98, p<0.001) for IUGR after adjustment for hCG.

Discussion

In our study higher AFP levels were found to be significantly associated with increased IUGR while higher hCG levels were shown to be associated with increased preterm labor rates, higher uE3 levels were associated with both decreased preterm labor and IUGR rates. We expected to observe an association between pregnancy complications caused by hyperemesis and increased hCG levels in HG. Association between hCG and uE3 levels with preterm labor and IUGR were shown in regression analysis, however they are not found to be significant predictors for IUGR or preterm labor in ROC analysis. These results led us to hypothesize that the second trimester screening test can give more information about pregnancy outcome in HG, but only the AFP was useful as a test to predict poor outcome. Patients

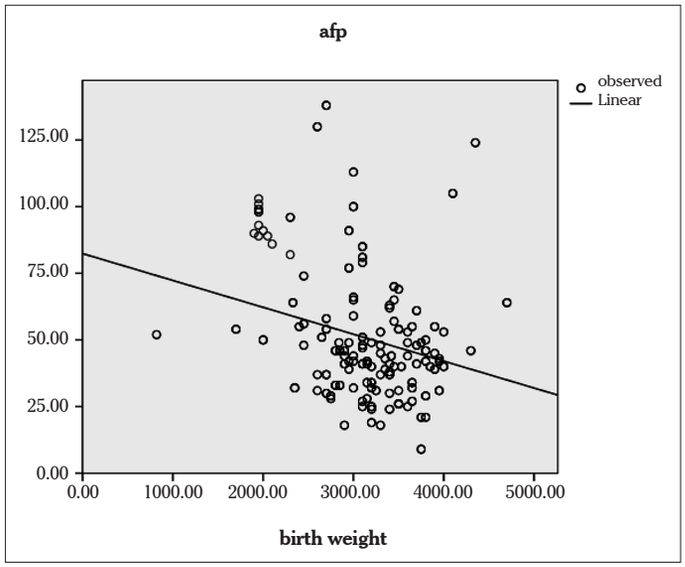


Figure 2. Relationship between AFP levels and birth weight

with different birth weight percentiles in the IUGR group were significantly different in terms of hCG, AFP and uE3 levels. The highest hCG and AFP levels were observed in patients with a birth weight lower than the 3rd percentile. These results are all thought to be due to increased abnormal placental tissue. Our literature search revealed that, while hyperemesis gravidarum has been found to be associated with high hCG levels (6), its effect on second trimester screening, AFP and unconjugated estriol levels in HG has not been studied. Hyperemesis occurs in 473 of 100.000 live births and is associated with significant changes. Infants of mothers with hyperemesis have lower birth weights and the mothers are more likely to have infants that are small for gestational age (8). Although elevated maternal serum AFP level usually shows an elevated risk for neural tube defects, they are also related to adverse pregnancy outcomes such as preeclampsia, spontaneous abortion, preterm delivery, low birth weight, IUGR, oligohydramnios, placental abruption and stillbirth (9-12), all of which are thought to be associated with abnormal placentation (13). In our study, a significant association between serum AFP values and IUGR was observed.

Patients with high AFP levels and bilateral uterine artery diastolic notch presence, were found to be candidates for pregnancy complications and these groups of patients were advised to be followed up more closely (14).

Multiparameter testing of placental function in the mid-trimester (uterine artery Doppler, placental morphology, AFP and hCG screening) may allow us to identify women with increased risk of developing severe placental insufficiency and pregnancy complications (15).

Increased maternal serum AFP >1.89 MoM were found to be significantly associated with IUGR, pregnancy induced hypertension (PIH) and placental pathology. In our study group, the cut off value was lower, and this might be due to the presence of hyperemesis with higher hCG levels compared to normal pregnancies. Increased beta-hCG >1.69MoM were significantly associated with PIH and IUGR. It was concluded that the triple test can be used not only for the detection of fetal chromosomal and neural tube defect abnormalities but also for the detection of high-risk pregnancies (16).

Recently published data has shown that serum unconjugated estriol, AFP or hCG values in triple test results may be associated with development of oligohydramnios, gestational diabetes and macrosomia in women with healthy and normal appearing fetuses (17).

Despite the increase in prematurity and low birth weight, unexplained raised levels of AFP were not associated with an increase in perinatal mortality (18). A retrospective trial found that increased antenatal surveillance for women with unexplained elevated AFP did not provide any benefit for detection of adverse events (19). Women with elevated second-trimester hCG values have been reported to have increased risks for preeclampsia, IUGR, small for gestational age (SGA) infants, preterm delivery, spontaneous abortion, stillbirth and placental abnormalities (9, 12, 20-22). However, Ashour et al. (23) suggested that the utility of hCG level as a screening test for preeclampsia was limited. Besides, the relationship between obstetric complications and elevated hCG levels was not confirmed in two other studies (10, 24). In another study, preterm delivery, preeclampsia and large for gestational age were found to be associated with only hCG levels of 3 MoM and the authors concluded that increased fetal surveillance is not warranted with lower values (25). Consistent with our results, a low uE3 level was suggested as an independent risk factor for adverse pregnancy outcome and is particularly associated with preeclampsia, in conjunction with IUGR, SGA, oligohydramnios, preterm delivery and stillbirth (9, 12, 26). In a meta-analysis by Morris et al. Down's serum screening analytes were found to have low predictive accuracy for pre-eclampsia and small for gestational age. The study concluded that they may be a useful means of risk assessment or of use in prediction when combined with other tests (27). This study was conducted specifically on patients with HG, and in our literature review we have not encountered a similar study. The relatively small sample size is a limitation of this study.

Our study suggests that the AFP level of the second trimester screening test of higher than 1.55 MoM is significantly associated with IUGR in HG. The highest hCG and AFP levels were seen in patients with birth weight below the 3rd percentile. Further studies are needed to establish the predictors of poor pregnancy outcome in HG.

Conflict of interest

No conflict of interest was declared by the authors.

References

1. Sheehan P. Hyperemesis gravidarum--assessment and management. Aust Fam Physician 2007; 36: 698-701.
2. Fejzo MS, Poursharif B, Korst LM, Munch S, MacGibbon KW, Romero R, et al. Symptoms and pregnancy outcomes associated with extreme weight loss among women with hyperemesis gravidarum. J Womens Health (Larchmt) 2009; 18: 1981-7. [CrossRef]
3. Pergament E, Stein AK, Fiddler M, Cho NH, Kupferminc MJ. Adverse pregnancy outcome after a false-positive screen for Down syndrome using multiple markers. Obstet Gynecol 1995; 86: 255-8. [CrossRef]

4. Morssink LP, de Wolf BT, Kornman LH, Beekhuis JR, van der Hall TP, Mantingh A. The relation between serum markers in the second trimester and placental pathology. A study on extremely small for gestational age fetuses. Br J Obstet Gynaecol 1996; 103: 779-83. [CrossRef]
5. Guven MA, Tanriverdi HA, Kılınç M, Sapmaz K, Usal D, Ademir G. Role of the Second Trimester Maternal Serum AFP Levels in Diagnosing Gestational Diabetes Mellitus, Low Birth Weight and Preterm Labor. J Turkish German Gynecol Assoc 2005; 6: 107-10.
6. Tan PC, Tan NC, Omar SZ. Effect of high levels of human chorionic gonadotropin and estradiol on the severity of hyperemesis gravidarum. Clin Chem Lab Med 2009; 47: 165-71. [CrossRef]
7. Furneaux EC, Langley-Evans AJ, Langley-Evans SC. Nausea and vomiting of pregnancy: endocrine basis and contribution to pregnancy outcome. Obstet Gynecol Surv 2001; 56: 775-82. [CrossRef]
8. Bailit JL. Hyperemesis gravidarium: Epidemiologic findings from a large cohort. Am J Obstet Gynecol 2005; 193: 811-4. [CrossRef]
9. Yaron Y, Cherry M, Kramer RL, O'Brien JE, Hallak M, Johnson MP, et al. Second-trimester maternal serum marker screening: maternal serum alpha-fetoprotein, beta-human chorionic gonadotropin, estriol, and their various combinations as predictors of pregnancy outcome. Am J Obstet Gynecol 1999; 181: 968-74. [CrossRef]
10. Spencer K. Second-trimester prenatal screening for Down syndrome and the relationship of maternal serum biochemical markers to pregnancy complications with adverse outcome. Prenat Diagn 2000; 20: 652-6. [CrossRef]
11. Wenstrom KD, Owen J, Boots LR, DuBard MB. Elevated second-trimester human chorionic gonadotropin levels in association with poor pregnancy outcome. Am J Obstet Gynecol 1994; 171: 1038-41.
12. Duric K, Skrablin S, Lesin J, Kalafatic D, Kuvacic I, Suchanek E. Second trimester total human chorionic gonadotropin, alpha-fetoprotein and unconjugated estriol in predicting pregnancy complications other than fetal aneuploidy. Eur J Obstet Gynecol Reprod Biol 2003; 110: 12-5. [CrossRef]
13. Boyd PA. Why might maternal serum AFP be high in pregnancies in which the fetus is normally formed? Br J Obstet Gynaecol 1992; 99: 93-5. [CrossRef]
14. Karsidag AY, Buyukbayrak EE, Kars B, Suyugul U, Unal O, Turan MC. The relationship between unexplained elevated serum markers in triple test, uterine artery Doppler measurements and adverse pregnancy outcome. J Pak Med Assoc 2010; 60: 181-6.
15. Androutsopoulos G, Gkogkos P, Papadopoulos V, Adonakis G, Tsapanos V, Vassilakos P, et al. Mid-trimester maternal serum markers in predicting adverse pregnancy outcome. Clin Exp Obstet Gynecol 2009; 36: 237-40.
16. Bas-Budecka E, Perenc M, Sieroszewski P. Abnormal second trimester screening for fetal chromosomal abnormalities as a predictor of adverse pregnancy outcome. Ginekol Pol 2007; 78: 877-80.
17. Sayin NC, Canda MT, Ahmet N, Arda S, Sut N, Varol FG. The association of triple-marker test results with adverse pregnancy outcomes in low-risk pregnancies with healthy newborns. Arch Gynecol Obstet 2008; 277: 47-53. [CrossRef]
18. Kiran TS, Bethel J, Bhal PS. Correlation of abnormal second trimester maternal serum alpha-fetoprotein (MSAFP) levels and adverse pregnancy outcome. J Obstet Gynaecol 2005; 25: 253-6. [CrossRef]
19. Huerta-Enochian G, Katz V, Erfurth S. The association of abnormal alpha-fetoprotein and adverse pregnancy outcome: does increased fetal surveillance affect pregnancy outcome? Am J Obstet Gynecol 2001; 184: 1549-53. [CrossRef]
20. Waller DK, Lustig LS, Cunningham GC, Feuchtbaum LB, Hook EB. The association between maternal serum alpha-fetoprotein and preterm birth, small for gestational age infants, preeclampsia, and placental complications. Obstet Gynecol 1996; 88: 816-22. [CrossRef]
21. Luckas MJ, Sandland R, Hawe J, Neilson JP, McFadyen IR, Meekins JW. Fetal growth retardation and second trimester maternal serum human chorionic gonadotrophin levels. Placenta 1998; 19: 143-7. [CrossRef]
22. Lepage N, Chitayat D, Kingdom J, Huang T. Association between second-trimester isolated high maternal serum maternal serum human chorionic gonadotropin levels and obstetric complications in singleton and twin pregnancies. Am J Obstet Gynecol 2003; 188: 1354-9. [CrossRef]
23. Ashour AM, Lieberman ES, Haug LE, Repke JT. The value of elevated second-trimester beta-human chorionic gonadotropin in predicting development of preeclampsia. Am J Obstet Gynecol 1997; 176: 438-42. [CrossRef]
24. Walton DL, Norem CT, Schoen EJ, Ray GT, Colby CJ. Second-trimester serum chorionic gonadotropin concentrations and complications and outcome of pregnancy. N Engl J Med 1999; 341: 2033-8. [CrossRef]
25. Towner D, Gandhi S, El Kady D. Obstetric outcomes in women with elevated maternal serum human chorionic gonadotropin. Am J Obstet Gynecol 2006; 194: 1676-81. [CrossRef]
26. Kowalczyk TD, Cabaniss ML, Cusmano L. Association of low unconjugated estriol in the second trimester and adverse pregnancy outcome. Obstet Gynecol 1998; 91: 396-400. [CrossRef]
27. Morris RK, Cnossen JS, Langejans M, Robson SC, Kleijnen J, Ter Riet G, et al. Serum screening with Down's syndrome markers to predict pre-eclampsia and small for gestational age: systematic review and meta-analysis. BMC Pregnancy Childbirth 2008; 8: 33. [CrossRef]

Normative values of fetal nasal bone lengths of Turkish singleton pregnancies in the first trimester

Türk tekil gebeliklerdeki fetal nazal kemik uzunluklarının birinci trimester normal değerleri

Murat Yayla[1], Rahime Nida Ergin[2], Gökhan Göynümer[3]

[1]Department of Gynecology and Obstetrics, International Hospital, İstanbul, Turkey

[2]Department of Gynecology and Obstetrics, Bayındır Hospital İçerenköy, İstanbul, Turkey

[3]Department of Gynecology and Obstetrics, Göztepe Education and Research Hospital, İstanbul, Turkey

Abstract

Objective: Evaluation of nasal bone improves the performance of first-trimester screening for trisomy 21. In this retrospective study we aimed to determine normative values related to the measurement of nasal bone length of the Turkish population during the first trimester ultrasonographic fetal screening.

Material and Methods: Medical records of singleton pregnancies, whose first trimester fetal screening was performed between 2004 and 2010, were evaluated retrospectively. Pregnancies with any detected/suspicious anatomical or genetic fetal anomalies, biochemical abnormalities, increased nuchal translucency measurements, and pregnancies of artificial reproduction techniques were excluded from data analyses. Mean±standard deviation, median and percentile values of the length of nasal bone were calculated separately for 11^{0-6}, 12^{0-6} and 13^{0-6} gestational weeks

Results: Nasal bone could be visualized in 99.6% of the included 1762 singleton pregnancies. In 16.5% of the cases nasal bones were only noted as present or absent. Mean maternal age was 29.67±4.50 years and mean gestational age was 12.54±0.61 weeks. Median values of nasal bone lengths were 1.7, 1.9, and 2.2 mm for 11^{0-6}, 12^{0-6} and 13^{0-6} gestational weeks respectively. Nasal bone length (NBL) increased linearly with advancing gestational age and CRL. NBL (mm)=[0.298xGestational Age (week)]-1.779, R^2=0.318; p<0.001; NBL (mm)= [0.023 x CRL (mm)] + 0.520, R^2=0.331; p<0.001

Conclusion: The present study presents normative values of nasal bone in the first trimester screening of normal singleton pregnancies of Turkish population. Nasal bone length increases with advancing gestational age and CRL. (J Turkish-German Gynecol Assoc 2011; 12: 225-8)

Key words: Screening, nasal bone, pregnancy, ultrasonography, fetus

Received: 11 August, 2011 **Accepted:** 26 September, 2011

Özet

Amaç: Birinci tirmester trizomi 21 taramasında nazal kemik değerlendirmesi performansı arttırmaktadır. Bu retrospektif çalışmada Türk popülasyonundaki tekil gebeliklerde birinci trimester fetal nazal kemik ölçümlerinin gebelik haftalarına göre persentil dağılımlarının saptanması amaçlandı.

Gereç ve Yöntemler: Retrospektif olarak 2004 ve 2010 tarihleri arasında taraması yapılmış olan anatomik anomali, biyokimyasal değer anormalliği, aile öyküsünde genetik hastalığı olmayan, nukal kalınlığı normal olan spontan tekil gebelikler değerlendirmeye alındı. 11^{0-6}, 12^{0-6} and 13^{0-6} gebelik haftaları için fetal nazal kemik ölçümlerinin ortlama±standart sapma, medyan ve persentil değerleri saptandı.

Bulgular: Dahil edilen 1762 tekil gebeliğin %99.6'sında nazal kemik görüntülenmişti; bunların %16.5'inde nazal kemik sadece var ya da yok olarak not edilmiştir. Ortalama anne yaşı 29.67±4.50 yıl ve ortalama gebelik haftası 12.54±0.61 idi. Nazal kemik ölçümlerinin medyan değerleri 11^{0-6}, 12^{0-6} ve 13^{0-6} gebelik haftaları için sırasıyla 1.7, 1.9, ve 2.2 mm olarak saptandı. Nazal kemik uzunluğu (NBL) ilerleyen gebelik haftası ve artan CRL ile artmaktaydı (NBL (mm)=[0.298xGebelik Haftası)] – 1.779, R^2=0.318; p<0.001 ve NBL (mm=[0.023xCRL (mm)]+0.520, R^2=0.331; p<0.001)

Sonuçlar: Mevcut çalışmada Türk popülasyonundaki tekil gebeliklerde birinci trimester fetal nazal kemik ölçümlerinin gebelik haftalarına göre persentil dağılımları gösterilmektedir. Nazal kemik uzunluğu ilerleyen gebelik haftası ve CRL ile orantılı olarak artmaktadır. (J Turkish-German Gynecol Assoc 2011; 12: 225-8)

Anahtar kelimeler: Tarama, nazal kemik, hamilelik, ultrason, fetus

Geliş Tarihi: 11 Ağustos 2011 **Kabul Tarihi:** 26 Eylül 2011

Introduction

The nasal bones, which begin to develop as collections of neural crest cells, can be histologically demonstrated when the fetal crown–rump length is 42 mm (10.9 weeks) (1). Absence of nasal bone ossification is one of the key skeletal features of trisomy 21 (2). Thus, determination of absence or presence of nasal bone is being used in the fetal sonographic screening for trisomy 21 (3-8). The evaluation of the nasal bone has been also shown to improve the performance of first-trimester screening for trisomy 21 (9). However, interobserver and intraobserver variability is a limitation for the measurement of length of the nasal bone and experience was shown to be important in the use of the nasal bones as an additional sonographic marker in first trimester screening (10-14).

Address for Correspondence: *Rahime Nida Ergin, Teşvikiye Cad. Bayer Apt. No: 63/11 Kat: 4 Nişantaşı, İstanbul, Turkey*
Phone: +90 505 373 71 33 e.mail: drnidaergin@gmail.com

doi:10.5152/jtgga.2011.56

In this retrospective study we aimed to determine normative values related to the measurement of nasal bone length of the Turkish population during the first trimester ultrasonographic fetal screening.

Materials and Methods

All medical records of singleton pregnancies, whose first trimester fetal screening was performed between January 2004 and December 2010, were evaluated retrospectively. Ultrasonographic fetal screening were performed by one of two sonographers using one of two ultrasound machines (Voluson 730 Pro (U.S.A) and Philips 4000 (U.S.A)). Pregnancies with any detected/suspicious anatomical or genetic fetal anomalies, biochemical abnormalities, increased nuchal translucency measurements, and pregnancies of artificial reproduction techniques were excluded from the data analyses. Ultrasonographic evaluation and measurement of fetal nasal bone had been performed mostly transabdominally (in case of inadequate fetal position transvaginally) in accordance with previously stated in the literature (5, 6).

Mean±standard deviation, median and percentile values of the length of nasal bone were calculated separately for 11^0-11^6, 12^0 -12^6, and 13^0-13^6 weeks of gestational age. The linear regression analyses were done between the length of nasal bone and gestational week and CRL. Statistical analyses were done with SPSS ver. 14.0.

Results

In accordance with the inclusion and exclusion criteria, data of 1762 singleton pregnancies were analyzed. The nasal bone could be visualized in 99.6% of these 1762 singleton pregnancies. In 16.5% of the cases nasal bones were only noted as present or absent. Therefore demographics and ranges of nasal bones were studied in 1465 fetuses. The mean maternal age was 29.67±4.50 years and the mean gestational age was 12.54±0.61 weeks. The mean and percentiles of ultrasonographic measurements of nasal bone according to the gestational weeks are shown in Table 1. The mean and percentiles of ultrasonographic measurements of nasal bone according to the measurements of CRL are shown in Table 2.

NBL increased linearly with advancing gestational age and was described by the following equation; NBL (mm)=[0.298 x Gestational Age (week)]-1.779, R^2=0.318; p<0.001. Again, a linear relationship was present between NBL and CRL and that was described by the following equation; NBL (mm)=[0.023xCRL (mm)]+0.520, R^2=0.331; p<0.001 (Figure 1 and Figure 2).

Discussion

Genetic sonography is an important tool in prenatal fetal evaluation. Evaluation of the nasal bone has been suggested to improve the performance of first-trimester screening for trisomy 21 (3-9). Experience has been shown to be an important factor for the use of the nasal bone as an additional sonographic marker in first trimester screening (10-14). In most of the previous studies determining the presence of nasal bone for screen-

Table 2. Nasal Bone Length Measurements (mm) according to CRL

	Percentiles		
CRL	5th	50th	95th
45-54 mm	1.3	1.7	2.1
55-64 mm	1.5	1.9	2.3
65-74 mm	1.6	2.1	2.6
75-84 mm	1.8	2.3	3

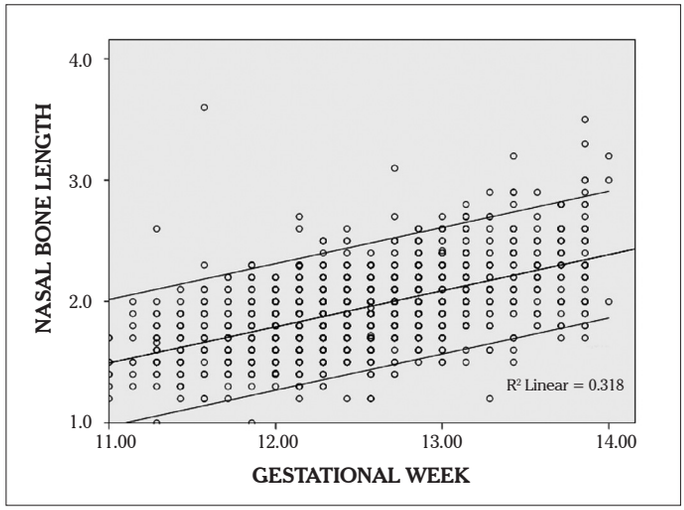


Figure 1. Nasal bone lengths according to gestational age (Lines indicating 95th, 50th, 5th percentiles above to bottom respectively)

Table 1. Nasal Bone Length Measurements (mm) according to gestational weeks

Gestational Age	Mean (mm)	Standard Deviation (mm)	Percentiles				
			5th	25th	50th (Median)	75th	95th
11^0-11^6 week (N=330)	1.73	0.27	1.30	1.50	1.70	1.90	2.15
12^0 -12^6 week (N=855)	1.95	0.26	1.50	1.80	1.90	2.10	2.40
13^0-13^6 week (N=280)	2.23	0.34	1.80	2.00	2.20	2.40	2.80

ing of trisomy 21 the ratio of successful examination varied between 83.2% to 100% (4, 8, 14-18). However, in these studies the determination ratio of trisomy 21 varied between 60% to 80% (4, 8, 14-18).

The ossification of the vomeral bone begins with two bilateral ossification centers before ossification of the nasal bone and then these two bilateral ossification centers fuse caudally below the cartilaginous nasal septum, changing into a U-shaped bone when observed in the coronal plane (1). The gap between these structures may sometimes be misinterpreted as absence of nasal bone (19).

In the literature, there are many studies indicating the normative values related to the length of nasal bone in different geographical parts of the world (12, 20-24). The median values of these nasal bone measurements vary from one study to another. In the present study, the sample size is larger and our results of nasal bone measurements were between the values of two other studies with a large sample size (20, 23). The values related to various previous studies indicating nasal bone measurements including the ones above mentioned are shown in Table 3. In all of these studies the reference values have different ranges. The examinations were commonly performed as transabdominal in the previous studies and as well our study (12, 20, 24). However, our mean NBL findings differ negatively at the 11th, 12th and 13rd gestational weeks from some of these studies (12, 22, 23) and positively from some others (19, 23). This difference might be due to ethnical difference (25) as well as interobserver and intraobserver variability in the measurement of length of the nasal bone (11, 13, 14). Variations may also be due to the quality of the machine as well, however it seems to be difficult to compare all these previous studies in this sense as most had different brand types of sonographic devices (12, 20-24). As a result, it seems to be impossible to clarify whether these differences are solely due to ethical difference, interobserver/intraobserver variability or systematic differences in these studies.

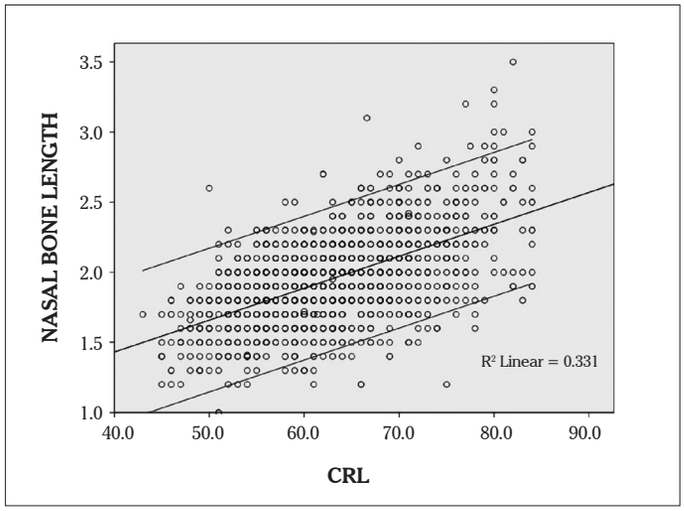


Figure 2. Nasal bone lengths according to CRL. (Lines indicating 95th, 50th, 5th percentiles above to bottom respectively)

The nasal bone length has been already found to increase linearly with advancing gestational week or CRL in the first trimester (13, 20, 22-24). In this study NBL of Turkish singleton pregnancies is also found to increase linearly with advancing gestational week and CRL in accordance with these previous studies in the literature.

Conclusion

This study presents normative values of nasal bone in the first trimester screening of normal singleton pregnancies of the Turkish population. In accordance with previous reports, nasal

Table 3. Different studies indicating nasal bone measurements in the first trimester of pregnancy

		Present Study	Casasbuena et al. (20)	Staboulidou et al. (21)	Chen et al. (22)	Sonek et al. (23)	Moon et al. (24)	Bekker et al. (12)
Gestational Age	Percentile	Turkey (N=1465)	Latin America (N=1040)	Germany (N=122)	China (N=2169)	USA + UK (N=3537)	Korea (N=982)	Netherlands (N=90)
11^{0-6} week	5	1,3	1	**	**	1.4	1,2	**
	50	1.7	1.5	1.73	**	2.3	1.5	2.3
	95	2.2	1.8	**	**	3.3	1.9	**
12^{0-6} week	5	1.5	1.2	**	1.7	1.7	1.4	**
	50	1.9	1.7	2.25	2.2	2.8	1.7	2.6
	95	2.4	2.2	**	2.8	4.2	2.1	**
13^{0-6} week	5	1.8	1.4	**	2.0	2.3	1.6	**
	50	2.2	1.9	**	2.5	3.1	1.9	2.9
	95	2.8	2.4	**	3.2	4.6	2.3	**
14^{0-6} week	5	**	**	**	2.2	2.5	1.7	**
	50	**	**	**	2.9	3.8	2.1	**
	95	**	**	**	3.5	5.3	2.6	**

**: data not given

bone length increases linearly with advancing gestational age and CRL. The values show variance similar to previous studies, which might be a consequence of ethnical difference or interobserver/intraobserver variability.

Acknowledgements
No financial or commercial interests from any drug company or others were involved and there is no relationship of authors that may pose a conflict of interest.

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

References

1. Sandikcioglu M, Molsted K, Kjaer I. The prenatal development of the human nasal and vomeral bones. J Craniofac Genet Dev Biol 1994; 14: 124-34.
2. Stempfle N, Huten Y, Fredouille C, Brisse H, Nessmann C. Skeletal abnormalities in fetuses with Down's syndrome: a radiographic post-mortem study. Pediatr Radiol 1999; 29: 682-8. [CrossRef]
3. Orlandi F, Bilardo CM, Campogrande M, Krantz D, Hallahan T, Rossi C, et al. Measurement of nasal bone length at 11-14 weeks of pregnancy and its potential role in Down syndrome risk assessment. Ultrasound Obstet Gynecol 2003; 22: 36-9. [CrossRef]
4. Zoppi MA, Ibba RM, Axiana C, Floris M, Manca F, Monni G. Absence of fetal nasal bone and aneuploidies at first-trimester nuchal translucency screening in unselected pregnancies. Prenat Diagn 2003; 23: 496-500. [CrossRef]
5. Cicero S, Curcio P, Papageorghiou A, Sonek J, Nicolaides K. Absence of nasal bone in fetuses with trisomy 21 at 11-14 weeks of gestation: an observational study. Lancet 2001; 358: 1665-7. [CrossRef]
6. Pandya PP, Snijders RJM, Johnson SP, De Lourdes Brizot M, Nicolaides KH. Screening for fetal trisomies by maternal age and fetal nuchal translucency thickness at 10 to 14 weeks' gestation. Br J Obstet Gynaecol 1995; 102: 957-62. [CrossRef]
7. Cicero S, Longo D, Rembouskos G, Sacchini C, Nicolaides KH. Absent nasal bone at 11-14 weeks of gestation and chromosomal defects. Ultrasound Obstet Gynecol 2003; 22: 31-5. [CrossRef]
8. Cicero S, Rembouskos G, Vandecruys H, Hogg M, Nicolaides KH. Likelihood ratio for trisomy 21 in fetuses with absent nasal bone at the 11-14-week scan. Ultrasound Obstet Gynecol 2004; 23: 218-23. [CrossRef]
9. Kagan KO, Cicero S, Staboulidou I, Wright D, Nicolaides KH. Fetal nasal bone in screening for trisomies 21, 18 and 13 and Turner syndrome at 11-13 weeks of gestation. Ultrasound Obstet Gynecol 2009; 33: 259-64. [CrossRef]
10. Cicero S, Dezerega V, Andrade E, Scheier M, Nicolaides KH. Learning curve for sonographic examination of the fetal nasal bone at 11-14 weeks. Ultrasound Obstet Gynecol 2003; 22: 135-7. [CrossRef]
11. Staboulidou I, Wüstemann M, Vaske B, Scharf A, Hillemanns P, Schmidt P. Interobserver variability of the measurement of fetal nasal bone length between 11+0 and 13+6 gestation weeks among experienced and inexperienced sonographers. Ultraschall Med 2009; 30: 42-6. [CrossRef]
12. Bekker MN, Twisk JW, van Vugt JM. Reproducibility of the fetal nasal bone length measurement. J Ultrasound Med 2004; 23: 1613-8.
13. Kanellopoulos V, Katsetos C, Economides DL. Examination of fetal nasal bone and repeatability of measurement in early pregnancy. Ultrasound Obstet Gynecol 2003; 22: 131-4. [CrossRef]
14. Senat MV, Bernard JP, Boulvain M, Ville Y. Intra- and interoperator variability in fetal nasal bone assessment at 11-14 weeks of gestation. Ultrasound Obstet Gynecol 2003; 22: 138-41. [CrossRef]
15. Otano L, Aiello H, Igarzabal L, Matayoshi T, Gadow EC. Association between first trimester absence of fetal nasal bone on ultrasound and Down's syndrome. Prenat Diagn 2002; 22: 930-2.
16. Viora E, Masturzo B, Errante G, Sciarrone A, Bastonero S, Campogrande M. Ultrasound evaluation of fetal nasal bone at 11 to 14 weeks in a consecutive series of 1906 fetuses. Prenat Diagn 2003; 23: 784-7. [CrossRef]
17. Wong SF, Choi H, Ho LC. Nasal bone hypoplasia: is it a common finding amongst chromosomally normal fetuses of southern Chinese women? Gynecol Obstet Invest 2003; 56: 99-101. [CrossRef]
18. Orlandi F, Rossi C, Orlandi E, Jakil MC, Hallahan TW, Macri VJ, et al. First-trimester screening for trisomy-21 using a simplified method to assess the presence or absence of the fetal nasal bone. Am J Obstet Gynecol 2005; 192: 1107-11. [CrossRef]
19. Peralta CF, Falcon O, Wegrzyn P, Faro C, Nicolaides KH. Assessment of the gap between the fetal nasal bones at 11 to 13 + 6 weeks of gestation by three-dimensional ultrasound. Ultrasound Obstet Gynecol 2005; 25: 464-7. [CrossRef]
20. Casasbuenas A, Wong AE, Sepulveda W. First-trimester nasal bone length in a normal Latin American population. Prenat Diagn 2009; 29: 108-12. [CrossRef]
21. Staboulidou I, Steinborn A, Schmidt P, Günter HH, Hillemanns P, Scharf A. References values for the fetal nasal bone in the first trimenon of pregnancy in a normal collective. A prospective study. Z Geburtshilfe Neonatol 2006; 210: 173-8. [CrossRef]
22. Chen M, Lee CP, Tang R, Chan B, Ou CQ, Tang MH. First-trimester examination of fetal nasal bone in the Chinese population. Prenat Diagn 2006; 26: 703-6. [CrossRef]
23. Sonek JD, McKenna D, Webb D, Croom C, Nicolaides K. Nasal bone length throughout gestation: normal ranges based on 3537 fetal ultrasound measurements. Ultrasound Obstet Gynecol 2003; 21: 152-5. [CrossRef]
24. Moon MH, Cho JY, Lee YM, Lee YH, Yang JH, Kim MY, et al. Nasal bone length at 11-14 weeks of pregnancy in the Korean population. Prenat Diagn 2006; 26: 524-7. [CrossRef]
25. Collado F, Bombard A, Li V, Julliard K, Aptekar L, Weiner Z. Ethnic variation of fetal nasal bone length between 11-14 weeks' gestation. Prenat Diagn 2005; 25: 690-2. [CrossRef]

Association of endothelial nitric oxide synthase gene polymorphisms with endometrial carcinoma: a preliminary study

Endometrium kanseri ile endotelyal nitrik oksit sentetaz gen polimorfizmi arasındaki ilişki: Pilot çalışma

Ebru Öztürk[1], Ebru Dikensoy[1], Özcan Balat[1], Mete Gürol Uğur[1], Sibel Oğuzkan Balcı[2], Abdullah Aydın[3], Ülkü Kazancı[3], Sacide Pehlivan[2]

[1]Department of Obstetrics and Gynecology, Faculty of Medicine, Gaziantep University, Gaziantep, Turkey
[2]Department of Medical Biology and Genetics, Faculty of Medicine, Gaziantep University, Gaziantep, Turkey
[3]Department of Pathology, Faculty of Medicine, Gaziantep University, Gaziantep, Turkey

Abstract

Objective: To investigate the relationship between specific endothelial nitric oxide synthase (eNOS) gene polymorphisms and endometrial cancer (ECa).

Material and Methods: The study group consisted of 89 patients histologically diagnosed with the endometrioid type of endometrial carcinoma. The control group consisted of 60 randomly selected individuals who had undergone total hysterectomy. Genomic DNA was isolated from paraffin-embedded endometrial tissues. We investigated the G894T polymorphisms (G894T) and variable number tandem repeats polymorphisms in intron 4 (VNTR intron 4) in the eNOS gene by using polymerase chain reaction (PCR) and/or restriction fragment length polymorphism (RFLP). The genotype distributions and allele frequencies of the two groups were compared.

Results: Analysis of the VNTR intron 4 polymorphisms in eNOS gene revealed that the frequency of the AA genotype was significantly higher in the control group, whereas the frequency of the BB genotype was significantly higher in the ECa group. Analysis of the G894T polymorphisms in eNOS gene revealed a significantly higher frequency of the GG genotype in the control group but a significantly higher frequency of the TT genotype in the endometrial cancer group.

Conclusion: The G894T and VNTR intron 4 polymorphisms in eNOS gene could be an intriguing susceptibility factor that modulates an individual's risk of ECa in the Turkish population.

(J Turkish-German Gynecol Assoc 2011; 12: 229-33)

Key words: eNOS gene polymorphisms, endometrial carcinoma

Received: 21 August, 2011 **Accepted:** 11 September, 2011

Özet

Amaç: Endometrium kanseri ile Endotelyal Nitrik Oksit Sentetaz (eNOS) gen polimorfizmi arasındaki ilişkinin ortaya konulması.

Gereç ve Yöntemler: Araştırmaya endometrioid tip endometrial karsinom tanısı almış 89 hasta çalışma grubu olarak, diğer benign sebeplerle total histerektomi uygulanmış 60 hasta kontrol grubu olarak dahil edilmiştir. Genomik DNA endometrial dokuların parafin bloklarından elde edilmiştir. Bu çalışmada eNOS geninin G894T polimorfizmleri ve intron 4'deki değişken tekrarlayan dizi polimorfizmleri (VNTR intron 4) polimeraz zincir reaksiyonu (PCR) ve/veya enzim kesimi (RFLP) ile değerlendirilmiştir.

Bulgular: eNOS geninin VNTR intron 4 gen polimorfizmi değerlendirildiğinde AA genotip sıklığı kontrol grubunda istatistiksel olarak anlamlı ölçüde yüksek saptanırken, BB genotip sıklığı endometrial kanser grubunda yüksek olarak izlenmiştir. eNOS geninin G894T polimorfizminde ise GG genotip sıklığı kontrol grubunda yüksek izlenirken TT genotip sıklığı hasta grubunda istatistiksel olarak anlamlı ölçüde yüksek olarak saptanmıştır.

Sonuç: Türk toplumunda eNOS geninin G894T ve VNTR intron 4 polimorfizmleri endometrium kanseri için risk grubunu belirleyebilecek kişisel faktörlerden biri olabilir.

(J Turkish-German Gynecol Assoc 2011; 12: 229-33)

Anahtar kelimeler: eNOS gen polimorfizmleri, endometrium kanseri

Geliş Tarihi: 21 Ağustos 2011 **Kabul Tarihi:** 11 Eylül 2011

Introduction

Endometrial cancer (ECa) is the most common pelvic malignancy. Each year, ECa develops in about 142,000 women worldwide and approximately 42,000 women die because of ECa (1). The majority of these tumors are of the endometrioid type that are typically hormone sensitive and have an excellent prognosis. Although hormonal and genetic association studies have been performed, the pathophysiology of ECa is still unclear (2-4).

Nitric oxide synthase (NOS), which has three isoforms, catalyzes the oxidation of L-arginine to nitric oxide (NO) and citrulline. Endothelial nitric oxide synthase (eNOS) is one of three isoforms of NOS that generates NO in vascular endo-

Address for Correspondence: *Ebru Öztürk, Department of Obstetrics and Gynecology, Faculty of Medicine, Gaziantep University, Longer Road Kilis, Şahinbey Application and Research Hospital, 27310, Şahinbey, Gaziantep, Turkey Phone: +90 533 344 17 02 e.mail: ebruozturkarslan@yahoo.com*

doi:10.5152/jtgga.2011.47

thelium and is encoded by the eNOS gene which was localized to chromosome 7q35-36 (5). The eNOS gene have about 1,500 base pairs of upstream promoter sequence similar to other NOS and include transcription factor-binding sites that mediate regulation by estrogens, shear stress and other cofactors (6).

Under normal physiological conditions, constitutively expressed NO is a very important intercellular messenger molecule. However, high concentrations of metabolic products of NO have been implicated in mutagenesis and carcinogenesis (7). Potentially cytotoxic oxygen and nitrogen metabolites of NO may directly damage DNA bases, resulting in point mutations, strand breaks and interactions with sulfhydryl groups, leading to carcinogenesis. Some of these metabolites can react with secondary amines and N-alkylamides to form nitroamines, which have been implicated in human carcinogenesis (8). Despite these negative effects on carcinogenesis, endothelial production of nitric oxide regulates blood flow and angiogenesis and reduces tumor cell adhesion to the endothelium and so positively affects tumor pathogenesis (9).

Recently, association studies on eNOS gene polymorphisms with different types of cancer including vulvar, prostate, colorectal and breast cancer have been performed (10-13).

Based on these data, the present study aimed to determine the relationship between eNOS gene polymorphisms and ECa. Although studies on Single Nucleotide Polymorphisms (SNPs) involved in DNA damage repair, steroid metabolism, carcinogen metabolism, cell-cycle control, apoptosis and steroid receptor activation pathways in ECa were performed, this is the first study in the English literature (14-18).

Although extensively different eNOS gene variants have been demonstrated, functional variation in the eNOS gene has yet to be completely characterized (19). This study has focused on two functional variants: a variant G to T conversion at nucleotide position 894 resulting in the replacement of glutamic acid with aspartic acid at codon 298 (G894T) and a variant variable number of 27 bp tandem repeats in intron 4 (VNTR intron 4) in a Turkish population.

Materials and Methods

Patients and Controls

The study was designed as a retrospective study. Formalin-fixed (10% neutral buffered formalin), paraffin-embedded surgical materials obtained between the years 2000 and 2010 were selected from the archives of the Department of Pathology of Gaziantep University Faculty of Medicine. The study group consisted of 89 patients histologically diagnosed with the endometrioid type of ECa. The control group consisted of 60 randomly selected individuals who had undergone total hysterectomy because of postmenopausal benign adnexal pathologies, dysfunctional uterine bleeding or myoma uteri at Gaziantep University. Hematoxylin and eosin-stained sections from each case were reviewed and representative sections for each case were selected. Genomic DNA was isolated from paraffin-embedded endometrial tissues. This study was approved by the local ethics committee of Gaziantep University.

The patients were diagnosed with ECa by fractional endometrial biopsy. Thereafter, total abdominal hysterectomy, bilateral salpingoophorectomy, bilateral pelvic and para-aortic lymphadenectomy, omentectomy, and peritoneal fluid sampling were performed in all ECa patients. Histopathological diagnosis and surgical staging were established according to the International Federation of Gynecology and Obstetrics (FIGO) criteria (20). All patients were operated by the same team of surgeons and all slides were reviewed by the same pathologist.

Genotyping

We investigated the following two polymorphisms of the eNOS gene: the G894T polymorphisms; and the VNTR polymorphisms in intron 4. Genomic DNA was isolated from paraffin-embedded endometrial tissues (21). In order to analyze the G894T polymorphisms, polymerase chain reaction (PCR) was used to amplify a 206-bp fragment. The resulting fragment was digested with MboI restriction endonuclease (Invitrogen CA, USA) overnight at 37°C. Digestion was resolved on a 3% agarose gel and visualized under ultraviolet light. For the analysis of the VNTR polymorphisms in intron 4, primers were designed to amplify a 393-bp and/or 420-bp segment of the polymorphic VNTR region containing the microsatellite repeat sequence. The products were then separated on 3% agarose gel (Figures 1, 2) (21, 22).

Statistical analysis

Statistical Package for the Social Sciences (SPSS) for Windows (version 9.0; SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Results were expressed as mean±standard deviation. Analysis of data regarding age was performed using the Student's t-test. A chi-square test was used to compare the two groups with

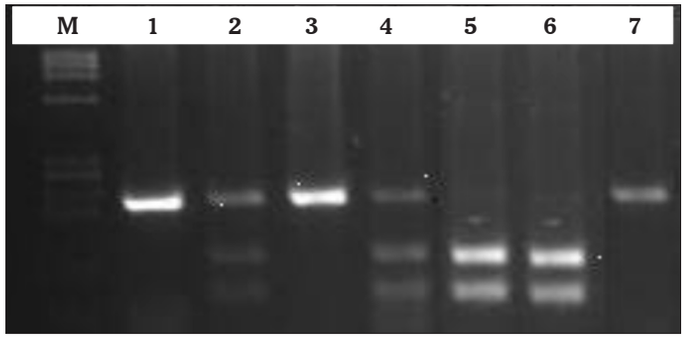


Figure 1. The G894T polymorphism. M: Marker, 1, 3, 7: The GG genotype, 2, 4: The GT genotype, 5, 6: The TT genotype

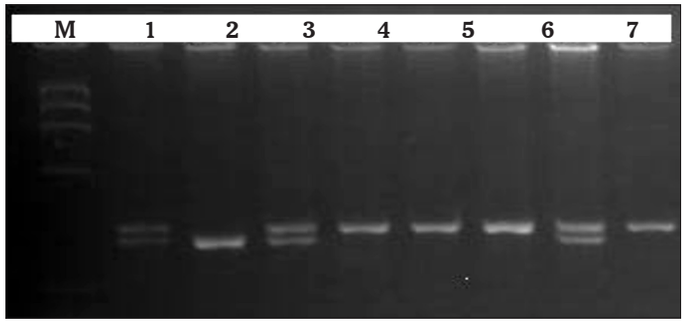


Figure 2. The variable number of tandem repeats (VNTR) polymorphism in intron 4. M: Marker, 4, 5, 6, 8: The AA genotype, 1, 3, 7: The AB genotype, 2: The BB genotype (27-kb tandem repeats)

respect to the genotype distributions and allele frequencies. The Hardy-Weinberg equilibrium (HWE) was calculated using the Finetti programme provided as an online source (23). Analysis of variance (ANOVA) and Kruskal-Wallis test were performed to compare genotypes of VNTR intron 4 or G894T polymorphisms in eNOS gene according to age and histopathological grade. A p value of <0.05 was considered statistically significant.

Results

The mean age in the ECa and control groups was 62.4±1.2 and 64±0.3, respectively. There were no significant differences between the ECa and control groups with respect to age (p=0.89).

In the patient group; of 89 patients, 74 (83.1%) were stage I, 7 (7.86%) were stage II, and 8 (10.0%) were stage III cancer patients. Of all patients, 39 (43.8%) had grade 1 (well-differentiated), 30 (37.8%) had grade 2 (moderately differentiated), and 20 (22.4%) had grade 3 (poorly differentiated) tumors.

The distribution of genotypes and allele frequencies are shown in Table 1 and Table 2. Regarding the VNTR intron 4 polymorphisms, while there was no deviation from HWE in control and patient groups, a significant difference existed between the two groups with respect to the genotype distribution. Comparison of the two groups revealed that the frequency of the AA genotype was significantly higher in the control group, while the frequency of the BB genotype was significantly higher in the ECa group (p=0.015 and p=0.015, respectively).

Regarding the G894T polymorphisms, there was a significant difference between the two groups with respect to the genotype distribution. There was no deviation from HWE in patient and control groups. Comparison of the two groups revealed a higher frequency of the GG genotype in the control group, whereas there was a significantly higher frequency of the TT genotype in the ECa group (p=0.003 and p=0.003, respectively).

No correlation was observed between patient age or histopatholgical grade and genotypes for the VNTR intron 4 polymorphisms (p=0.991, p=0.719) and for the G894T polymorphisms (p=0.560, p=0.178).

Discussion

Although ECa is the most common gynecologic cancer worldwide and numerous studies have been performed on risk factors or prognostic factors, the underlying carcinogenic mechanisms remain unknown (24, 25). Unopposed and prolonged estrogen stimulation, including stimulation by nulliparity, late menopause, and obesity, has been identified as a risk factor for the development of ECa. Estrogen acts in two different ways in the carcinogenesis of ECa; as a hormone that stimulates cell proliferation and as a procarcinogen that induces genetic damage by the action of free radicals. Cellular and molecular vascular studies demonstrated the estrogen-induced rapid, membrane-initiated activation of numerous signal transduction cascades (26). These effects include estrogen-stimulated, rapid activation of eNOS, resulting in

Table 1. Comparison of eNOS/VNTR intron 4 and eNOS/G894T gene polymorphisms genotype frequencies between patients with endometrial cancer and healthy controls

	Genotypes	Endometrial cancer		Control		p
		n^a	(%)	n^b	(%)	
eNOS/ VNTR intron 4	AA	48	(53.9)	43	(71.7)	0.015
	AB	31	(34.8)	16	(26.7)	0.101
	BB	10	(11.3)	1	(1.6)	0.015
HWE p		0.162		0.723		
eNOS/ G894T	GG	47	(52.8)	42	(70)	0.003
	GT	31	(34.8)	18	(30)	0.235
	TT	11	(12.4)	0	(0)	0.003
HWE p		0.114		(0.171)		

n^a=89, n^b=60, *:OR (95% CI) was adjusted by age, &Fisher's Exact Test, HWE: Hardy-Weinberg Equilibrium

Table 2. Comparison of eNOS/VNTR intron 4 and eNOS/G894T gene polymorphisms allele frequencies between patients with endometrial cancer and healthy controls

	Alleles	Endometrial cancer		Control		p
		n^a	(%)	n^b	(%)	
eNOS/ VNTR intron 4	A	127	(71.3)	102	(85)	0.006
	B	51	(28.7)	18	(15)	
eNOS/ G894T	G	125	(70.2)	102	(85)	0.003
	T	53	(29.8)	18	(15)	

n^a=178, n^b=120, *:OR (95% CI) was adjusted by age, &Fisher's Exact Test

production of NO which has implications in carcinogenesis, tumour progression, invasion, angiogenesis and modulation of therapeutic responses (27, 28).

In the literature, several SNPs of the eNOS gene have been reported in various cancers with increased risk of developing malignancy (11, 29) and prognosis after developing malignancy (30, 31); however, there is no study in the literature investigating the relationship of SNPs of the eNOS gene with ECa.

Recently, Hao et al. performed a meta-analysis about G894T polymorphisms of eNOS gene in breast cancer which has similar risk factors such as nulliparity, late menopause, and obesity to ECa (11). They concluded that there was a significant association between the eNOS polymorphism and the risk of breast cancer. The result of our study on G894T polymorphisms of eNOS gene in ECa suggest that, while the homozygote T variant of G894T polymorphisms in eNOS could be a predisposing factor for ECa, the homozygote G variant could be a protective factor for ECa in Turkish population.

In the literature, only a few case-control studies have been performed to evaluate the role of intron 4 polymorphisms in cancer. Although VNTR intron 4/eNOS variant is less likely to be functional, recently Yeh et al. demonstrated the association between VNTR intron 4/eNOS polymorphisms and early-onset colorectal cancer (13). In this study, we observed the association between VNTR intron 4/eNOS polymorphisms and ECa.

To the best of our knowledge, this is the first report of a relationship between eNOS gene polymorphisms and ECa.

In conclusion, Glu298Asp and VNTR intron 4 polymorphisms in the eNOS gene could be an intriguing factor that modulates an individual's risk of ECa in Turkish population. However, further studies with a large number of patients to clarify the association between eNOS gene polymorphisms and risk of developing ECa and to evaluate the relationship between prognosis in ECa and eNOS gene polymorphisms are needed.

Conflict of interest

No conflict of interest was declared by the authors.

References

1. Amant F, Moerman P, Neven P, Timmerman D, Van Limbergen E, Vergote I. Endometrial cancer. Lancet 2005; 366: 491-505. [CrossRef]
2. Meydanlı M, Karadag N, Kose F, Tulunay G, Ozfuttu A. Body Mass Index is Associated with Immunohistochemical Nuclear Phosphatase and Tensin Homolog Deleted on Chromosome 10 (PTEN) Expression in Stage IB-IC Endometrioid Endometrial Carcinoma. J Turkish-German Gynecol Assoc 2007; 8: 408-14.
3. Ozler A, Kuscu NK, Temiz P, Kandiloglu AR, Koyuncu FM. Leptin expression in proliferative, secretory and hyperplastic endometrial tissues. J Turkish-German Gynecol Assoc 2011; 12: 157-61. [CrossRef]
4. Ozturk HB, Vural B, Caliskan E, Solakoglu S. Effect of GnRH analogues and octreotide treatment on apoptosis and the cell proliferation of endometrium adenocarcinoma cell lines. J Turkish-German Gynecol Assoc 2010; 11: 131-6. [CrossRef]
5. Marsden PA, Heng HH, Scherer SW, Stewart RJ, Hall AV, Shi XM, et al. Structure and chromosomal localization of the human constitutive endothelial nitric oxide synthase gene. J Biol Chem 1993; 268: 17478-88.
6. Hingorani AD. Polymorphisms in endothelial nitric oxide synthase and atherogenesis: John French Lecture 2000. Atherosclerosis 2001; 154: 521-7. [CrossRef]
7. Mirvish SS. Role of N-nitroso compounds (NOC) and N-nitrosation in etiology of gastric, esophageal, nasopharyngeal and bladder cancer and contribution to cancer of known exposures to NOC. Cancer Lett 1995; 93: 17-48. [CrossRef]
8. Bentz BG, Barnes MN, Haines GK, Lurain JR, Hanson DG, Radosevich JA. Cytoplasmic localization of endothelial constitutive nitric oxide synthase in endometrial carcinomas. Tumour Biol 1997; 18: 290-300. [CrossRef]
9. Charles IG, Scorer CA, Moro MA, Fernàndez C, Chubb A, Dawson J, et al. Expression of human nitric oxide synthase isozymes. Methods Enzymol 1996; 268: 449-60. [CrossRef]
10. Riener EK, Hefler LA, Grimm C, Galid A, Zeillinger R, Tong-Cacsire D, et al. Polymorphisms of the endothelial nitric oxide synthase gene in women with vulvar cancer. Gynecol Oncol 2004; 93: 686-90. [CrossRef]
11. Hao Y, Montiel R, Huang Y. Endothelial nitric oxide synthase (eNOS) 894 G>T polymorphism is associated with breast cancer risk: a meta-analysis. Breast Cancer Res Treat 2010; 124: 809-13. [CrossRef]
12. Lee KM, Kang D, Park SK, Berndt SI, Reding D, Chatterjee N, et al. Nitric oxide synthase gene polymorphisms and prostate cancer risk. Carcinogenesis 2009; 30: 621-5. [CrossRef]
13. Yeh CC, Santella RM, Hsieh LL, Sung FC, Tang R. An intron 4 VNTR polymorphism of the endothelial nitric oxide synthase gene is associated with early-onset colorectal cancer. Int J Cancer 2009; 124: 1565-71. [CrossRef]
14. Meyer LA, Westin SN, Lu KH, Milam MR. Genetic polymorphisms and endometrial cancer risk. Expert Rev Anticancer Ther 2008; 8: 1159-67. [CrossRef]
15. Esinler I, Aktas D, Alikasifoglu M, Tuncbilek E, Ayhan A. CYP1A1 gene polymorphism and risk of endometrial hyperplasia and endometrial carcinoma. Int J Gynecol Cancer 2006; 16: 1407-11. [CrossRef]
16. Junqueira MG, da Silva ID, Nogueira-de-Souza NC, Carvalho CV, Leite DB, Gomes MT, et al. Progesterone receptor (PROGINS) polymorphism and the risk of endometrial cancer development. Int J Gynecol Cancer 2007; 17: 229-32. [CrossRef]
17. Xu L, Hu Y, Chen B, Tang W, Han X, Yu H, et al. Mitochondrial polymorphisms as risk factors for endometrial cancer in southwest China. Int J Gynecol Cancer 2006; 16: 1661-7. [CrossRef]
18. Saffari B, Bernstein L, Hong DC, Sullivan-Halley J, Runnebaum IB, Grill HJ, et al. Association of p53 mutations and a codon 72 single nucleotide polymorphism with lower overall survival and responsiveness to adjuvant radiotherapy in endometrioid endometrial carcinomas. Int J Gynecol Cancer 2005; 15: 952-63. [CrossRef]
19. Casas JP, Cavalleri GL, Bautista LE, Smeeth L, Humphries SE, Hingorani AD. Endothelial nitric oxide synthase gene polymorphisms and cardiovascular disease: a HuGE review. Am J Epidemiol 2006; 164: 921-35. [CrossRef]
20. Mikuta JJ. International Federation of Gynecologic and Obstetrics staging of endometrial cancer. Cancer 1988; 71: 1460-3. [CrossRef]
21. Erciyas K, Pehlivan S, Sever T, Igci M, Pehlivan M, Arslan A, et al. Endothelial nitric oxide synthase gene polymorphisms associated with periodontal diseases in Turkish adults. African J Biotechnology 2010; 9: 3042-7.
22. Erciyas K, Pehlivan S, Sever T, et al. Endothelial nitric oxide synthase gene polymorphisms associated with periodontal diseases in Turkish adults. African J Biotechnology 2010; 9: 3042-7.
23. Mendell NR, Simon GA. A general expression for the variance-covariance matrix of estimates of gene frequency: the effects of departures from Hardy-Weinberg equilibrium. Ann Hum Genet 1984; 48: 283-6. [CrossRef]
24. Turan AT, Dundar B, Gundogdu B, Boztosun A, Özgül N, Boran N, et al. The effect of cell type on surgico-pathologic risk factors in

endometrial cancer. J Turkish-German Gynecol Assoc 2011; 12: 9-14. [CrossRef]
25. Turan T, Ortac F, Güngör M, Heper A, Ensari A. Angiogenesis is not a prognostic marker in endometrial carcinoma. J Turkish-German Gynecol Assoc 2000; 1: 13-6.
26. Emons G, Fleckenstein G, Hinney B, Huschmand A, Heyl W. Hormonal interactions in endometrial cancer. Endocr Relat Cancer 2000; 7: 227-42. [CrossRef]
27. Duckles SP, Miller VM. Hormonal modulation of endothelial NO production. Pflugers Arch 2010; 459: 841-51. [CrossRef]
28. Singh S, Gupta AK. Nitric oxide: role in tumour biology and iNOS/NO-based anticancer therapies. Cancer Chemother Pharmacol 2011; 67: 1211-24. [CrossRef]
29. Cheon KT, Choi KH, Lee HB, Park SK, Rhee YK, Lee YC. Gene polymorphisms of endothelial nitric oxide synthase and angiotensin-converting enzyme in patients with lung cancer. Lung 2000; 178: 351-60. [CrossRef]
30. Ghilardi G, Biondi ML, Cecchini F, DeMonti M, Guagnellini E, Scorza R. Vascular invasion in human breast cancer is correlated to T-786C polymorphism of NOS3 gene. Nitric Oxide 2003; 9: 118-22. [CrossRef]
31. Mozet C, Marin DG, Bertolini J, Tannapfel A, Wichmann G, Dietz A. Nitric oxide synthase (NOS2/3) expression in head and neck squamous cell carcinomas in correlation with clinical patterns. Onkologie 2009; 32: 655-60.[CrossRef]

Nitric oxide levels and endothelial nitric oxide synthase gene polymorphisms in Turkish women with idiopathic recurrent miscarriage

Açıklanamayan tekrarlayan gebelik kaybı izlenen Türk kadınlarında endotelyal nitrik oksit sentetaz gen polimorfizmleri ve serum nitrik oksit seviyeleri

Ebru Öztürk[1], Özcan Balat[1], Sacide Pehlivan[2], Mete Gürol Uğur[1], Yelda Özkan[1], Tuğçe Sever[2], Emine Namıduru[3], Seval Kul[4]

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

[2]Department of Medical Biology and Genetics, Faculty of Medicine, Gaziantep University, Gaziantep, Turkey

[3]Department of Biochemistry, Faculty of Medicine, Gaziantep University, Gaziantep, Turkey

[4]Department of Biostatistics, Faculty of Medicine, Gaziantep University, Gaziantep, Turkey

Abstract

Objective: To determine whether endothelial nitric oxide synthase (eNOS) gene polymorphisms are associated with an increased risk for Idiopathic Recurrent Miscarriage (IRM) in the Turkish population and to evaluate the association between Nitric Oxide (NO) levels and eNOS gene polymorphisms in women with IRM.

Material and Methods: A total of 120 Turkish women were enrolled in this study in four groups. Of these, 30 women were first trimester pregnant who had IRM (Group I). Thirty healthy multipara women were in the first trimester of pregnancy with no history of abortion (Group II). Thirty women were non pregnant with a history of IRM (Group III). The remaining 30 subjects were healthy multipara non-pregnant women with no history of abortion (Group IV). DNA analysis of four groups were performed for the two polymorphisms using the PCR and/or PCR-RFLPs method and NO levels were measured spectrophotometrically.

Results: We observed statistically significant decreased NO levels in the pregnant patient group (p=0.001) while elevated NO levels were measured in the non pregnant patient group (p=0.004). We demonstrated that,while there was no significant difference in terms of VNTR 4/eNOS genotype, there was a marginally significant difference in terms of Glu298Asp/eNOS genotype frequency (p=0.055) in patients with IRM in the Turkish population. We observed no association between NO levels and Glu298Asp/eNOS or VNTR 4/eNOS genotypes in any of the groups.

Conclusion: The Glu298Asp polymorphism of eNOS could be an intriguing susceptibility factor that modulates an individual's risk of IRM in Turkish population. Further studies to explain the role of the NO pathway in the pathophysiology of IRM are needed.

(J Turkish-German Gynecol Assoc 2011; 12: 234-8)

Key words: Idiopathic recurrent miscarriage, nitric oxide, gene polymorphisms

Received: 28 August, 2011 **Accepted:** 11 September, 2011

Available Online Date: 28 September, 2011

Özet

Amaç: Türk toplumunda Endotelyal Nitrik Oksit Sentetaz (eNOS) gen polimorfizmlerinin açıklanamayan tekrarlayan gebelik kayıpları (ATGK) için bir risk faktörü olup olmadığının değerlendirilmesi, ATGK izlenen kadınlarda nitrik oksid (NO) seviyeleri ile eNOS gen polimorfizmleri arasındaki ilişkinin ortaya konulması.

Gereç ve Yöntemler: Bu çalışmaya 120 Türk kadını dört grup halinde dahil edilmiştir. Bu kadınlardan 30'u ATGK izlenen ilk trimester gebeliği bulunan kadın (Grup I), 30'u hiç abortusu bulunmayan, ilk trimester gebeliği bulunan multipar kadın (Grup II), 30'u ATGK hikayesi bulunan gebe olmayan kadın, geri kalan 30'u ise hiç abortus hikayesi bulunmayan sağlıklı multipar kadın (Grup IV) dır. Dört grubun iki polimorfizminin analizi PCR ve/veya PCR-RFLP metodları kullanılarak gerçekleştirilmiş ve NO seviyeleri spektrofotometrik olarak ölçülmüştür.

Bulgular: Gebe hasta grubunda NO seviyeleri istatistiksel olarak anlamlı ölçüde azalmış olarak izlenirken, gebe olmayan hasta grubunda NO seviyeleri anlamlı ölçüde artmış olarak izlenmiştir. Hasta ve kontrol grupları arasında VNTR 4/eNOS genotipi açısından fark saptanmazken, Glu298Asp/eNOS genotip sıklığı açısndan istatistiksel olarak sınırda anlamlı ölçüde bir fark saptanmıştır. Tüm gruplarda NO seviyeleri ile Glu298Asp/eNOS veya VNTR 4/eNOS genotipleri arasında bir korelasyon izlenmemiştir.

Sonuç: Türk toplumunda ATGK izlenen gebelerde eNOS geninin Glu298Asp polimorfizmleri kişisel bir risk faktörü olabilir. ATGK patofizyolojisinde NO yolaklarının yerinin ortaya konulabilmesi için yeni çalışmalara ihtiyaç vardır.

(J Turkish-German Gynecol Assoc 2011; 12: 234-8)

Anahtar kelimeler: Açıklanamayan tekrarlayan gebelik kayıpları, nitrik oksit, gen polimorfizmleri

Geliş Tarihi: 28 Ağustos 2011 **Kabul Tarihi:** 11 Eylül 2011

Çevrimiçi Yayın Tarihi: 28 Eylül 2011

Address for Correspondence: *Ebru Öztürk, Department of Obstetrics and Gynecology, Faculty of Medicine, Gaziantep University, Longer Road Kilis, Şahinbey Application and Research Hospital, 27310, Şahinbey, Gaziantep, Turkey Phone: +90 533 344 17 02 e.mail: ebruozturkarslan@yahoo.com*

doi:10.5152/jtgga.2011.48

Introduction

Recurrent miscarriage (RM), which is defined as 3 or more consecutive pregnancy losses before 20 weeks of gestation, affects 0.5%-2% of women in the reproductive age group (1). Although chromosomal and uterine abnormalities, thyroid dysfunction, coagulation and immunological alterations cause RM, 50%-60% of recurrent pregnancy losses are idiopathic (2).

Nitric oxide (NO), which is synthesized from L-arginine in endothelial cells by endothelial nitric oxide synthase (eNOS), provides a tonic dilator tone and regulates the adhesion of white blood cells and platelet aggregation. The NO pathway activation has been clearly demonstrated during normal pregnancy (3). It has been shown that, in the early stage of pregnancy, trophoblasts express high amounts of eNOS activity and placental production of gonadotrophic hormone is modulated by eNOS expressions (4). In an animal model, it was demonstrated that placental NO production mediated lipopolysaccharide induced abortion (5).

In the literature there are some reports on the association between eNOS gene polymorphisms and IRM. However, the results of these studies have been controversial among different ethnic groups (6-11).

In this study we aimed to determine whether eNOS gene polymorphisms are associated with an increased risk for IRM in the Turkish population and to evaluate the association between NO levels and eNOS gene polymorphisms in women with IRM. This study has focused on two functional variants of eNOS gene: a variant G to T conversion at nucleotide position 894 resulting in the replacement of glutamic acid with aspartic acid at codon 298 (Glu298Asp) and a variant variable number of 27 bp tandem repeats in intron 4 (VNTR intron 4).

Material and Methods

Subjects

The protocol of this prospective controlled study was approved by the Ethics Committee for Clinical Research of Gaziantep University. Subjects were selected among women attending the Obstetrics and Gynecology Department of Gaziantep University. One hundred twenty Turkish women living in southeastern Anatolia were enrolled in this study in four groups. Because of increasing of NO production in pregnancy, NO levels of patients were compared with pregnant and non pregnant control groups which were Group II and Group IV respectively.

Group I consisted of 30 pregnant women in the first trimester with a history of IRM, and Group II consisted of 30 multipara pregnant women in the first trimester with no history of abortion. Group III consisted of 30 non-pregnant women with a history of IRM, Group IV consisted of 30 multipara non-pregnant women with no history of abortion.

IRM was defined after all the known causes were excluded by routinely performed investigations, including hysterosalpingography or sonohysterography, karyotype analysis, measurement of anticardiolipin antibody (IgG and IgM), lupus anticoagulant and thyroid stimulating hormone. Women with multiple pregnancy, or any concurrent medical complications before or developing during pregnancy, such as diabetes mellitus or inflammatory diseases, were excluded from the study. Smoking and alcohol or any drug use were also considered exclusion criteria.

Genotyping Procedure

DNA isolation

Genomic DNA was extracted from EDTA-treated peripheral venous blood using the salting-out method (13).

eNOS/Glu298Asp genotyping

A polymerase chain reaction (PCR) was used to amplify a 206 bp fragment. The resulting fragment was digested with MboI restriction endonuclease (Invitrogen,Carlsbad, CA, USA) overnight at 37°C. Digestion was resolved on 3% agarose gel and visualized using ultraviolet light. The 206 bp PCR products had a consistent restriction site resulting in a 119 bp and an 87 bp fragment. Twenty percent of the samples were duplicated as an internal quality control to avoid sampling or reading errors (14).

eNOS /VNTR intron 4 genotyping

Primers were designed to amplify a 393 bp and/or 420 bp segment of the eNOS intron 4 VNTR region containing the microsatellite repeat sequence (14). The products were then separated on 4% NuSieve GTG agarose. The experimental process was repeated twice for each sample.

Measurement of NO Levels

NO released by the cells was measured in the plasma as nitrite/nitrate, by the Griess reaction, after incubation of plasma samples with copperized cadmium (Cd) granules to convert NO_3 to NO_2. Griess reagent (1 ml of 1% sulfanilamide, 0.1% naphtylethylenediamine hydrochloride and 2.5% phosphoric acid; Sigma Chemical Co.) was then added to 1 ml of supernatant (12). The absorbance was read at 545 nm after 30 min of incubation. Standard curves were prepared with known concentrations (1–100 μmol/L) of sodium nitrite. The results were given in μmol/L.

Statistical Analysis

Data were analyzed using SPSS for Windows version 13.0; (SPSS, Chicago, IL, USA). Statistical significance of the differences between the patient and control groups was estimated by logistic regression analysis. The Hardy-Weinberg equilibrium (HWE) was used to calculate estimated genotype frequency and experienced genotype frequency. A p value less than 0.05 was considered statistically significant.

Results

There was no significant difference between the groups studied in terms of maternal age and BMI. There was no significant difference between Group I and Group II in terms of gestational age.

Genotype frequency of eNOS Glu298Asp and VNTR intron 4 polymorphisms of patients who had IRM (Group I+III) and controls who had no abortion (Group II+IV) are presented in Table 2. There was no deviation from HWE between the patient

and control groups in terms of Glu298Asp and VNTR Intron 4 in eNOS (Table 1). While there was no significant difference in patients with IRM in terms of eNOS/VNTR intron 4 genotype frequency, a marginally significant difference was determined in patients with IRM in terms of eNOS/Glu298Asp genotype frequency (p=0.055). G/G homozygotes of Glu298Asp among IRM groups were marginally significantly frequent (Table 1).
NO levels were significantly different between Group I and Group II (p=0.001); Group III and Group IV (p=0.004) (Figure 1).
There was no association between NO levels and Glu298Asp/eNOS or VNTR 4/eNOS genotypes in any of the groups (Table 2).

Discussion

Despite extensive researches to explain the causative effects of RM, about 50%-60% of recurrent pregnancy losses are still idiopathic (15-17). Endothelial damage, impaired placental vascularization and resultant oxidative stress have been proposed to play a role in the pathophysiology of IRM (2). eNOS has been regarded as the source of endothelial NO, which has a critical role in vascular physiology and impaired placental vascularization.
Even though extensively different eNOS gene variants have been demonstrated, functional variation in the eNOS gene has yet to be completely characterized (18). This study has focused on two functional variants: Glu298Asp and VNTR intron 4 polymorphisms of eNOS gene. This is the first study to evaluate the association between eNOS polymorphisms with IRM in a Turkish population.

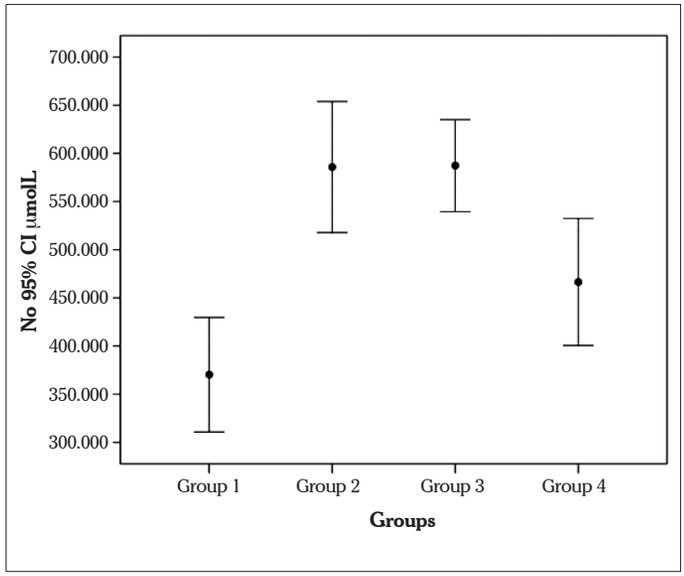


Figure 1. Comparison of Nitric Oxide Levels between patients with Idiopathic Recurrent Miscarriage (IRM) and healthy controls

Table 1. Comparison of eNOS/VNTR intron 4 and eNOS/Glu298Asp gene polymorphisms frequencies between patients with Idiopathic Recurrent Miscarriage (IRM) and healthy controls

	Genotypes	IRM (n=60)	Control (n=60)	OR*	95% CI	P Value
Glu298Asp n (%)	GG (reference)	42 (70.0)	28 (46.7)			
	GT+TT	18 (30.0)	32 (36.7)	2.743	0.980-7.675	0.055*
HWE p		0.287	0.314			
VNTR Intron 4 n (%)	AA	38 (63.3)	54 (79.4)	0.469	0.071-3.084	0.431
	AB	16 (26.7)	10 (14.7)	0.469	0.071-3.084	0.952
	BB (reference)		6 (10.0)	4 (5. 9)		
HWE p		0.309	0.272			

HWE: Hardy-Weinberg Equilibrium, * marginally significant

Table 2. Correlation of eNOS/VNTR intron 4 and eNOS/Glu298Asp gene polymorphisms and nitric oxide levels

Nitric Oxide Levels					
	Genotypes	N	Mean	Std. Deviation	p value
eNOS/Glu298Asp	GG	70	529.39	159.12	0.395
	GT	42	474.17	141.80	
	TT	8	552.47	241.41	
eNOS/ VNTR 4	AA	88	519.32	167.15	0.302
	AB	33	462.00	150.88	
	BB	9	586.38	88.29	

In the literature, there are some reports on the association between eNOS gene polymorphisms and IRM. However, the results of these studies have been controversial among different ethnic groups.

Although Tempfer et al. demonstrated an association between VNTR intron 4/eNOS polymorphisms and IRM, in Austria (6), Karvela et al. (10) showed no association between VNTR intron 4 polymorphisms in eNOS gene and IRM in Greece. Our results were similar to those of Karvela et al., with no association between VNTR intron 4/eNOS polymorphisms and IRM in the Turkish population. In contrast to Karvela et al., we observed a marginally significant association between Glu298Asp/eNOS gene polymorphisms and IRM.

In this study we evaluated NO levels in four groups. To the best of our knowledge this is the first study evaluating both NO levels and eNOS gene polymorphisms with IRM simultaneously in the English literature.

Interestingly we observed statistically significant decreased NO levels in pregnant patient groups when elevated NO levels were measured in non pregnant patient groups and we demonstrated no association between NO levels and Glu298Asp/eNOS or VNTR 4/eNOS genotypes in any of the groups.

Defective placentation and resultant oxidative stress are believed to be largely responsible for preeclampsia and early pregnancy loss (19). In the literature, although some studies have demonstrated elevated NO levels, others have showed decreased NO levels in preeclampsia (20-22). This difference could be the result of complexity of NO pathways. Although in the past, it was believed that the increase in NO output was simply a result of eNOS expression, today it is known that the process of NO is more complex and flexible. Recent studies suggest that pregnancy-associated changes in NO output occur through reprogramming at the level of post-receptor cell signaling (23). The difference between pregnant and non pregnant groups in this study could be explained with reprogramming at the level of post-receptor cell signaling.

The results of our study suggest that impaired placental NO production play a key role in the pathophysiology of IRM and elevated NO levels in the non pregnant patient group could be a maternal response to compensate for placental deficiency. Indeed, recently Raffaelli et al demonstrated elevated NO production by platelets in patients with recurrent spontaneous miscarriage (24). This kind of maternal reponse could be persistant after pregnancy.

In conclusion, Glu298Asp polymorphism in the eNOS gene could be an intriguing factor that modulates an individual's risk of IRM in the Turkish population. However, further studies to explain the role of NO pathway in the pathophysiology of IRM are needed.

Conflict of interest

No conflict of interest was declared by the authors.

References

1. Wilcox AJ, Weinberg CR, O'Connor JF. Incidence of early loss of pregnancy. N Engl J Med 1988; 319: 189-94. [CrossRef]
2. Gupta S, Agarwal A, Banerjee J, Alvarez JG. The role of oxidative stress in spontaneous abortion and recurrent pregnancy loss: a systematic review. Obstet Gynecol Surv 2007; 62: 335-47. [CrossRef]
3. Lopez-Jaramillo P, Narvaez M, Calle A et al. Cyclic guanosine 3,5 monophosphate concentrations in pre- eclampsia: Effects of hydralazine. Br J Obstet Gynaecol 1996; 103: 33-8. [CrossRef]
4. Sanyal M, Nag TC, Das C. Localization of nitric oxide synthase in human trophoblast cells: role of nitric oxide in trophoblast proliferation and differentiation. Am J Reprod Immunol 2000; 43: 70-7. [CrossRef]
5. Haddad EK, Duclos AJ, Baines MG. Early embryo loss is associated with local production of nitric oxide by decidual mononuclear cells. J Exp Med 1995; 182: 1143-51. [CrossRef]
6. Tempfer C, Unfried G, Zeillinger R, Hefler L, Nagele F, Huber JC. Endothelial nitric oxide synthase gene polymorphism in women with idiopathic recurrent miscarriage. Hum Reprod 2001; 16: 1644-7. [CrossRef]
7. Hefler LA, Tempfer CB, Bashford MT, Unfried G, Zeillinger R, Schneeberger C, et al. Polymorphisms of the angiotensinogen gene, the endothelial nitric oxide synthase gene, and the interleukin-1beta gene promoter in women with idiopathic recurrent miscarriage. Mol Hum Reprod 2002; 8: 95-100. [CrossRef]
8. Suryanarayana V, Rao L, Kanakavalli M, Padmalatha V, Deenadayal M, Singh L. Recurrent early pregnancy loss and endothelial nitric oxide synthase gene polymorphisms. Arch Gynecol Obstet 2006; 274: 119-24. [CrossRef]
9. Zammiti W, Mtiraoui N, Mahjoub T. Lack of consistent association between endothelial nitric oxide synthase gene polymorphisms, homocysteine levels and recurrent pregnancy loss in Tunisian women. Am J Reprod Immunol 2008; 59: 139-45. [CrossRef]
10. Karvela M, Papadopoulou S, Tsaliki E, Konstantakou E, Hatzaki A, Florentin-Arar L, et al. Endothelial nitric oxide synthase gene polymorphisms in recurrent spontaneous abortions. Arch Gynecol Obstet 2008; 278: 349-52. [CrossRef]
11. Al Sallout RJ, Sharif FA. Polymorphisms in NOS3, ACE and PAI-1 genes and risk of spontaneous recurrent miscarriage in the Gaza Strip. Med Princ Pract 2010; 19: 99-104. [CrossRef]
12. Granger DL, Taintor RR, Boockvar KS, Hibbs JB Jr. Measurement of nitrate and nitrite in biological samples using nitrate reductase and Griess reaction. Methods Enzymol 1999; 268: 142-51. [CrossRef]
13. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988; 16: 1215. [CrossRef]
14. Erciyas K, Pehlivan S, Sever T, et al. Endothelial nitric oxide synthase gene polymorphisms associated with periodontal diseases in Turkish adults. J Biotechnol 2010; 9: 3042-7.
15. Zeteroglu S, Ustun Y, Engin Ustun Y, Zeteroglu U, Karayel M. Serum folic acid levels in women with recurrent early pregnancy loss. Artemis, 2003;4: 36-7.
16. Tripathi P, Naik S, Agrawal S. HLA-E*0101 associated with recurrent spontaneous abortion. J Turkish-German Gynecol Assoc 2007; 8: 278-82.
17. Saatci C, Oner G, Tasdemir S, Kiraz A, Ozkul Y, Dündar M, et al. Parental karyotype and genetic markers for thrombophilia in recurrent miscarriage. J Turkish-German Gynecol Assoc 2008; 9: 139-43.
18. Casas JP, Cavalleri GL, Bautista LE, Smeeth L, Humphries SE, Hingorani AD. Endothelial nitric oxide synthase gene polymorphisms and cardiovascular disease: A HuGE review. Am J Epidemiol 2006; 164: 921-35. [CrossRef]
19. Harma M, Harma M. Defective placentation and resultant oxidative stress play a similar role in complete hydatidiform mole to that in preeclampsia and early pregnancy loss. Med Hypotheses 2006; 66: 100-2. [CrossRef]
20. Teran E, Chedraui P, Vivero S, Villena F, Duchicela F, Nacevilla L. Plasma and placental nitric oxide levels in women with and with-

out pre-eclampsia living at different altitudes. Int J Gynaecol Obstet 2009; 104: 140-2. [CrossRef]

21. D'Anna R, Baviera G, Corrado F, Crisafulli A, Ientile R, Buemi M, et al. Neurokinin B and nitric oxide plasma levels in pre-eclampsia and isolated intrauterine growth restriction. BJOG 2004; 111: 1046-50. [CrossRef]
22. Mao D, Che J, Li K, Han S, Yue Q, Zhu L, et al. Association of homocysteine, asymmetric dimethylarginine, and nitric oxide with preeclampsia. Arch Gynecol Obstet 2010; 282: 371-5. [CrossRef]
23. Boeldt DS, Yi FX, Bird IM. eNOS activation and NO function: Pregnancy adaptive programming of capacitative entry responses alters nitric oxide (NO) output in vascular endothelium-new insights into eNOS regulation through adaptive cell signaling. J Endocrinol 2011; 210: 243-58. [CrossRef]
24. Raffaelli F, Nanetti L, Vignini A, Mazzanti L, Giannubilo SR, Curzi CM, et al. Nitric oxide platelet production in spontaneous miscarriage in the first trimester. Fertil Steril 2010; 93: 1976-82. [CrossRef]

Use of lactobacilli and estriol combination in the treatment of disturbed vaginal ecosystem: a review

Laktobasil ve estriol kombinasyonunun bozulmuş vajina ekosisteminin tedavisinde kullanımı: Bir derleme

Cihat Ünlü[1], Gilbert Donders[2]

[1]Department of Obstetrics and Gynaecology, Acıbadem University, Bakırköy Hospital, İstanbul, Turkey,
[2]Department of Obstetrics and Gynaecology, Regional Hospital Heilig Hart Tienen, University Hospital Gasthuisberg Leuven, and University Hospital Citadelle Liège, Liège, Belgium

Abstract

To maintain a healthy vaginal ecosystem or to restore any disturbance, sufficient estrogen levels, an intact mature vaginal epithelium, and physiological lactobacillary microflora are essential. Thus, a combination of beneficial lactobacilli and estrogen is an appealing treatment option. This article reviews the published data on the use of viable *Lactobacillus acidophilus* KS400 and a low dose of estriol (0.03 mg E3) in the form of vaginal tablets (Gynoflor®). In vitro studies demonstrated that *L. acidophilus* KS400 produces lactic acid and hydrogen peroxide (H_2O_2), inhibits the growth of relevant vaginal pathogens, and inhibits adherence of pathogens to epithelial cells. Topical administration of E3 for treatment of vaginal diseases is generally preferred, as this route of application of hormones produces a more significant local proliferative response and has no stimulating effect on the endometrium. Overall, 16 clinical studies have been published with the combination of L. acidophilus KS400 and 0.03 mg E3. The results of these trials have demonstrated that the combination improves the vaginal epithelium and the restoration of the lactobacillary microflora with an excellent safety profile, even during pregnancy. The combination can be used in pre- and postmenopausal women for the restoration of the vaginal flora after anti-infective therapy, for treatment of symptomatic vaginal atrophy, and for abnormal vaginal flora therapy. It can be also considered in repetitive therapy courses for the long-term prevention of recurrences of bacterial vaginosis, even though further clinical studies are needed to substantiate the benefit of this application. (J Turkish-German Gynecol Assoc 2011; 12: 239-46)

Key words: *Lactobacillus acidophilus*, estriol, abnormal vaginal flora, restoration of vaginal ecosystem, recurrence prevention, vaginal atrophy

Received: 22 August, 2011 **Accepted:** 19 October, 2011

Özet

Sağlıklı bir vajinal ekosistemi sürdürmek veya bozulmuş olanı düzeltmek için, yeterli östrojen düzeyleri, sağlam olgun bir vajina epiteli ve fizyolojik bir laktobasiler mikroflora esastır. Bu nedenle, yararlı laktobasiller ve östrojen kombinasyonu cazip bir tedavi seçeneğidir. Bu makale vajinal tablet (Gynoflor®) formundaki düşük doz estriol (0.03 mg E3) ve canlı laktobasillus asidofilus KS400'ün kullanımı üzerine yayınlanmış verileri derlemektedir. İn vitro çalışmalar L. asidofilus KS400'ün laktik asit ve hidrojen peroksit (H_2O_2) **ürettiğini, ilgili vajinal patojenlerin** çoğalmasını baskıladığını ve patojenlerin epitel hücrelerine tutunmasını baskıladığını göstermiştir. Vajinal hastalıkların tedavisi için E3'ün genellikle topikal uygulanması tercih edilmektedir; çünkü hormonların bu şekilde uygulanması daha belirgin bir proliferatif yanıt oluşturmakta ve endometriyumda uyarıcı bir etki olmamaktadır. L. asidofilus KS400 ve 0.03 mg E3 kombinasyonu ile ilgili toplam 16 klinik çalışma yayınlanmıştır. Bu çalışmaların sonuçları kombinasyonun vajinal epiteli ve laktobasiler mikroflora restorasyonunu iyileştirdiğini, güvenlilik profilinin ise gebelik dahil mükemmel olduğunu göstermiştir. Kombinasyon menopoz öncesi ve sonrası kadınlarda anti-enfektif tedaviden sonra vajinal floranın restorasyonu için, semptomatik vajinal atrofinin tedavisi ve anormal vajinal floranın tedavisi için kullanılabilir. Bakteriyel vajinozis rekürenslerinin uzun dönemde önlenmesi için tekrarlanan tedavi kürlerinde de düşünülebilir ancak bu uygulamanın geçerliliğini göstermek için daha fazla klinik çalışmaya gerek duyulmaktadır.
(J Turkish-German Gynecol Assoc 2011; 12: 239-46)

Anahtar kelimeler: *Laktobasillus asidofilus*, estriol, anormal vajinal flora, vajinal ekosistemin restorasyonu, rekürenslerin önlenmesi, vajinal atrofi

Geliş Tarihi: 22 Ağustos 2011 **Kabul Tarihi:** 19 Ekim, 2011

Introduction

The vagina and its healthy microflora form a balanced vaginal ecosystem, with the environment controlling the microbial strains present and the flora in turn controlling the vaginal environment and engaging in the natural defence against pathogens (1, 2). Sufficient estrogen levels leading to an intact mature vaginal epithelium, as well as the physiological lactobacillary microflora are essential (3). Additionally, the vaginal immune response also influences the vaginal ecosystem and is important for overall defence (4).

During the last decade, the vaginal microflora has been intensively studied, and additionally in the framework of the human microbiome project-analysis of both the human and microbial genome (5). The microorganisms living in symbiosis with the human body can be mutualists (benefiting themselves and the host), commensals (benefiting themselves only) or pathogens (benefiting themselves by harming the host) (6). *Lactobacillus* species are the dominant vaginal mutualists (7) and reach lev els of 10^7 to 10^8 colony forming units (cfu)/g of vaginal fluid in healthy premenopausal woman (8). The vaginal flora is in a dynamic state-the abundance and bacterial type of vaginal

Address for Correspondence: *Cihat Ünlü, Women's Health Clinic, Abdi Ipekci Caddesi 2/7, 34357 Nişantaşı, İstanbul, Turkey*
Phone: +90 212 241 45 45 Fax: +90 212 241 44 08 e.mail: cunlu@ada.net.tr

doi:10.5152/jtgga.2011.57

microbiota can change rapidly within months or sometimes days (5, 6, 9). The predominant species of lactobacilli found in the vagina of healthy women remains controversial. Organisms previously collectively known as *Lactobacillus* acidophilus were currently shown to form a number of separate species within the *L. acidophilus* complex. Depending on the study, *L. crispatus, L. jensenii, L. gasseri,* and *L. iners* were predominantly identified in the vagina (5, 9, 10). Beneficial lactobacilli (1) produce antibacterial compounds, (2) have the ability to colonize the vaginal epithelium, and (3) seem to influence the regulation of local vaginal immunity (4, 5, 10).

Another crucial element to be considered in the microecology of the vagina is the functional condition of the stratified, squamous nonkeratinised vaginal epithelium (1). The fluctuating sex hormones, particularly estrogens (1, 3) induce the proliferation and maturation of the vaginal epithelium (1). The breakdown of proliferated superficial vaginal epitheliocytes liberates glycogen, which is metabolised to glucose by either enzymes secreted by the vaginal or cervical cells or by lactobacilli. Hence, a mature vaginal epithelium is a prerequisite for establishing and maintaining physiological lactobacillary microflora (11). Vaginal atrophy is associated with reduced efficacy of the barrier function of the vaginal epithelial lining and, as a consequence facilitate the penetration of pathogenic micro-organisms (12, 13).

Risk factors for disturbing the vaginal ecosystem can be (1) endogenous (variations in hormone levels, contraceptives, menstruation, pregnancy and lactation, diabetes mellitus, systemic diseases) or (2) exogenous. The exogenous factors are either life-style related (smoking, stress, unprotected sex, etc.), infectious, or medical (antibacterial therapy, radiation, etc.) (1, 8, 14) It still remains unknown whether alterations of the *Lactobacillus* flora, the vaginal epithelium or the pathogens are the initial cause of an abnormal vaginal flora (15). It was found that some healthy women (7-33%) lack the *Lactobacillus* species in the vagina and have other lactic acid-producing bacteria such as Atopobium vaginae, Megasphaera, and Leptotrichia species instead to maintain vaginal health, i.e. remain asymptomatic (5). The studies do not address whether such 'healthy' women are in a transitional phase towards or from BV, or whether it should be called asymptomatic BV, i.e. abnormal flora but no symptoms (5).

The Gynoflor® vaginal tablet (*L. acidophilus*-0.03 mg estriol-combination) contains both lyophylised, viable *Lactobacillus* acidophilus KS400 bacteria (100 million colony forming units [cfu] per tablet) and a very low dose of estriol (0.03 mg E3). The goal of the current article is to review the published evidence in order to provide a rationale for the use of the L. acidophilus-0.03 mg estriol-combination vaginal tablets in daily clinical practise.

In vitro properties of Lactobacillus acidophilus KS400

There are numerous mechanisms by which probiotic lactobacilli ensure their ability to colonize the host and to competitively exclude the pathogens (6, 8). Firstly, vaginal lactobacilli inhibit the growth of pathogens by the production of lactic acid, or by the production of hydrogen peroxide (H_2O_2) and other antimicrobial substances, i.e. bacteriocins and bacteriocin-like substances (6, 8). Furthermore, beneficial lactobacilli strains compete with other microorganisms, such as *Escherichia coli, Salmonella typhimurium, Candida albicans, Staphylococcus aureus, Gardnerella vaginalis, Prevotella bivia, Mobiluncus hominis,* etc. for adherence to the vaginal epithelium and for nutrients. Additionally, other mechanisms, especially immune modulation, although yet less well understood, are at play (4, 6, 8).

Not every *Lactobacillus* strain of a single species produces a beneficial effect for the vaginal ecosystem, emphasising the importance of careful selection, study and assessment of the different properties of a specific strain in order to consider it for the prophylactic or therapeutic use as probiotic in humans. The lactobacilli strain *L. acidophilus* KS400 has been originally isolated from humans and is well characterised with regard to its in vitro properties.

The production of lactic acid by lactobacilli, via the fermentation of glycogen from desquamated vaginal epithelial cells, induces vaginal milieu acidification resulting in a normal (<4.5) vaginal pH. The acidic environment provides optimal conditions for the lactobacilli and unfavourable growth conditions for the pathogenic micro-organisms (1, 16). Lactic acid has been also shown to stimulate the immune response (17). In vitro experiments with *L. acidophilus* KS400 have demonstrated that the strain is able to produce substantial amounts of lactic acid (16). The DL-lactic acid concentration in the nutrient medium increased by about 5 mg/mL within 6-12 h and the pH of the culture decreased by ≥2 units within 6-12 hours of incubation. The ability of *L. acidophilus* KS400 to produce lactic acid has been confirmed also in clinical studies: a decrease and thus normalization of the vaginal pH has been observed (see below).

A further important defence mechanism of beneficial lactobacilli strains involves the production of H_2O_2-a well-recognised mechanism of bacterial antagonism (1, 8). H_2O_2 is an oxidizing agent killing pathogens through the production of free radicals. It is thought that lactobacilli produce Fe^{3+}-activated extracellular peroxidase to protect themselves (6). The important role of H_2O_2-producing lactobacilli is also demonstrated by clinical evidence: the vagina of 96% of healthy women was colonised by H_2O_2-producing lactobacilli strains, compared to only 6% in women with bacterial vaginosis (7). *L. acidophilus* KS400 has been shown to produce H_2O_2, in vitro, thus enabling it to inhibit the growth of relevant vaginal pathogens (16).

Bacteriocins are protein-like substances, usually with limited bacterial killing activity, which inhibit strains of the same or closely-related species (11). Bacteriocin-like substance is a term applied to antagonistic substances which are incompletely defined and have a broader spectrum of activity. However, the role of these substances in vivo remains to be elucidated. Bacteriocins and bacteriocin-like substances have been identified for some strains of *L. acidophilus, L. helveticus, L. fermenti* and *L. plantarum*, and shown to inhibit a wide range of both Gram-positive and -negative bacteria as well as fungi (11). Whether *L. acidophilus* KS400 produces a bacteriocin or bacteriocin-like substances has not been fully elucidated.

The overall growth inhibition activity of lactobacilli strains was also investigated with in vitro co-culture experiments. Kanne et al. (18) incubated the cultures of *E. coli, S. aureus* and *C.*

albicans either with or without *L. acidophilus* KS400, and have determined the growth of the respective pathogens. After 6 hours of co-incubation, the numbers of all three pathogens were significantly decreased, and *E. coli* and *S. aureus* were no longer detectable. Servin [Servin A.L., 2004, data on file] further investigated the ability of *L. acidophilus* KS400 to inhibit vaginal pathogens. Again, *S. aureus*, uropathogenic *E. coli, G. vaginalis*, and *P. bivia* were incubated with or without *L. acidophilus* KS400. In the presence of the lactobacilli strain, the numbers of pathogens in the culture was significantly reduced for all tested bacteria (Figure 1), confirming the earlier results and also demonstrating inhibitory activity against relevant urogenital pathogens.

Beneficial vaginal lactobacilli strongly adhere to epithelial cells and thereby prevent colonization of the vagina by pathogens by competitive exclusion. A number of studies have demonstrated the ability of lactobacilli to adhere to human epithelial cells and to diminish the pathogens (19, 20). In experiments using cervical HeLa cells and intestinal Caco-2/TC7 cells, it was shown that *L. acidophilus* KS400 adhered well to these epithelial cells. [Servin A.L., 2004, data on file] Later, *L. acidophilus* KS400 efficiently inhibited the adherence of *E. coli, G. vaginalis*, and *P. bivia* to epithelial cells.

Lactobacilli also have immune modulation properties. Through the production of bacterial signalling factors, lactobacilli induce human antimicrobial factors such as defensins, lysozyme and haemocidins (6, 21) and modulate the expression of 70-kDa human shock protein (hsp70) and Mannose-Binding Lectin (MBL) (4). It seems that the immune system accurately calibrates responses to pathogens and differentiates microorganisms through pattern recognition receptors, mediated by epithelial trans-membrane proteins called Toll-like receptor (TLR) signalling (4). It is thought that bacteria present in the vagina of healthy women maintain epithelial cell TLR activation at a steady level, resulting in increasing anti-inflammatory cytokines, such as IL-10, and decreasing the proinflammatory cytokines IL-1β, TNF-α, and IL-6, thus creating a T-helper-like response in the vaginal milieu to inhibit the proliferation of pathogens (1, 21).

More recently, additional defense mechanisms, such as the production of biosurfactants, co-aggregation and lactobacilli biofilm formation, have been discussed (6, 22).

Key characteristics of Estriol (E3)

The estrogens, estrone (E1), estradiol (E2), and estriol (E3), are female sex hormones occurring naturally in humans. E3 is specific for humans and does not occur in rodents (2). Whereas E2 and E1 can be reversibly metabolised to each other, E3 cannot be transformed back. Like all estrogens, E3 stimulates the proliferation and maturation of the vaginal epithelium. However, E3 has a lower receptor affinity (about 10 times) than E2, and thus is not able to induce estrogenic effects on the endometrium, bone and breast tissue at physiological concentrations (2). Unlike E2, after single-dose oral or vaginal applications of E3 in normal doses (oral: ≤8-10 mg; vaginal: ≤0.5 mg), there is no, or only a weak proliferative, effect on the endometrium (23).

Estrogens administered vaginally are absorbed in a dose-dependent, bypass hepatic metabolism and are biologically active (24, 25). Vaginal estrogens are more effective in relieving urogenital symptoms than oral preparations as (1) lower doses are required due to the absence of hepatic metabolism, and (2) high local estrogen level induces direct vaginal response (2, 26). Thus, topical administration of E3 for treatment of vaginal diseases is generally preferred, as this hormone applied locally is safer than other estrogens and produces a more significant proliferation response than after oral intake (2). It is particularly important if systemic hormone replacement with estrogen is not required.

Synergy of the combination

As mentioned, both sufficient estrogen levels inducing a mature vaginal epithelium and colonisation by lactobacilli are essential to maintain or restore a healthy vaginal ecosystem. Estrogen level fluctuations, with accompanying alterations in the proliferation and maturation of the epithelium, can alter bacterial

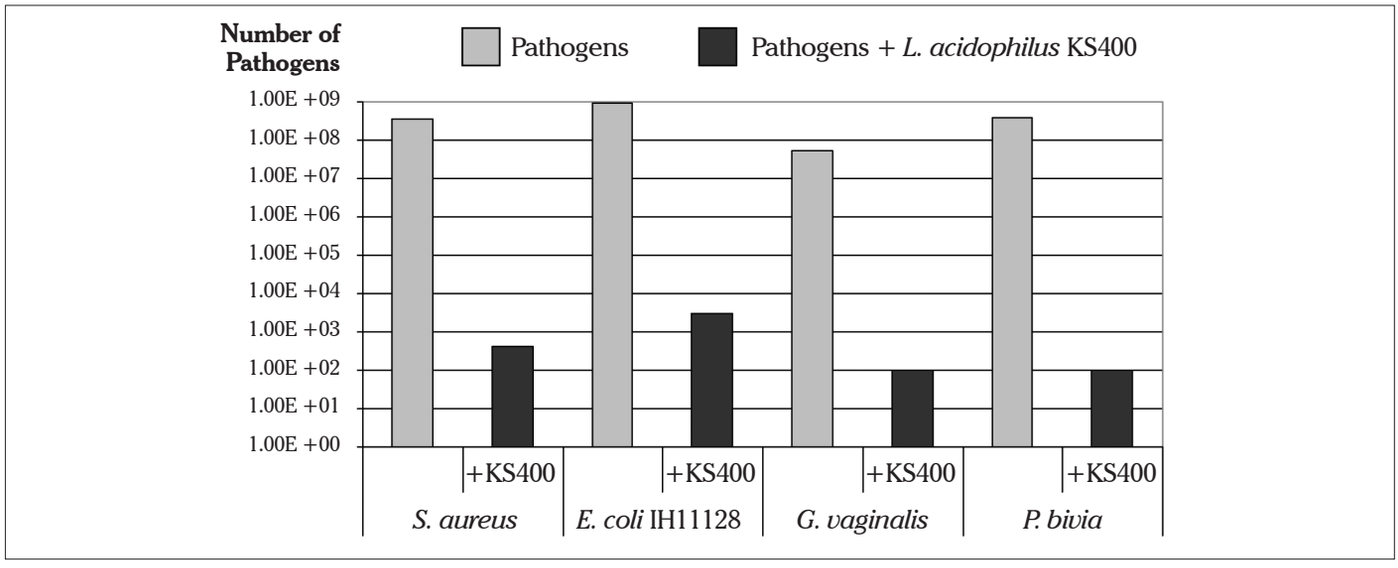


Figure 1. Inhibition of pathogens by Lactobacillus acidophilus KS400 [Servin A.L., 2004, data on file

adherence and other properties and hence affect the composition of the vaginal microflora (1, 19). It has been shown that the in vitro adherence of lactobacilli to vaginal epithelial cells is stronger on days of high concentrations of estrogens, and that administration of estrogen is able to restore vaginal colonisation in post-menopausal women (27).

A disturbed vaginal ecosystem is characterized by reduced or non-existing *Lactobacillus* flora and a more or less damaged epithelium (15). It is not always evident whether alterations of the vaginal epithelium or pathogenic micro-organisms are the primary cause of a disturbed vaginal ecosystem. Thus, treatment with a combination of beneficial lactobacilli and low-dosed estriol to support the restoration of the vaginal ecosystem on the level of the vaginal epithelium and microflora makes sense, not only in postmenopausal women, but also in women of reproductive age.

Clinical data

Pharmacokinetic studies

In a pharmacokinetic study, Kaiser et al. (28) demonstrated that the plasma level of unconjugated E3 transiently increased after the first application of *L. acidophilus*-0.03 mg estriol-combination, but was still within the normal range for untreated post-menopausal women. After the 12th daily application lactobacilli-estriol-combination however, no increase of the E3 plasma level could be observed. This was explained by the presence of a more proliferated and matured vaginal epithelium, preventing the E3 from crossing the vaginal mucosal layers. Basal plasma concentrations of unconjugated E3 during the 12 day treatment remained at the same level, indicating indirectly that no accumulation of E3 had taken place and that systemic effects were hence extremely unlikely. Thus, in contrast to the standard dose of 0.5 mg E3 (2), the *L. acidophilus*-0.03 mg estriol-combination does not result in a significant absorption and increase in systemic estriol levels

Restoration of disturbed vaginal ecosystem

For the treatment of vaginal infections or abnormal vaginal flora, as well as for the restoration therapy after use of anti-infective to prevent relapses, the same defense mechanisms induced by lactobacilli-estriol-combination are important to achieve a healthy vaginal ecosystem, i.e. the epithelial maturation, the growth inhibition of pathogens and the prevention of pathogenic vaginal colonisation. Following treatment of vaginal infections with antibiotics, a slow spontaneous recovery of the vaginal flora composition is usually seen, probiotic lactobacilli can be administered to support this restoration process.

The promising clinical data of probiotic lactobacilli treatment have been previously reviewed by other authors (29, 30). In this section, the published clinical studies of *L. acidophilus*-0.03 mg estriol-combination that have been performed to investigate the restoration of the vaginal ecosystem in different clinical indications are summarized (Table 1). The first clinical studies with lactobacilli-estriol-combination were carried out in the 1980's as open, uncontrolled trials in the treatment of vaginal infections (18, 31). Further open clinical studies demonstrating a positive effect and benefit of *L. acidophilus*-0.03 mg estriol-combination have been published in the treatment of bacterial vaginosis (32, 33), in the perioperative treatment for gynaecological operations (34), and in the prophylaxis of infectious complications during intravaginal and intrauterine operations (35).

Parent et al. (36) performed a randomized, placebo-controlled, double-blind, multicentre study to compare *L. acidophilus*-0.03 mg estriol-combination (n=17) with placebo (n=15) in pre-menopausal women with bacterial vaginosis according to Amsel (37). Women were randomly assigned to a 6-day therapy protocol with verum or placebo. Follow-up took place 2 and 4 weeks after the therapy. Cure rate (defined as ≤1 of the 4 Amsel criteria positive) was 77% in the lactobacilli-estriol-combination group vs. 25% in the placebo group at 2 weeks follow-up visit. The corresponding numbers at 4 weeks were 88% and 22%, respectively. At both visits, the cure rate was higher for the test group than for the placebo group. In addition, a significant increase in the number of lactobacilli was observed in the vaginal smear of women treated with the test product as compared to the placebo. The therapeutic cure rate observed in this study was similar to cure rates of metronidazole and clindamycin.

Donders et al. (38) evaluated the efficacy of *L. acidophilus*-0.03 mg estriol-combination in comparison to metronidazole in the treatment of bacterial vaginal infections in a multi-centre, randomised, active-controlled pilot study. Forty six, pre-menopausal women between 18 to 50 years of age with a disrupted vaginal flora due to a bacterial vaginal infection (bacterial vaginosis and/or aerobic vaginitis) were included. Diagnosis was based on fresh phase contrast microscopy of vaginal fluid showing either lactobacillary grades IIb (decreased number of lactobacillary morphotypes overgrown by other bacterial morphotypes) or III (disappearance of lactobacilli due to overgrowth by other bacteria). Patients were randomized for treatment either with 12 vaginal tablets of lactobacilli-estriol-combination or with 6 vaginal suppositories containing 500 mg metronidazole (high dose). The treating physician was blinded to the medication type patients received. Eight efficacy variables were studied to assess the status of the vaginal flora at entry, at one week, one month and 4 months after the end of therapy. The combination of *L. acidophilus* KS400 and 0.03 mg E3 had equivalent efficacy to metronidazole in the short-term treatment, but was slightly less efficacious after one month. The authors suggested that continued repetitive use of the lactobacilli-estriol-combination should be considered and advised more studies to address this.

In a randomised, placebo-controlled, double-blind study, Özkinay et al. (20) investigated the efficacy of L. acidophilus-0.03 mg estriol-combination to restore the physiological vaginal ecosystem in patients who had received specific anti-infective therapy for vaginal infections of various aetiology (bacterial vaginosis, candidiasis, trichomoniasis, and mixed infections). In total, 354 of 360 randomized pre- and post-menopausal women were included in the statistical analysis. Two to three days after the end of the anti-infective therapy, they were randomly assigned to treatment with either lactobacilli-estriol-combination or placebo. The Normal Flora Index (NFI), based on the number of lactobacilli versus the number of pathogens, vaginal pH-value, and number of leukocytes, increased signifi-

Table 1. Overview of main published clinical studies with 10^7 cfu/g Lactobacillus acidophilus KS400 and 0.03 mg estriol (E3) combination (Gynoflor®)

Study	Design	Patients	Intervention	Outcome
Kaiser et al., 2000 (28)	Pharmacokinetic, open, monocentric, multiple-dose	8 menopausal volunteers aged 57-65	*L. acidophilus* -0.03 mg E3 1 tablet daily for 12 days	Vaginal Maturation Index (VMI) improved strongly, plasma concentration of E3 remained at the same level, no accumulation
Parent et al., 1996 (36)	Multicentric (3 centres), randomised, placebo-controlled, parallel group	32 women with bacterial vaginosis intermediate cases aged 20-52	*L. acidophilus*-0.03 mg E3 1-2 tablet daily for 6 days or placebo	Clinical cure rate (defined as ≤1 of 4 Amsel criteria) was significantly higher in the test group
Donders et al., 2010 (38)	Multicentric, active-controlled, randomised, single-blind study, parallel	46 premenopausal women with vaginitis or vaginosis with disrupted microflora aged 18-50	*L. acidophilus*-0.03 mg E3 1 tablet for 12 days or metronidazole 500 mg for 6 days	Short-term efficacy of L. acidophilus-0.03 mg E3 comparable to metronidazole, long-term efficacy was lower, repeated treatment is required
Özkinay et al., 2005 (20)	Monocentric, randomised, double-blind, placebo-controlled, parallel	360 women, restoration of flora after anti-infective therapy, aged 19-70	*L. acidophilus*-0.03 mg E3 or placebo after anti-infective therapy	Normal Flora Index (NFI) significantly improved in test group; restoration of the flora significantly enhanced
Unzeitig & Al Awad, 2006 (39)	Open, monocentric	98 women of childbearing age women with at least 4 episodes of vaginal discomfort in past year	*L. acidophilus*-0.03 mg E3; benzydamide irrigation (various schemes, 3 months)	Most of the unsuccessfully treated women reported a marked improvement and the remainder - a partial improvement
Kanne & Jenny, 1991 (45)	Double-blind, active- and placebo-controlled, randomised, parallel-group, dose-finding	15 women with atrophic vaginitis aged 51-65	*L. acidophilus*-0.03 mg E3 2 tablets daily for 6 days or E3 0.5 mg + lactobacilli or lactobacilli	No significant difference between the test medication (0.03 mg E3) and active control (0.5 mg E3)
Feiks & Grünberger, 1991 (46)	Double-blind, randomised, active-controlled, parallel groups	48 women with atrophic vaginitis aged 49-69	*L. acidophilus*-0.03 mg E3 1 tablet daily for 12 days and E3 0.5 mg 1 ovula for 12 days	No significant difference between the L. acidophilus-0.03 mg E3 and 0.5 mg E3
Hengst et al., 1992 (47)	Open, monocentric, perspective	Preterm prevention during pregnancy, 161 pregnant women	*L. acidophilus*-0.03 mg E3 1 tablet daily for 6-12 days	The lowest pre-term delivery rate observed in the group with *L. acidophilus*-0.03 mg E3; no adverse events
Hoyme et al., 1998 (48) Hoyme et al., 2004 (49)	Pilot multicentre (16 centres) cohort	314 pregnant women, preterm prevention during pregnancy	*L. acidophilus*-0.03 mg E3 1 tablet daily for 6 days	Reduction in preterm birth rate, delivery before week 32 and premature rupture; no adverse events
Melczer et al., 2002 (33)	Open, monocentric, before-after	100 women with bacterial vaginosis 3 of 4 Amsel criteria positive	*L. acidophilus*-0.03 mg E3 1 tablet daily for 6 days	Overall, 81% became completely free of complaints and 17 % showed substantial improvement; no adverse events

cantly more in the lactobacilli-estriol group than in the placebo group and remained at the higher level after 4 to 6 weeks. The vaginal flora in the women of the test group improved significantly more than in the placebo group, both directly after restoration therapy and at the follow-up examination, as measured by the degree of purity and number of lactobacilli. Furthermore, the number of relapses at follow-up was considerably lower in the lactobacilli-estriol-combination group (19/239, 7.9%) than in the placebo group (15/119, 12.6%).

In an open, monocentric clinical study, Unzeitig and Al Awad (39) treated 98 women of childbearing age women with at least 4 episodes of vaginal symptoms in the previous year (yeast or bacterial origin, recurrent bacterial vaginosis, etc.). The women received a single benzydamide irrigation (anti-inflammatory drug for local use) and subsequently *L. acidophilus*-0.03 mg estriol-combination for 6 consecutive days during one menstrual cycle, and during the following cycle they were again treated with the single benzydamide irrigation, but the lactobacilli-estriol-combination therapy was given in a 2-weeks interval (4 and 2 days, i.e. repetitive dosing). Two thirds of the previously unsuccessfully treated women with chronic vaginal discharge and vulvodynia reported a marked improvement, and about one fifth showed a partial improvement. Similar to the study by Donders et al., also in this study it was evident that a single 6-day therapy with lactobacilli-estriol-combination is not sufficient to achieve a long-term cure in women with recurrent disease and that a long-term maintenance therapy probably would be needed. The efficacy of repeated, probiotic lactobacilli therapy has been investigated in the long-term prevention of bacterial vaginosis recurrence, providing promising results (29, 40, 41).

Treatment of vaginal atrophy

An important quality of life issue in the management of women's menopause is an effective and safe treatment of vaginal atrophy and atrophic vaginitis. In recent years, the use of locally applied vaginal low-dose estrogen has been advocated in preference to systemic treatment and is considered to be the best therapy for vaginal atrophy (3, 12, 13). Another broadly discussed question is the possible use of low-dosed vaginal estrogens for the treatment of the symptomatic vaginal atrophy (vaginal dryness, dyspareunia, etc.) in breast cancer survivors taking aromatase inhibitors. Due to lack of proper safety data, the available clinical experience remains controversial and requires further investigation (42, 43).

The clinical studies for E3 have been reviewed by Head (2). She concluded that intravaginal E3 appears to be effective in controlling urogenital symptoms of menopause and found no incidence of endometrial hyperplasia for a conventional vaginal E3 dose of 0.5 mg. From a meta-analysis of clinical studies, Vooijs and Geurts (23) concluded that once daily treatment with intravaginal 0.5 mg E3 in post-menopausal women is safe and has no increased risk of endometrial proliferation or hyperplasia.

The first evidence of efficacy of *L. acidophilus*-0.03 mg estriol-combination in the treatment of atrophic vaginitis has been reported in an open, uncontrolled study (44) Kanne and Jenny (45) used vaginal *L. acidophilus*-0.03 mg E3-combination in a randomised, double-blind, controlled, dose finding study, including 14 post-menopausal women with atrophic vaginitis. Women received twice daily vaginal therapy during 6 days with *L. acidophilus* supplemented with either a 0.03 mg or 0.5 mg E3 dose. Both therapies led to a significant improvement in proliferation and maturation of the vaginal epithelium, but without relevant differences between both groups.

Feiks and Grünberger (46) treated 48 post-menopausal women (49-83 years) with clinical findings of atrophic vaginitis in a randomized, blinded and controlled study. The patients were assigned to daily vaginal therapy for 12 days with either *L. acidophilus*-0.03 mg estriol-combination or with conventional dose of 0.5 mg E3. The degree of proliferation significantly improved in both treatment groups; from 1.44 to 2.19 ($p<0.0001$) and 1.35 to 2.62 ($p<0.0001$), in the first and second treatment group respectively. Interestingly, despite a 16-fold higher dose in the conventional dosed product, efficacy between the groups did not differ ($p=0.094$), i.e. results were equivalent.

In a recent, unpublished controlled study Jaisamrarn et al. have demonstrated that the low-dose 0.03 mg E3 combined with *L. acidophilus* is sufficient to treat symptomatic atrophic vaginitis adequately and that a twice weekly maintenance therapy is on average sufficient to prevent relapse of vaginal atrophy.

Safety and tolerability

In a total of 16 published clinical studies, 1,715 patients have been treated with *L. acidophilus*-0.03 mg estriol-combination, and only 46 adverse drug reactions were observed (2.7%), none of which were serious. Most of the adverse events were local reactions (burning, irritation, pruritus, local allergic reactions, and reddening), which were mostly mild and usually occurred temporarily at the start of therapy. The typical estrogen side effects have not been reported.

The use of *L. acidophilus*-0.03 mg estriol-combination during pregnancy can also be considered as safe, because these substances are present physiologically in the human vagina, and systemic absorption of E3 is negligible. Furthermore, the blood level of E3 increases during pregnancy up to 1000 times compared to non-pregnant women. A total of 151 pregnant women have been intentionally treated with *L. acidophilus*-0.03 mg E3-combination in clinical studies, and no adverse effects on pregnancy, the foetus or new-born have been observed.

Hengst et al. (47). screened 443 pregnant women for an increase in vaginal pH (>4.5) within a pre-term birth prevention programme. In the prospective patient group, 161 pregnant women were included. Forty-nine of the 102 women with an increased vaginal pH received lactobacilli-estriol-combination once daily for 12 days to normalise the vaginal pH. Pre-term delivery rates were compared, with results indicating that the lowest one was observed in lactobacilli-estriol combination group in all studies. No adverse events were reported for the lactobacilli-estriol-combination.

Within the pre-term prevention program in Erfurt, Germany by Hoyme et al., (48) 314 pregnant women checked their vaginal pH periodically. Fifty-nine of these women were identified as having a higher risk of pre-term delivery. Subsequently, 52 of these cases were treated with lactobacilli-estriol-combination, and in 19 cases clindamycin cream was also used for therapy.

No adverse events were reported, indicating the safe use of lactobacilli-estriol-combination during pregnancy.
Melczer et al. (33) treated 50 pregnant (12 in first, 25 in second, and 13 in third trimester of pregnancy) and 50 non-pregnant women with bacterial vaginosis with *L. acidophilus*-0.03 mg estriol-combination once daily for 6 days. The pregnant women tolerated the lactobacilli-estriol vaginal tablets well and no adverse effects on pregnancy, the foetus or new-born have been observed.

Conclusions

The available data on *L. acidophilus* KS400 demonstrate that this human lactobacilli strain possesses beneficial properties to inhibit the growth of vaginal pathogens by means of production of lactic acid, hydrogen peroxide and possibly yet unidentified antimicrobial substances. Furthermore, *L. acidophilus* KS400 adheres to epithelial cells and prevents the adherence of pathogens. E3 has about a 10-times lower receptor affinity than E2, and thus is regarded as a short-acting estrogen. Vaginal E3 doses of up to 0.5 mg daily have a full vaginotrophic activity resulting in the proliferation and maturation of the epithelium, but show a negligible uterotrophic and systemic effect. The published clinical studies with *L. acidophilus*-0.03 mg estriol-combination have demonstrated its efficacy in improving the vaginal epithelium and the restoration of the lactobacillary microflora. The matured vaginal epithelium is a prerequisite for the lactobacilli colonization and depends on the estrogen level, which fluctuates during the menstrual cycle and with age. Thus, a combination of beneficial lactobacilli and a low-dose of estriol is beneficial for women of any age.
The unique combination of viable *L. acidophilus* KS400 and low dose 0.03 mg E3 is efficient in establishing and maintaining a healthy vaginal ecosystem, with an excellent safety profile, including also during pregnancy. The combination can be used for restoring the vaginal flora after local and/or systemic treatment with anti-infective agents, for treatment of symptomatic vaginal atrophy due to estrogen deficiency, and for women with a vaginal discharge of unknown origin, when use of antibiotic therapy is not necessary. It can be also considered in repetitive therapy courses for the prevention of long-term recurrences of bacterial vaginosis, even though further clinical studies are needed to support this application.

Acknowledgments
The authors would like to thank Dr Philipp Grob, Dr Valdas Prasauskas, and Gabriela Zwyssig, Medinova AG, Switzerland, for their valuable comments on the manuscript.

Conflict of interest
CU&GD are members of Global Advisory Board of Medinova AG, Switzerland.

References

1. Redondo-Lopez V, Cook RL, Sobel JD. Emerging role of lactobacilli in the control and maintenance of the vaginal bacterial microflora. Rev Infect Dis 1990; 12: 856-72. [CrossRef]
2. Head KA. Estriol: safety and efficacy. Altern Med Rev 1998; 3: 101-13.
3. Sturdee DW, Panay N. Recommendations for the management of postmenopausal vaginal atrophy. Climacteric 2010; 13: 509-22. [CrossRef]
4. Witkin SS, Linhares IM, Giraldo P. Bacterial flora of the female genital tract: function and immune regulation. Best Pract Res Clin Obstet Gynaecol 2007; 21: 347-54. [CrossRef]
5. Lamont RF, Sobel JD, Akins RA, Hassan SS, Chaiworapongsa T, Kusanovic JP, et al. The vaginal microbiome: new information about genital tract flora using molecular based techniques. BJOG 2011; 118: 533-49. [CrossRef]
6. Reid G, Younes JA, van der Mei HC, Gloor GB, Knight R, Busscher HJ. Microbiota restoration: natural and supplemented recovery of human microbial communities. Nat Rev Microbiol 2011; 9: 27-38. [CrossRef]
7. Eschenbach DA, Davick PR, Williams BL, Klebanoff SJ, Young-Smith K, Critchlow CM, et al. Prevalence of hydrogen peroxide-producing Lactobacillus species in normal women and women with bacterial vaginosis. J Clin Microbiol 1989; 27: 251-6. [CrossRef]
8. Barbes C, Boris S. Potential role of lactobacilli as prophylactic agents against genital pathogens. AIDS Patient Care STDS 1999; 13: 747-51. [CrossRef]
9. Fredricks DN. Molecular methods to describe the spectrum and dynamics of the vaginal microbiota. Anaerobe 2011; 17: 191-5. [CrossRef]
10. Fredricks DN, Fiedler TL, Marrazzo JM. Molecular identification of bacteria associated with bacterial vaginosis. N Engl J Med 2005; 353: 1899-911. [CrossRef]
11. McGroarty JA. Probiotic use of lactobacilli in the human female urogenital tract. FEMS Immunol Med Microbiol 1993; 6: 251-64. [CrossRef]
12. Al-Baghdadi O, Ewies AA. Topical estrogen therapy in the management of postmenopausal vaginal atrophy: an up-to-date overview. Climacteric 2009; 12: 91-105. [CrossRef]
13. Simon JA. Vulvovaginal atrophy: new and upcoming approaches. Menopause 2009; 16: 5-7. [CrossRef]
14. Donders G. Diagnosis and management of bacterial vaginosis and other types of abnormal vaginal bacterial flora: a review. Obstet Gynecol Surv 2010; 65: 462-73. [CrossRef]
15. Sobel JD. Bacterial vaginosis. Annu Rev Med 2000; 51: 349-56.
16. Schöni M, Graf F, Meier B. Behandlung von Vaginalstörungen mit Döderlein-Keimen. SAZ 1988; 126: 139-42. [CrossRef]
17. Witkin SS, Alvi S, Bongiovanni AM, Linhares IM, Ledger WJ. Lactic acid stimulates interleukin-23 production by peripheral blood mononuclear cells exposed to bacterial lipopolysaccharide. FEMS Immunol Med Microbiol 2011; 61: 153-8. [CrossRef]
18. Kanne B, Beyerle-Müller HL, Patz B, Wackerle L. Die antibiotische Aktivität der Döderleinflora zur lokalen Behandlung vaginaler Infektionen mit lebensfähigen Döderleinkeimen und Estriol. Jatros Gynäkologie 1986; 2: 3-28.
19. Boris S, Barbes C. Role played by lactobacilli in controlling the population of vaginal pathogens. Microbes Infect 2000; 2: 543-6. [CrossRef]
20. Ozkinay E, Terek MC, Yayci M, Kaiser R, Grob P, Tuncay G. The effectiveness of live lactobacilli in combination with low dose oestriol (Gynoflor) to restore the vaginal flora after treatment of vaginal infections. BJOG 2005; 112: 234-40. [CrossRef]
21. Witkin SS, Linhares IM, Giraldo P, Ledger WJ. An altered immunity hypothesis for the development of symptomatic bacterial vaginosis. Clin Infect Dis 2007; 44: 554-7. [CrossRef]
22. McMillan A, Dell M, Zellar MP, Cribby S, Martz S, Hong E, et al. Disruption of urogenital biofilms by lactobacilli. Colloids Surf B Biointerfaces 2011; 86: 58-64. [CrossRef]
23. Vooijs GP, Geurts TBP. Review of the endometrial safety during intravaginal treatment with estriol. Eur J Obstet Gynecol Reprod Biol 1995; 62: 101-6. [CrossRef]

24. Englund DE, Elamsson KB, Johansson EDB. Bioavailability of oestriol. Acta Endocrinol (Copenh) 1982; 99: 136-40.
25. Keller PJ, Riedmann R, Fischer M. Estrone, estradiol and estriol content after intravaginal application of estriol in postmenopause. Gynäkol Rundsch 1980; 20: 77-9.
26. Trinkaus M, Chin S, Wolfman W, Simmons C, Clemons M. Should urogenital atrophy in breast cancer survivors be treated with topical estrogens? Oncologist 2008; 13: 222-31. [CrossRef]
27. Reid G, Bruce AW. Selection of lactobacillus strains for urogenital probiotic applications. J Infect Dis 2001; 183: 77-80. [CrossRef]
28. Kaiser RR, Michael-Hepp J, Weber W, Graf F, Lauritzen C. Absorption of estriol from vaginal tablets after single and repeated application in healthy, postmenopausal women. Therapiewoche 2000.
29. Abad CL, Safdar N. The role of lactobacillus probiotics in the treatment or prevention of urogenital infections--a systematic review. J Chemother 2009; 21: 243-52.
30. Barrons R, Tassone D. Use of Lactobacillus probiotics for bacterial genitourinary infections in women: a review. Clin Ther 2008; 30: 453-68. [CrossRef]
31. Lauritzen C, Graf F, Mucha M. Restoration of the physiological vaginal environment with Doederlein bacteria and estriol. Frauenarzt 1984; 4.
32. Horvath B, Hadarits F, Szabo L, Serenyi P, Godo G. Probiotic treatment of vaginal infections. A prospective study of the treatment of 144 patient with Gynoflor vaginal tablets. Journal of Hungarian Association for Obstetrics and Gynecology [Magyar Nöorv.Labj] 2004; 67: 85-91.
33. Melczer Z, Langmar Z, Paulin F. Experience with local ecotherapy in the treatment of bacterial vaginosis in pregnant and non-pregnant women. Journal of Hungarian Association for Obstetrics and Gynecology [Magyar Nöorv.Labj] 2002; 65: 319-23.
34. Grishchenko OV, Dudko VL, Lakchno IV, Storchak AV. Gynoflor application in preoperative preparation complex for women suffering from colpoptosis. Women Reproductive Health (WHR) 2007; 2.
35. Tatarchuk TF, Zacharenko NF, Kosey NV, Suhorebraya EI. Function of vaginal ecosystem in postoperative complications prophylaxis. "Women Reproductive Health" (WHR) 2006; #4.
36. Parent D, Bossens M, Bayot D, Kirkpatrick C, Graf F, Wilkinson FE, et al. Therapy of bacterial vaginosis using exogenously-applied Lactobacilli acidophili and a low dose of estriol. Arzneimittelforschung 1996; 46: 68-73.
37. Amsel R, Totten PA, Spiegel CA, Chen KCS, Eschenbach DA, Holmes KK. Nonspecific vaginitis. Diagnostic criteria and microbial and epidemiological associations. Am J Med 1983; 74: 14-22. [CrossRef]
38. Donders GG, Van Bulck B, Van de Walle P, Kaiser RR, Pohlig G, Gonser S, et al. Effect of Lyophilized Lactobacilli and 0.03 mg Estriol (Gynoflor(R)) on Vaginitis and Vaginosis with Disrupted Vaginal Microflora: A Multicenter, Randomized, Single-Blind, Active-Controlled Pilot Study. Gynecol Obstet Invest 2010; 70: 264-72. [CrossRef]
39. Unzeitig V, Al Awad H. New options of stabilization of disturbed vaginal environment balance. Prakticka Gynekologia 2006; 10: 170-3.
40. Larsson PG, Stray-Pedersen B, Ryttig KR, Larsen S. Human lactobacilli as supplementation of clindamycin to patients with bacterial vaginosis reduce the recurrence rate; a 6-month, double-blind, randomized, placebo-controlled study. BMC Womens Health 2008; 8: 3. [CrossRef]
41. Larsson PG, Brandsborg E, Forsum U, Pendharkar S, Andersen KK, Nasic S, et al. Extended antimicrobial treatment of bacterial vaginosis combined with human lactobacilli to find the best treatment and minimize the risk of relapses. BMC Infect Dis 2011; 11: 223. [CrossRef]
42. Biglia N, Peano E, Sgandurra P, Moggio G, Panuccio E, Migliardi M, et al. Low-dose vaginal estrogens or vaginal moisturizer in breast cancer survivors with urogenital atrophy: a preliminary study. Gynecol Endocrinol 2010; 26: 404-12. [CrossRef]
43. Pfeiler G, Glatz C, Königsberg R, Geisendorfer T, Fink-Retter A, Kubista E, et al. Vaginal estriol to overcome side-effects of aromatase inhibitors in breast cancer patients. Climacteric 2011; 14: 339-44. [CrossRef]
44. Kanne B, Patz B, Wackerle L. Lokale Behandlung vaginaler Infektionen mit Döderlein-Keimen und Estriol im Klimakterium und Senium. Frauenarzt 1986; 3: 35-40.
45. Kanne B, Jenny J. Local administration of low-dose estriol and vital Lactobacillus acidophilus in postmenopause. Gynäkol Rundsch 1991; 31: 7-13. [CrossRef]
46. Feiks A, Grünberger W. Therapie der atrophen Kolpitis - Ist eine Reduktion der Östrogendosis bei lokaler Anwendung möglich? Gynäkol Rundsch 1991; 31: 268-71. [CrossRef]
47. Hengst P, Uhlig B, Bollmann R, Kokott T. Uses of vaginal pH measurement for prevention of premature labor. Results of a prospective study. Z Geburtshilfe Perinatol 1992; 196: 238-41.
48. Hoyme UB, Grosch A, Roemer VM, Saling E. Erste Resultate der Erfurter Frühgeburten-Vermeidungs-Aktion. Zeitschrift für Geburtshilfe & Neonatologie 1998; 6: 247-50.
49. Hoyme UB, Saling E. Efficient prematurity prevention is possible by pH-self measurement and immediate therapy of threatening ascending infection. Eur J Obstet Gynecol Reprod Biol 2004; 115: 148-53. [CrossRef]

Pregnancy associated breast cancer and pregnancy after breast cancer treatment

Gebelik ile ilişkili meme kanseri ve meme kanseri tedavisi sonrası gebelik

Emek Doğer[1], Eray Çalışkan[1], Peter Mallmann[2]

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

[2]Department of Obstetrics and Gynecology, Universitäts-Frauenklinik, Köln, Germany

Abstract

Breast cancer is one of the most common cancers diagnosed during pregnancy and its frequency is increasing as more women postpone their pregnancies to their thirties and forties. Breast cancer diagnosis during pregnancy and lactation is difficult and complex both for the patient and doctors. Delay in diagnosis is frequent and treatment modalities are difficult to accept for the pregnant women. The common treatment approach is surgery after diagnosis, chemotherapy after the first trimester and radiotherapy after delivery. Even though early stage breast cancers have similar prognosis, advanced stage breast cancers diagnosed during pregnancy and lactation have poorer prognosis than similar stage breast cancers diagnosed in non-pregnant women. Women who desire to become pregnant after treatment of breast cancer will have many conflicts. Although the most common concern is recurrence of breast cancer due to pregnancy, the studies conducted showed that pregnancy has no negative effect on breast cancer prognosis. In this review we search for the frequency of breast cancer during pregnancy, the histopathological findings, risk factor, diagnostic and treatment modalities. We reviewed the literature for evidence based findings to help consult the patients on the outcome of breast cancer diagnosed during pregnancy and lactation, and also inform the patients who desire to become pregnant after breast cancer according to current evidences. (J Turkish-German Gynecol Assoc 2011; 12: 247-55)

Key words: Breast cancer, pregnancy

Received: 8 September, 2011 **Accepted:** 11 September, 2011

Özet

Meme kanseri, gebelik sırasında en sık tanı alan kanserlerden biridir ve daha fazla kadın gebeliklerini otuzlu, kırklı yaşlara erteledikçe sıklığı artmaktadır. Gebelik ve emzirme sürecinde meme kanseri tanısı hem hasta hem de doktoru için zor ve karmaşıktır. Tanıda gecikmeye sık rastlanır ve gebeler için tedavi yöntemlerini kabul etmek zordur. Tedavi yaklaşımı sıklıkla, tanı sonrası cerrahi, ilk trimester sonrası kemoterapi ve doğum sonrası radyoterapi şeklindedir. Erken evre meme kanserleri benzer prognoza sahip olmasına rağmen, gebelik ve emzirme sırasında ileri evrede tanı alan meme kanserleri, aynı evredeki gebe olmayan meme kanserli hastalardan daha kötü prognoza sahiptir. Meme kanseri tedavisinden sonra gebe kalmak isteyen kadınlar da pek çok çelişki ile yüzleşirler. Her ne kadar en sık endişelenilen, gebelik sırasında meme kanseri rekürrens göstermesi olsa da çalışmalar gebeliğin meme kanseri prognozu üzerine olumsuz etkisi olmadığını göstermiştir. Bu derlemede, gebelik sırasında meme kanseri sıklığını, histopatolojik bulgularını, risk faktörlerini, tanı ve tedavi yöntemlerini araştırdık. Literatürü, kanıta dayalı bulgular ile, gebelik ve emzirme sırasında tanı alan meme kanserinin seyrini ortaya koymak, ayrıca meme kanseri sonrası gebe kalmak isteyen hastaların güncel kanıtlarla uyumlu olarak bilgilendirilmesini sağlamak amacıyla derledik.

(J Turkish-German Gynecol Assoc 2011; 12: 247-55)

Anahtar kelimeler: Meme kanseri, gebelik

Geliş Tarihi: 08 Eylül 2011 **Kabul Tarihi:** 11 Eylül 2011

Introduction

Among the cases with breast cancer, 0.2-3.8% are diagnosed during pregnancy and lactation (1). The frequency increases as the patients' age become younger and among women younger than 30 years of age 10-20% of the breast cancer cases are diagnosed during pregnancy or wthin one year after delivery (2). The breast cancer diagnosed during pregnancy or within one year after delivery is known to be pregnancy associated breast cancer (PABC) and its incidence is 1.3 in 10.000 births (3).

Breast cancer frequency is also increasing in Turkey (4). More women are postponing pregnancy to older ages as they have increasing social participation. The mean age of first pregnancy in Europe increased from 26.2 years in 1970 to 29.8 years in 2005 and approached the mean age of breast cancer diagnosis during pregnancy, which is 33 years (Range: 22-43) (5-7). All these data point out that we will encounter more cases of PABC in the future. On the other hand, improvements in breast cancer therapy lead to more women desiring pregnancy in fertile ages after completing the cancer therapy (8). In population based studies, pregnancy after completing breast cancer therapy among women younger than 45 years of age is 3.6-5% (9, 10).

Most of the signs of PABC are seen as normal consequences of pregnancy and breast feeding and usually doctors do not take the complaints into account. Due to concerns of radiation based diagnostic modalities during pregnancy, both the patients and doctors delay screening, and most patients with PABC are diagnosed in advanced stages (11). As large randomized controlled trials are lacking, an evidence based management algorithm of PABC cases is still lacking. The use and long term safety of chemotherapeutics in pregnancy

Address for Correspondence: *Emek Doğer, Department of Obstetrics and Gynecology, Faculty of Medicine, Kocaeli University, Kocaeli, Turkey*
Phone: +90 262 303 84 33 Fax: +90 262 303 80 03 e.mail: emekdoger@hotmail.com

doi:10.5152/jtgga.2011.58

and lactation is still a matter of debate and most patients reject proper treatment because of inadequate consultation. The questions to be answered are the prognosis of PABC, the effect of pregnancy after breast cancer on recurrence and mortality rates and deciding on the appropriate time to delay a new pregnancy after PABC.

In this review we tried to summarize the findings in the literature about treatment modalities of PABC, effect of pregnancy on prognosis and provide the evidence to inform the women accordingly.

1. Pregnancy Associated Breast Cancer (PABC)

1. 1. Pathgological Findings of Pregnancy Associated Breast Cancer

The most common histological type of PABC is invasive ductal carcinoma in 75-90% of the cases (12). Invasive lobular carcinoma and inflammatory carcinoma are less frequent. Most of the breast carcinoma diagnosed in pregnant women are grade 2 or 3 tumors with frequent invasion of the lymphovascular space (13).

Estrogen and progesterone receptors are found in 20-40% of the PABC cases (12-14). The estrogen and progesterone receptors are also frequently negative in non-pregnant women younger than 40 years of age and the presence of receptors is more frequent in breast cancers diagnosed after the menopause (15). Ishida and colleagues found that estrogen receptor was negative in 70% of the tumors in pregnant women while this was 39% in non-pregnant women of similar ages (16). This might be due to negative feedback of high hormone levels during pregnancy or high hormone levels might lead to false negative findings (17). In fact the presence of hormone receptors might give conflicting results with different techniques. For example, cases with a previous diagnosis of negative estrogen receptor were re-evaluated with pS2-trefoil factor 1 immunohistochemical staining and were found to have the estrogen receptors (18). It is probable that the presence of estrogen receptors is underestimated and evolving techniques might help to decide accurately on which patients would need anti-hormonal therapy.

In general, HER2/neu oncoprotein is expressed in 25-30% of the cases with breast cancer, which is present in 28-50% of pregnant women with breast cancer who are younger than 35 years of age (12, 19). Within the same age groups, some studies report higher HER2/neu expression in PABC than non-pregnant women (58% vs 16% respectively) while others report a similar expression of around 28% (12, 19).

1. 2. Pregnancy and risk of breast cancer

Age and pregnancy related risk: The cancer risk is higher within 3-15 years of term delivery (20, 21). This increased risk is specific for women whose first delivery occurred after 30 years of age. In women who delivered their first baby at ages younger than 25, none or a very small increase in breast cancer risk is observed (22). Accordingly, age is accepted as the main risk factor. A transient increase in breast cancer is seen within 10 to 15 years of delivery and after that a protective effect of delivery is seen. Among women who delivered their first baby before 25 years of age, the life time breast cancer risk is decreased by 36% after the period of transient increase (23). Among women who delivered their first baby after 30 years of age, the transient increase in breast cancer risk is higher and may last 30-50 years whereas the protective effect after that time is low or none (24, 25). Every year of increase in the age of first delivery leads to a 3.5-5% increase in lifetime breast cancer risk (24).

Family history and parity related risk: Both the familial history and advancing age have a synergistic increase in breast cancer risk. If the age at first pregnancy is older than 30 years, women with a familial history of breast cancer have a 2-3 fold higher incidence of breast cancer risk than women without a familial history (26). Even in women who gave birth at a younger age, familial history increases the risk of breast cancer (26).

In the study of Albrektsen et al. every increase in parity was shown to decrease the total risk of breast cancer compared to nulliparous women; second birth RR=0.91, third birth RR=0.81, fourth birth RR=0.64 and fifth birth RR=0.50) (22). After the birth of the second child, the risk of breast cancer is decreased and if it develops, it occurs at later ages compared to nulliparous women (21, 27). Multiparity decreases the estrogen and progesterone receptor positive breast cancer risk if breast feeding is carried out (23). On the other hand, if breast feeding is not carried out, parity does not decrease the risk and parity does not affect the incidence of hormone receptor negative breast cancer incidence (23).

Breast feeding: Lactation decreases the life time risk of breast cancer. This decrease is 4.3% for every 12 months of lactation and 7% for every additional birth (28). Within five years after birth, the risk of breast cancer is 1.64 in non breast feeding women and 1.24 in breast feeding women (29). Although the decrease in risk in BRCA 2 mutation carriers is controversial, in BRCA 1 mutation carriers breast feeding for more than one year significantly decreases the risk compared to non breast feeding carriers (OR=0.55, 95% CI=0.38-0.80, p=0.001) (30). The importance of breast feeding should be stressed in women with BRCA mutations.

BRCA 1 and BRCA 2 mutations: BRCA 1 and BRCA 2 related breast cancer is diagnosed in younger women whose probability of becoming pregnant is high. In BRCA 1 mutation carriers, the OR of PABC is 3.9, in BRCA 1 carriers the odds ratio is 1.9 and when combined BRCA 1 and 2 mutation is present the odds ratio for PABC is 4.5 (31). Culliane et al. showed that the risk of breast cancer decreases significantly in BRCA 1 carriers after four or more births while parity increases the risk of breast cancer in BRCA 2 carriers which is 1.5 fold of nulliparous women after two or more births (32). In BRCA 2 mutation carriers, breast cancer risk within two years after delivery is 70% higher than for nulliparous women (32). These studies point out the importance of follow up of BRCA 2 carriers during and after breast feeding.

The cause of increased breast cancer risk after pregnancy: The probable causes of increase in breast cancer risk after pregnancy are increased malignant transformation of breast cancer cells due to estrogen, progesterone and growth hormones secreted during pregnancy, the immune suppressive effects of pregnancy and breast involution after pregnancy (24, 27, 33). Once the pregnancy reaches term, terminal differentiation of breast glands are induced and the breast becomes less vulnerable to tumorigenic effects. The shorter the time to first pregnancy after puberty the faster the induction of breast glands to terminal differentiation. On the other hand, when the first pregnancy is delayed the breast will be more vulnerable to

oncogenic effects and will have a higher risk of development of premalignant lesions. During pregnancy, very high levels of estrogen and its metabolites will have genotoxic, mutagenic and carcinogenic potential and may cause progression of premalignant lesions. The proliferation of breast cancers cells are enhanced by estrogen, progesterone and insulin like growth factor-1 (24).

Breast involution after pregnancy may also enhance tumorigenesis. In fact, breast involution resembles wound healing and is pre-tumorigenic in the presence of immune suppression. The microenvironment during involution has immune cell interaction, active trophobasts, increased extracellular matrix deposition, increased matrix metalloproteinase levels and bioactive matrix fragments like a pretumorigenic micro environment (33, 34).

Current studies showed that parity decreases stem cells in the breast in animals (35). Stem cells in the breast are the main targets for transformation and the decrease in their number also decreases the lifetime risk of breast cancer.

1. 3. The diagnosis of pregnancy associated breast cancer (PABC)

Breast cancer diagnosed during pregnancy and breast feeding is usually advanced in stage with a bulky tumor and a lymph node. PABC is diagnosed in stage II-III in 65-90% of cases, while these numbers are 45-66% in other breast cancers (12, 16). The average tumor diameter is 3.5 cm and lymph node metastasis is 56-89% in pregnant women, while it is 2 cm and 38-54% respectively in non-pregnant women (1, 16, 36, 37). The reasons for delayed diagnosis in PABC are increased vascularity in breast, high hormone levels and immunosupression during pregnancy. Normal breast hyperplasia during pregnancy and lactation masks the diagnosis of palpable masses. Both the doctor and the patient detain radiographic and invasive procedures at this time, which contributes to the diagnostic delay in PABC. A one month delay in the diagnosis of primary tumor increases the axillary lymph node metastasis by 0.9%, three month delay by 2.6% and a six month delay by 5.1% (38). An average delay in definitive diagnosis after detection of a mass in pregnant women is 0.8 to 8.1 months (16, 39). In contrast, Ives et al found an average of one month (1-104 weeks) from symptom to diagnosis in pregnant breast cancer cases and Ibrahim et al found symptom to diagnosis time to be shorter in pregnant breast cancer cases than the nonpregnant cases (5.6 months versus 9.4 months respectively) (40, 41). The summary of studies on this issue is presented in Table 1 (13, 16, 39-44).

For early diagnosis of PABC, all pregnant women should have a breast examination during the first antenatal visit. Palpable breast masses and bloody breast discharge should be evaluated carefully and it should be kept in mind that the newborn may not suck the breast with cancer. In order to prevent diagnostic delay in PABC all masses persisting for more than two weeks should be biopsied although 80% of them will be benign (1). Atypical cytological findings are common in pregnant women with normal breasts so biopsy is the preferred method rather than aspiration cytology (45). Biopsy increases the frequency of milk fistulas and infection but stopping breast feeding before biopsy, careful hemostasis and prophylactic antibiotics will decrease the risk of complications (46).

1. 4. Imaging in pregnant women with breast cancer

Many imaging modalities expose the fetus to ionizing radiation. If the fetus is exposed to radiation within two weeks after conception, it results in the loss of pregnancy, while developing organ systems are affected 8 weeks following this period. If the radiation dose is more than 0.05 Gy during organogenesis it results in major malformations and if the radiation dose is more than 0.28 Gy it might result in mental retardation (47, 48). In pregnant women if the abdomen is protected, thoracic radiation exposes the fetus to less than 0.0001 Gy radiation and this dose is considered to be safe (49). In suspected cases, mammography can be performed but its sensitivity is 63-78% during pregnancy (16, 42). Besides these limitations, mammography is useful for detecting microcalcifications. Fetal radiation dose due to mammography is 0.01-0.004 Gy (11, 50). Upper abdominal or thoracic computed tomography delivers 0.0036 Gy radiation and is considered to be safe for the fetus, while lower abdominal tomography scan delivers a unwanted amount of 0.089 Gy radiation (49).

Breast ultrasonography can be used to diagnose breast cancer cases with high sensitivity and specificity. In their study on 20 preoperative breast cancer cases, Yang et al. found that a cancer lesion was detected in 18 cases using mammography compared to 20 detected cases with ultrasonography and furthermore ultrasonograhy detected axillary lymph node metastasis in 15 out of 18 cases (50). Although some concerns about

Table 1. The time interval from the initial symptoms to the diagnosis among the patients with the diagnosis of pregnancy associated breast cancer and other breast cancer patients

	Year	PABC (month)	(case)	(month)	Non-pregnancy (control)	Assoc.	Difference
Applewhite et al.[a]	1973	13.2	(48)	5.1	(2689)		8.1
King et al.	1985	1.4	(63)	-			-
Tretli et al.[a]	1988	2.5/6[b]	(20/35[b])	-		-	
Ishida et al.[a]	1992	6.2	(72/120[c])	5.4	(191)		0.8
Libermann et al.	1994	8.2	(12/19[b])	1.9	(11)		6.3
Bonnier et al.[a]	1997	2.2	(154)	1.2	(308)		1
Ibrahim et al.	2001	5.6	(72)	9.4	(216)		3.8

[a]Include postpartum patient, [b]lactation group [c]include first 2 years of postpartum period, PABC: Pregnancy associated breast cancer

magnetic resonance imaging were mentioned, it can be used to detect breast cancer and its metastasis to liver, bone and brain (51). Godolinium contrast is not used in pregnant women as it was shown to pass the placenta and cause malformations in mice (52). As in non-pregnant women, screening the bones is not necessary in stage I and II tumors (37).

1. 5. Surgical treatment modalities in pregnant breast cancer patients

An individualized approach should be planned for every case to provide the most appropriate treatment and protect the ongoing pregnancy. Surgery should be scheduled in every case without considering the trimester. Usually, modified radical mastectomy or breast sparing surgeries with axillary lymph node dissections are performed. In the case of stage I and II tumors, total survival and disease free survival is similar between breast sparing surgery and modified radical mastectomy (53). Although survival is similar, radiotherapy is not necessary after mastectomy for early stage breast cancer, whereas after breast sparing surgery radiotherapy should be applied after delivery to avoid local recurrences (54). Although radiotherapy can be postponed after delivery in cases with breast cancer diagnosed at the second or third trimester, if the patient is far from term, starting chemotherapy after surgery and postponing radiotherapy until after delivery is advised (55). Sentinel lymph node biopsies are found to be safe and sufficient but it is not part of the routine surgery during pregnancy (56). Isosulfane mapping during sentinel lymph node biopsy carries a risk of anaphylaxis and its possible effects on the fetus are yet to be determined. There is also no data regardind the safety of radioactive probes on the fetus.

After surgery, if the pregnancy is in its first trimester, chemotherapy should be postponed until the second trimester and if the woman is in the second or third trimester, chemotherapy is started immediately after surgery, if indicated. If surgery was performed near term chemotherapy can also be postponed until after delivery. On the other hand, if indicated neoadjuvant chemotherapy can be given in the second and third trimester and surgery can be performed after (57). General anesthesia has little risk for the pregnant women and the fetus, also complication rates after breast and axillary surgery are not increased in pregnant women. On the other hand, the rate of low birth weight, premature delivery and intrauterine growth restriction is increased in breast cancer cases (58).

1. 6. Radiotherapy in pregnant women with breast cancer

Although radiation has dose and gestational week dependent effects on the fetus, radiotherapy can be applied during pregnancy after a careful dose adjustment and if not targeting the pelvis or abdomen (59). Breast, chest wall and lymph nodes should be the primary targets at radiotherapy. Radiotherapy is relatively safer during the first and second trimester when the fetus is far from the targeted area, but during the third trimester the fetus comes closer to the radiotherapy area and can receive a dangerous amount of radiotherapy (60). Radiation can cause intrauterine growth restriction, mental retardation and childhood cancers (47, 48). As this age group,of women have poorer prognosis, their management should include chemotherapy after surgery and radiotherapy postponed until after delivery.

1. 7. Systemic chemotherapy in pregnant women with breast cancer

The standard approach is applying chemotherapy to all breast cancer cases if the tumor size is larger than one centimeter or if there is lymph node metastasis. When planning chemotherapy to pregnant women with breast cancer, increased plasma volume, increased glomerular filtration rate, liver metabolism, and changes in plasma protein concentrations should be kept in focus, whereas body mass index is the only important parameter in non-pregnant breast cancer cases (61). Also the placental transfer and fetal effects of the chemotherapeutics should be considered.

Cyclophosphamide, adriamycine and fluorouracil combination is frequently used during pregnancy and is well tolerated. Anthracycline based chemotherapy can be used in pregnant women after the first trimester (62). Current evidence showed that taxanes do not pose any risk to the fetus beyond the first trimester (63). Taxanes can also be appropriate for neoadjuvant chemotherapy before surgery or as a second line drug for anthracycline resistant cases. Alkylating agents and methotrexate are not used during pregnancy (62). HER2/neu is synthesized from many embryonic tissues during pregnancy. In a limited series of case reports Trastuzumab, a monoclonal antibody that interferes with the HER2/neu receptor, was shown to reverse anhydramnios, fetal renal and heart failure and in one case had no effect on the fetus (64, 65). In the presence of limited data anti-HER2 therapies are not recommended during pregnancy and if there is medical necessity for their use, a close follow up of the amniotic fluid is mandatory. Selective estrogen receptor modulator tamoxiphene used in cases of hormone receptor positive breast cancers is teratogenic and may cause craniofacial malformations and ambiguous genitalia (66). Aromatase inhibitors were reported to be teratogenic in animals but there is insufficient data in humans (67).

The possible harmful effects of chemotherapeutics on the fetus largely rely on the week of gestation. If used during the first trimester chemotherapeutics cause abortion and malformation at a rate greater than 17%, on the other hand during the second and third trimester pregnancy loss and malformation is seen in 1.3% of the cases while preterm delivery, intrauterine growth restriction, neurologic developmental delay, cardiotoxicity and carcinogenesis are more commonly encountered (57). Whether these complications result from breast cancer, chemotherapy or surgery is yet to be determined. In their study, Berry et al found that in 24 women receiving cyclophosphamide, adriamycine and 5-flourouracil, no malformation was seen but three preterm births, two transient tachypnea, one low birth weight occurred with hyaline membrane disease and transient leucopenia (68). In their study of locally invasive breast cancer during the second and third trimester of pregnancy, Hahn et al applied adjuvant chemotherapy to 32 cases and neoadjuvant chemotherapy to 25 cases, with a mean of four cycles of 5-FU, doxurubicin and cyclophosphamide combination (69). The patients delivered at a median gestational age of 37 weeks with one maternal death due to pulmonary embolism after cesarean delivery but no intrauterine or perinatal fetal death, only 10% need for ventilation and one fetus with neutropenia, thrombocytopenia and subarachnoid bleeding (69). In the light of the literature, chemotherapy can be applied safely after the first trimester of pregnancy. If indicated, chemotherapy should not

be postponed in the second and third trimesters of pregnancy. Also inducing the pregnancy early to apply chemotherapy is not right, as prematurity related problems will cause more problems than the chemotherapy itself (60). However, if the pregnant woman refuses to receive chemotherapy, labor can be induced at 32-34 weeks of gestation. After chemotherapy myelosuppression may increase the risk of sepsis and postpartum hemorrhage, while low blood counts can also be seen in the newborn (70). In order to avoid myelosupppression during delivery, it is better to discontinue chemotherapy after 34th week of gestation, three weeks earlier from delivery. Vaginal birth is the preferred method rather than cesarean section as maternal morbidity is lower.

Long term effect of chemotherapy is still a matter of debate. In a large study, 82 fetuses subjected to chemotherapy during maternal treatment of hematological malignancies were followed up until a mean of 18.7 years of age: no malignancies were encountered and normal physical, neurological and psychological development was observed (71).

Breast feeding is not recommended in mothers receiving chemotherapy, biological therapy, endocrine therapy or radiotherapy (25). Almost all therapeutics are secreted in the milk, and breast feeding should start four weeks after the last cure.

1. 8. Prognosis

The prognosis of pregnancy associated breast cancer is similar to breast cancers unrelated to pregnancy when matched according to age and stage of the disease (13, 33, 72-74). On the other hand, several existing data indicate that advaced stage cancer prognosis is worst in the pregnancy associated group when compared to stage matched cases unrelated to pregnancy (13, 16, 75, 76). In fact, the diagnosis in PABC cases is almost always delayed (13, 16, 39, 76). PABC also have unfavorable biological features related to poor prognostic outcome such as high grade tumor, low hormone receptors, increased HER2/neu expression and high ki-67 nuclear antigen (12, 24, 77). The risk of being in an advanced stage at the time of diagnosis is 2.5 times higher in PABC compared to other cases (24). Overall survival rate in PABC is 40-73% compared to 48-97% in other breast cancer cases. Five years and 10 years survival rates among pregnancy associated breast cancer and other breast cancer patients is presented in Table 2 (13, 16, 18, 43, 53, 63, 73, 74, 76, 78-84).

Petrek et al. showed that the five year survival rate is 82% in cases of PABC and other cases if lymph node metastasis is not present (79). On the other hand, they also showed that if lymph node metastasis is present the five year survival rate in PABC cases is 47% which is lower than the 59% in pregnancy unrelated breast cancer cases (79). Similar findings have also been confirmed by other studies showing the worst prognosis of PABC in stage II-IIIa cases (13, 16, 76).

Poor prognosis was reported in breast cancer cases diagnosed in the postpartum period which was independent of age, stage and additional biological features of the tumor (80, 81). In a study focusing on the cases of postpartum diagnosed breast cancer, Whiteman et al. showed that the poorest survival rate of 38% was seen in cases diagnosed within 12 months after delivery, which was 51% in cases diagnosed at 13-48 months postpartum, and 60% in cases diagnosed after 48 months postpartum compared to the 65% survival among age matched

Table 2. Five year and 10 year survival rates among pregnancy associated breast cancer and other breast cancer patients

	Year	Number of Patient Cases/Control (n)	PABC Survival Rate 5 years (%)	10 years (%)	Other breast cancer Survival Rate 5 years (%)	10 years (%)
Nugent ve O'Connel	1985	19/157	56	-	56	-
King et al.	1985	63	75[b]/33[c]	-	-	-
Pertek et al.	1991	63	61	45	73	62
Ishida et al.[a]	1992	192/191	-	55	-	7
Zemlickis et al.	1992	118/269	-	40	-	48
Chang et al.	1994	21/199	57	-	70	-
Guinee et al.[a]	1994	66	40	-	70	-
Anderson et al.	1996	22/205	-	73[d]/17[e]	-	74[d]/47[e]
Bonnier et al.a	1997	114/280	61	-	75	-
Kuerer et al.	1996	26	60[b]/45[c]	-	-	-
Bladström et al.[a]	2003	94/7799	52	44	80	69
Whiteman et al.	2004	60	-	%38[f]	-	65f
Mathelin et al.[a]	2008	40/61	-	72[g]/63[h]	-	97
Beadle et al.	2009	104/668	-	63[g]/65[h]	-	65
Stensheim et al.	2009	105	-	56[i]	-	69i
Cardonick et al.	2010	130	100[j]/86[k]/0l	-	-	-

[a]Statistically significant survival difference (p<0.01), [b]lymph node involvement negative, [c]lymph node involvement positive, [d]Stage I-IIa, [e]Stage IIb-III, [f]15 years survival, [g]Group includes only pregnant patients, [h]Group includes only postpartum patients, [i]Survival rate of 60th years. [jkl]After 3.14±2.5 years follow up survival rate according to stage, [j]Stage I, [k]Stage II ve III, [l]Stage IV, PABC: Pregnancy associated breast cancer

nulliparous women (80). Steinsheim et al also showed that the highest breast cancer related death rate of 67% was seen in breast cancer cases diagnosed within six months after delivery and the overall death rate was 44% in pregnant group compared to 31% in the control group (81).
There is no evidence that termination of pregnancy favorably affects the prognosis (85). On the other hand, if stage II or IV disease was diagnosed during the first trimester, if there is an aggressive primary tumor and if the expected survival is shorter than the duration of pregnancy, then pregnancy termination can be an option (11).
Despite these findings, there is still uncertainty if the pregnancy or postpartum period is an independent prognostic factor on breast cancer. In most studies, case and control groups are extracted from national registries which causes methodological problems in comparisons due to age and stage matching, inadequate data on biological features of the tumor, different treatment modalities and postponing of chemotherapy to the postpartum period by many patients.

2. Pregnancy After Breast Cancer Treatment

Fertility history of the women is a well known risk factor for breast cancer and estrogen is a known growth factor for breast cancer (24, 27, 33, 86, 87). It was hypothesized that the induction of tumor growth was caused by the hormonal changes of pregnancy which results in the poor prognosis of advanced stage breast cancer diagnosed during pregnancy or the postpartum period. It is not clear whether a similar relationship exists in pregnancies occurring after breast cancer treatment. The possibility that high hormonal levels during pregnancy might induce dormant micrometastases is the main source of anxiety for the patient and her doctor.

2. 1. The effect of pregnancy after breast cancer treatment on survival, recurrence and distant metastases

Studies targeting the effect of pregnancy after breast cancer treatment on the survival of the patients generally agreed that it has no adverse effect on the prognosis of the disease and survival rate. The first population based studies showed that the relative risk of pregnancy after breast cancer on survival and distant metastases is 0.2 and 0.4 respectively (88, 89). The subgroup analysis of term deliveries, preterm deliveries and abortions after breast cancer treatment showed that none worsens the prognosis even after considering age at diagnosis, tumor size, lymph node metastasis, time from prior pregnancy to breast cancer diagnosis and time from breast cancer diagnosis to last pregnancy (9, 86). These studies statistically lower breast cancer related death risk (RR=0.73) if a term delivery is present in low risk tumors (86). Mueller et al. found that pregnancy after breast cancer treatment lowers the death risk (RR=0.54) which was statistically lower in cases younger than 35 years, white ethnicity, tumor size larger than 2 cm (90). In another study, age and stage adjusted analysis revealed a lower relative risk for death (0.8) in cases who became pregnant after breast cancer treatment in which the survival in cases and controls were similar according to tumor size, the extent of surgery, need for chemotherapy or radiotherapy (91). Blakely et al. showed that, in 370 breast cancer cases younger than 35 years of age, 47 became pregnant after adjuvant chemotherapy and pregnancy did not increase the recurrence or death risk (92). Ives et al. also found that pregnancy after breast cancer treatment did not adversely effect survival, but better survival rates were found in cases delaying pregnancy for 24 months or more after the end of breast cancer treatment (10). Table 3 summarizes the case control and population based studies calculating survival rate considering whether the patient got pregnant after breast cancer treatment (9, 10, 86, 88-93, 95).
In most case control studies, providing a multivariate analysis including presence of estrogen receptor, age at diagnosis, tumor size, histological grade, pregnancy after breast cancer treatment has a favourable effect on 5 to 10 years of survival (88, 93-95). This favourable effect of pregnancy on survival after breast cancer treatment was explained with the `healthy mother effect` which was first proposed by Sankila et al stating that women who can plan pregnancy are more likely to have local disease without recurrence during follow up and who are in a good general health (88). It is obvious that women with advanced tumor, early recurrence and poor general health will not consider pregnancy as an option. Comparison of these women with poorer prognosis to women who feel healthy enough to plan a pregnancy may cause a selection bias.
Besides the `healthy mother effect` theory, immune mediated `fetal antigen hypothesis` is also worth attention. Fetal antigen hypothesis is based on the assumption that fetal and maternal tissues share common antigens and isoimmunisation during pregnancy might be protective against breast cancer (96). According to the fetal antigen hypothesis, pregnancy after breast cancer treatment provokes an immune memory against fetal antigens which keeps subclinical metastases under control through humoral and specific cellular immune system (97). Also, it was shown that pregnancy after breast cancers creates high estrogen and progesterone values together with hCG which induce apoptosis in breast cancer cells with receptors for these hormones (98). The presence of estrogen receptor son breast cancer cells is mainly seen in postmenopausal women and premenopausal women becoming pregnant after breast cancer treatment have 23-34% estrogen receptor (9, 10, 92). However, the estrogen receptor status was not proven to have any effect

Table 3. RR ratios for survival if the patient became pregnant after breast cancer treatment

	Year	n (case)	RR
Population based trials			
Sankila et al.	1994	91	0.20
Von Schoultz.	1995	50	0.42-0.48[a]
Kroman et al.	1997	173	0.55
Ives et al.	2007	123	0.59
Kroman et al.	2008	371	0.73
Cohort based trials			
Malamos et al.	1996	21	0.48
Velentgas et al.	1999	53	0.80
Gelber et al.	2001	94	0.44
Müller et al.	2003	438	0.54
Blakely et al.	2004	47	0.70

[a]RR for distant metastasis after adjustment to lymph node status

on the prognosis of breast cancer if the women become pregnant (9, 10, 92).
The effect of pregnancy after breast cancer treatment is still an open question because the studies conducted on the subject are mostly retrospective case control studies depending on hospital records or patient responses. Also the unknown spontaneous or induced abortion rate after breast cancer renders exact comparisons impossible.

2. 2. The effect of abortion after breast cancer treatment on the prognosis

The pregnancies after breast cancer treatment have a 70% higher rate of both spontaneous and induced abortions compared to the general population (91). In the study of Kroman et al, among 465 pregnancies after breast cancer treatment, 41% ended with induced abortion and 7.7% ended with spontaneous abortion (9). The overall abortion rate was found to be 24-29% in several studies (91, 92). Abortion rate is higher if the women become pregnant within two years of therapy and is especially high in the first six months after therapy. Although chemotherapy triggered hormonal changes might be a reason, the main determinant of decision on abortion is the doctors' and patients' negative attitude to the pregnancy. Gelber et al showed that two thirds of induced abortions were done according to the doctors' advice (95). It is possible that in a few studies, women whose pregnancies end with abortion have a poorer prognosis than women carrying the fetus to term (86, 91). Selection bias is possible as doctors advise abortion to women with poorer prognosis more commonly. Most of the studies show no negative effect of abortion on the prognosis of breast cancer cases (9, 10, 86, 91, 92, 97). It was concluded that it is not necessary to advise abortion to breast cancers cases becoming pregnant after therapy unless there is lymph node involvement.

2. 3. What is the optimum time for pregnancy after breast cancer therapy?

As recurrences after breast cancer therapy are mostly seen in the first two years and pregnancy may interrupt the therapy, it is generally advised that patients should wait for two years to become pregnant (98, 99). In addition, due to the poorer prognosis, higher recurrence rate in young women, those younger than 35 years of age were advised to wait for three years and women with lymph node involvement were advised to wait for five years before becoming pregnant (97). In their study, Clark and Chua showed that women who became pregnant within the first six months of therapy have a 59% survival rate compared to the 92% survival rate in women who become pregnant between 6 to 24 months (100). This was not confirmed in other studies (10, 101).
Breast cancer cases who would consider pregnancy are usually at the end of their thirties or at the beginning of their forties. Chemotherapy may further decrease their ovarian reserve so cases who desire a baby but have advanced disease or critical ovarian reserve might consider surrogate motherhood as an option.
In conclusion, women experience a transient increase in breast cancer risk after pregnancy. This risk is more evident in women with a familial history of breast cancer, mutation in BRCA genes and who have their first pregnancy after 30 years of age. Pregnancy associated breast cancers might have a poorer prognosis in advanced stages compared to other advanced cases unrelated to pregnancy. Also, breast cancers which were diagnosed in the postpartum period have a poorer prognosis. If breast cancer is suspected in pregnant women, the diagnostic work-up should be similar to non-pregnant women as diagnostic delays are unacceptable costs. Surgery is acceptable in pregnant women but radiotherapy and hormonal therapy should not be used. Chemotherapy is an acceptable alternative after the first trimester but longterm effects are still obscure. Terminating the pregnancy has no favorable effect on survival.
In the light of current literature, pregnancy after stage I or II breast cancer therapy has no negative effect on the overall or five year survival rate. Also considering pregnancies after breast cancer therapy the time elapsing until pregnancy, number of pregnancies, spontaneous abortions, induced abortions, and term pregnancies have no negative effect on survival rates. Although recurrent stage I and II breast cancer cases and women with advanced stage tumor were not advised to become pregnant or to postpone their pregnancy there is scarce data in the literature to support these.
A multidisciplinary approach to pregnancy associated breast cancer is important and all decisions should include the opinion of the patient. A patient tailored approach should be designed considering disease status, desire for pregnancy, ovarian reserve and survival.

Conflict of interest

No conflict of interest was declared by the authors.

References

1. Vinatier E, Merlot B, Poncelet E, Collinet P, Vinatier D. Breast cancer during pregnancy. Eur J Obstet Gynecol Reprod Biol 2009; 147: 9-14. [CrossRef]
2. Anders CK, Johnson R, Litton J, Phillips M, Bleyer A. Breast cancer before age 40 years. Semin Oncol 2009; 36: 237-49. [CrossRef]
3. Smith L, Dalrymple J, Leiserowitz G, Danielsen B, Gilbert W. Obstetrical deliveries associated with maternal malignancy in California, 1992 through 1997. Am J Obstet Gynecol 2001; 184: 1504-12. [CrossRef]
4. Ozmen V, Ozcinar B, Karanlik H, Cabioglu N, Tukenmez M, Disci R, et al. Breast cancer risk factors in Turkish women: a University Hospital based nested case control study. World J Surg Oncol 2009; 8: 37-45. [CrossRef]
5. Loibl S, Kohl J, Kaufmann M. Reproduction after breast cancer: what advice do we have for our patients? Zentralbl Gynakol 2005; 127: 120-4. [CrossRef]
6. Upponi SS, Ahmad F, Whitaker IS, Purushotham AD. Pregnancy after breast cancer. Eur J Cancer 2003; 39: 736-41. [CrossRef]
7. Loibl S. Breast cancer during pregnancy: a prospective and retrospective European registry. In: Proceedings of the European Breast Cancer Conference: 2008; 2008.
8. Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15 year survival: an overview of the randomised trials. Lancet 2005; 365: 1687-717. [CrossRef]
9. Kroman N, Jensen MB, Wohlfahrt J, Ejlertsen B, Danish Breast Cancer Cooperative Group. Pregnancy after treatment of breast cancer--a population-based study on behalf of Danish Breast Cancer Cooperative Group. Acta Oncol 2008; 47: 545-9. [CrossRef]
10. Ives A, Saunders C, Bulsara M, Semmens J. Pregnancy after breast cancer: population based study. BMJ 2007; 334: 194. [CrossRef]

11. Navrozoglou I, Vrekoussis T, Kontostolis E, Dousias V, Zervoudis S, Stathopoulos EN, et al. Breast cancer during pregnancy: a mini-review. Eur J Surg Oncol 2008; 34: 837-43. [CrossRef]
12. Middleton LP, Amin M, Gwyn K, Theriault R, Sahin A. Breast carcinoma in pregnant women: assessment of clinicopathologic and immunohistochemical features. Cancer 2003; 98: 1055-60. [CrossRef]
13. Bonnier P, Romain S, Dilhuydy JM, Bonichon F, Julien JP, Charpin C, et al. Influence of pregnancy on the outcome of breast cancer: a case-control study. Societe Francaise de Senologie et de Pathologie Mammaire Study Group. Int J Cancer 1997; 72: 720-7. [CrossRef]
14. Ring AE, Smith IE, Ellis PA. Breast cancer and pregnancy. Ann Oncol 2005; 16: 1855-60. [CrossRef]
15. Kothari AS, Fentiman IS. 11. Breast cancer in young women. Int J Clin Pract 2002; 56: 184-7.
16. Ishida T, Yokoe T, Kasumi F, Sakamoto G, Makita M, Tominaga T, et al. Clinicopathologic characteristics and prognosis of breast cancer patients associated with pregnancy and lactation: analysis of case-control study in Japan. Jpn J Cancer Res 1992; 83: 1143-9. [CrossRef]
17. Elledge RM, Ciocca DR, Langone G, McGuire WL. Estrogen receptor, progesterone receptor, and HER-2/neu protein in breast cancers frompregnant patients. Cancer 1993; 71: 2499-506. [CrossRef]
18. Mathelin C, Annane K, Treisser A, Chenard MP, Tomasetto C, Bellocq JP, et al. Pregnancy and post-partum breast cancer: a prospective study. Anticancer Res 2008; 28: 2447-52.
19. Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, Keith DE, et al. Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. Science 1989; 244: 707-12. [CrossRef]
20. Lambe M, Hsieh C, Trichopoulos D, Ekbom A, Pavia M, Adami HO. Transient increase in the risk of breast cancer after giving birth. N Engl J Med 1994; 331: 5-9. [CrossRef]
21. Liu Q, Wuu J, Lambe M, Hsieh SF, Ekbom A, Hsieh CC. Transient increase in breast cancer risk after giving birth: postpartum period with the highest risk(Sweden). Cancer Causes Control 2002; 13: 299-305. [CrossRef]
22. Albrektsen G, Heuch I, Hansen S, Kvale G. Breast cancer risk by age at birth, time since birth and time intervals between births: exploring interaction effects. Br J Cancer 2005; 92: 167-75. [CrossRef]
23. Lord SJ, Bernstein L, Johnson KA, Malone KE, McDonald JA, Marchbanks PA, et al. Breast cancer risk and hormone receptor status in older women by parity, age of first birth, and breastfeeding: a case-control study. Cancer Epidemiol Biomarkers Prev 2008; 17: 1723-30. [CrossRef]
24. Lyons TR, Schedin PJ, Borges VF. Pregnancy and breast cancer: when they collide. J Mammary Gland Biol Neoplasia 2009; 14: 87-98. [CrossRef]
25. Litton JK, Theriault RL, Gonzalez-Angulo AM. Breast cancer diagnosis during pregnancy. Womens Health (Lond Engl) 2009; 5: 243-9. [CrossRef]
26. Albrektsen G, Heuch I, Thoresen S, Kvåle G. Family history of breast cancer and short-term effects of childbirths on breast cancer risk. Int J Cancer 2006; 119: 1468-74. [CrossRef]
27. Lambe M, Hsieh C, Trichopoulos D, Ekbom A, Pavia M, Adami HO. Transient increase in the risk of breast cancer after giving birth. N Engl J Med 1994; 331: 5-9. [CrossRef]
28. Collaborative Group on Hormonal Factors in Breast Cancer. Breast cancer and breastfeeding: collaborative reanalysis of individual data from 47 epidemiological studies in 30 countries, including 50302 women with breast cancer and 96973 women without the disease. Lancet 2002; 360: 187-95. [CrossRef]
29. Peterson NB, Huang Y, Newcomb PA, Titus-Ernstoff L, Trentham-Dietz A, Anic G, et al. Childbearing recency and modifiers of premenopausal breast cancer risk. Cancer Epidemiol Biomarkers Prev 2008; 17: 3284-7. [CrossRef]
30. Jernstrom H, Lubinski J, Lynch HT, Ghadirian P, Neuhausen S, Isaacs C, et al. Breast-feeding and the risk of breast cancer in BRCA1 and BRCA2 mutation carriers. J Natl Cancer Inst 2004; 96: 1094-8. [CrossRef]
31. Johannsson O, Loman N, Borg A, Olsson H. Pregnancy-associated breast cancer in BRCA1 and BRCA2 germline mutation carriers. Lancet 1998; 352: 1359-60. [CrossRef]
32. Cullinane CA, Lubinski J, Neuhausen SL, Ghadirian P, Lynch HT, Isaacs C, et al. Effect of pregnancy as a risk factor for breast cancer in BRCA1/BRCA2 mutation carriers. Int J Cancer 2005; 117: 988-91. [CrossRef]
33. Schedin P. Pregnancy-associated breast cancer and metastasis. Nat Rev Cancer. 2006; 6: 281-91. [CrossRef]
34. Schedin P, O'Brien J, Rudolph M, Stein T, Borges V. Microenvironment of the involuting mammary gland mediates mammary cancer progression. J Mammary Gland Biol Neoplasia 2007; 12: 71-82. [CrossRef]
35. Siwko SK, Dong J, Lewis MT, Liu H, Hilsenbeck SG, Li Y. Evidence that an early pregnancy causes a persistent decrease in the number of functional mammary epithelial stem cells-implications for pregnancy-induced protection against breast cancer. Stem Cells 2008; 26: 3205-9. [CrossRef]
36. Clark RM, Chua T. Breast cancer and pregnancy: the ultimate challenge. Clin Oncol (R Coll Radiol) 1989; 1: 11-8. [CrossRef]
37. Woo JC, Yu T, Hurd TC. Breast cancer in pregnancy: a literature review. Arch Surg 2003; 138: 91-8. [CrossRef]
38. Nettleton J, Long J, Kuban D, Wu R, Shaefffer J, El-Mahdi A. Breast cancer during pregnancy: quantifying the risk of treatment delay. Obstet Gynecol 1996; 87: 414-8. [CrossRef]
39. Applewhite RR, Smith LR, DiVincenti F. Carcinoma of the breast associated with pregnancy and lactation. Am Surg 1973; 39: 101-4.
40. Ives AD, Saunders CM, Semmens JB. The Western Australian gestational breast cancer project: a population-based study of the incidence, management and outcomes. Breast 2005; 14: 276-82. [CrossRef]
41. Ibrahim EM, Ezzat AA, Baloush A, Hussain ZH, Mohammed GH. Pregnancy-associated breast cancer: a case-control study in a young population with a high-fertility rate. Med Oncol 2000; 17: 293-300. [CrossRef]
42. Liberman L, Giess CS, Dershaw DD, Deutch BM, Petrek JA. Imaging of pregnancy-associated breast cancer. Radiology 1994; 191: 245-8.
43. King RM, Welch JS, Martin JK Jr, Coulam CB. Carcinoma of the breast associated with pregnancy. Surg Gynecol Obstet 1985; 160: 228-32.
44. Tretli S, Kvalheim G, Thoresen S, Høst H. Survival of breast cancer patients diagnosed during pregnancy or lactation. Br J Cancer 1988; 58: 382-4. [CrossRef]
45. Novotny DB, Maygarden SJ, Shermer RW, Frable WJ. Fine needle aspiration of benign and malignant breast masses associated with pregnancy. Acta Cytol 1991; 35: 676-86.
46. Petrek JA. Breast cancer during pregnancy. Cancer 1994; 74: 518-27. [CrossRef]
47. Greskovich JF Jr, Macklis RM. Radiation therapy in pregnancy: risk calculation and risk minimization. Semin Oncol 2000; 27: 633-45.
48. Otake M, Schull WJ, Lee S. Threshold for radiation-related severe mental retardation in prenatally exposed A-bomb survivors: a re-analysis. Int J Radiat Biol 1996; 70: 755-63. [CrossRef]
49. Osei EK, Faulkner K. Fetal doses from radiological examinations. Br J Radiol 1999; 72: 773-80.
50. Yang WT, Dryden MJ, Gwyn K, Whitman GJ, Theriault R. Imaging of breast cancer diagnosed and treated with chemotherapy during pregnancy. Radiology 2006; 239: 52-60. [CrossRef]
51. Nicklas AH, Baker ME. Imaging strategies in the pregnant cancer patient. Semin Oncol 2000; 27: 623-32.
52. Shellock FG, Kanal E. Safety of magnetic resonance imaging contrast agents. J Magn Reson Imaging 1999; 10: 477-84. [CrossRef]
53. Kuerer HM, Cunningham JD, Brower ST, Tartter PI. Breast carcinoma associated with pregnancy and lactation. Surg Oncol 1997; 6: 93-8. [CrossRef]
54. Kuerer HM, Gwyn K, Ames FC, Theriault RL. Conservative surgery and chemotherapy for breast carcinoma during pregnancy. Surgery 2002; 131: 108-10. [CrossRef]
55. Gwyn K, Theriault R. Breast cancer during pregnancy. Oncology (Williston Park) 2001; 15: 39-46.

56. Keleher A, Wendt R 3rd, Delpassand E, Stachowiak AM, Kuerer HM. The safety of lymphatic mapping in pregnant breast cancer patients using Tc-99m sulfur colloid. Breast J 2004; 10: 492-5. [CrossRef]
57. Ring AE, Smith IE, Jones A, Shannon C, Galani E, Ellis PA. Chemotherapy for breast cancer during pregnancy: an 18-year experience from five London teaching hospitals. J Clin Oncol 2005; 23: 4192-7. [CrossRef]
58. Mazze RI, Källén B. Reproductive outcome after anesthesia and operation during pregnancy: a registry study of 5405 cases. Am J Obstet Gynecol 1989; 161: 1178-85.
59. Antypas C, Sandilos P, Kouvaris J, Balafouta E, Karinou E, Kollaros N, et al. Fetal dose evaluation during breast cancer radiotherapy. Int J Radiat Oncol Biol Phys 1998; 40: 995-9. [CrossRef]
60. Amant F, Deckers S, Van Calsteren K, Loibl S, Halaska M, Brepoels L, et al. Breast cancer in pregnancy: Recommendations of an international consensus meeting. Eur J Cancer 2010; 46: 3158-68. [CrossRef]
61. Wiebe VJ, Sipila PE. Pharmacology of antineoplastic agents in pregnancy. Crit Rev Oncol Hematol 1994; 16: 75-112. [CrossRef]
62. Janni W, Hepp P, Nestle-Kraemling C, Salmen J, Rack B, Genss E. Treatment of pregnancy-associated breast cancer. Expert Opin Pharmacother 2009; 10: 2259-67. [CrossRef]
63. Potluri V, Lewis D, Burton GV. Chemotherapy with taxanes in breast cancer during pregnancy: case report and review of the literature. Clin Breast Cancer 2006; 7: 167-70. [CrossRef]
64. Bader AA, Schlembach D, Tamussino KF, Pristauz G, Petru E. Anhydramnios associated with administration of trastuzumab and paclitaxel for metastatic breast cancer during pregnancy. Lancet Oncol 2007; 8: 79-81. [CrossRef]
65. Shrim A, Garcia-Bournissen F, Maxwell C, Farine D, Koren G. Favorable pregnancy outcome following Trastuzumab (Herceptin) use during pregnancy. Case report and updated literature review. Reprod Toxicol 2007; 23: 611-3. [CrossRef]
66. Tewari K, Bonebrake RG, Asrat T, Shanberg AM. Ambiguous genitalia in infant exposed to tamoxifen in utero. Lancet 1997; 350: 183. [CrossRef]
67. Tiboni GM, Marotta F, Rossi C, Giampietro F. Effects of the aromatase inhibitor letrozole on in utero development in rats. Hum Reprod 2008; 23: 1719-23. [CrossRef]
68. Berry DL, Theriault RL, Holmes FA, Parisi VM, Booser DJ, Singletary SE, et al. Management of breast cancer during pregnancy using a standardized protocol. J Clin Oncol 1999; 17: 855-61.
69. Hahn KM, Johnson PH, Gordon N, Kuerer H, Middleton L, Ramirez M, et al. Treatment of pregnant breast cancer patients and outcomes of children exposed to chemotherapy in utero. Cancer 2006; 107: 1219-26. [CrossRef]
70. Giacalone PL, Laffargue F, Bénos P. Chemotherapy for breast carcinoma during pregnancy: A French national survey. Cancer 1999; 86: 2266-72. [CrossRef]
71. Avilés A, Neri N. Hematological malignancies and pregnancy: a final report of 84 children who received chemotherapy in utero. Clin Lymphoma 2001; 2: 173-7. [CrossRef]
72. Nugent P, O'Connell TX. Breast cancer and pregnancy. Arch Surg 1985; 120: 1221-4.
73. Beadle BM, Woodward WA, Middleton LP, Tereffe W, Strom EA, Litton JK, et al. The impact of pregnancy on breast cancer outcomes in women<or=35 years. Cancer 2009; 115: 1174-84. [CrossRef]
74. Cardonick E, Dougherty R, Grana G, Gilmandyar D, Ghaffar S, Usmani A. Breast cancer during pregnancy: maternal and fetal outcomes. Cancer J 2010; 16: 76-82. [CrossRef]
75. Keleher AJ, Theriault RL, Gwyn KM, Hunt KK, Stelling CB, Singletary SE, et al. Multidisciplinary management of breast cancer concurrent with pregnancy. J Am Coll Surg 2002; 194: 54-64. [CrossRef]
76. Anderson BO, Petrek JA, Byrd DR, Senie RT, Borgen PI. Pregnancy influences breast cancer stage at diagnosis in women 30 years of age and younger. Ann Surg Oncol 1996; 3: 204-11. [CrossRef]
77. Reed W, Hannisdal E, Skovlund E, Thoresen S, Lilleng P, Nesland JM. Pregnancy and breast cancer: a population-based study. Virchows Arch 2003; 443: 44-50. [CrossRef]
78. Bladström A, Anderson H, Olsson H. Worse survival in breast cancer among women with recent childbirth: results from a Swedish population-based register study. Clin Breast Cancer 2003; 4: 280-5. [CrossRef]
79. Petrek JA, Dukoff R, Rogatko A. Prognosis of pregnancy-associated breast cancer. Cancer 1991; 67: 869-72. [CrossRef]
80. Whiteman MK, Hillis SD, Curtis KM, McDonald JA, Wingo PA, Marchbanks PA. Reproductive history and mortality after breast cancer diagnosis. Obstet Gynecol 2004; 104: 146-54. [CrossRef]
81. Stensheim H, Møller B, van Dijk T, Fosså SD. Cause-specific survival for women diagnosed with cancer during pregnancy or lactation: a registry-based cohort study. J Clin Oncol 2009; 27: 45-51. [CrossRef]
82. Zemlickis D, Lishner M, Degendorfer P, Panzarella T, Burke B, Sutcliffe SB, et al. Maternal and fetal outcome after breast cancer in pregnancy. Am J Obstet Gynecol 1992; 166: 781-7.
83. Chang YT, Loong CC, Wang HC, Jwo SC, Lui WY. Breast cancer and pregnancy. Zhonghua Yi Xue Za Zhi (Taipei) 1994; 54: 223-9.
84. Guinee VF, Olsson H, Möller T, Hess KR, Taylor SH, Fahey T, et al. Effect of pregnancy on prognosis for young women with breast cancer. Lancet 1994; 343: 1587-9. [CrossRef]
85. Royal College of Obstetricians and Gynaecologists. Guideline No. 12; Pregnancy and Breast Cancer; 2004.
86. Kroman N, Jensen MB, Melbye M, Wohlfahrt J, Mouridsen HT. Should women be advised against pregnancy after breast-cancer treatment? Lancet 1997; 350: 319-22. [CrossRef]
87. Kelsey JL, Gammon MD, John EM. Reproductive factors and breast cancer. Epidemiol Rev 1993; 15: 36-47.
88. Sankila R, Heinävaara S, Hakulinen T. Survival of breast cancer patients after subsequent term pregnancy: "healthy mother effect". Am J Obstet Gynecol 1994; 170: 818-23.
89. von Schoultz E, Johansson H, Wilking N, Rutqvist LE. Influence of prior and subsequent pregnancy on breast cancer prognosis. J Clin Oncol 1995; 13: 430-4.
90. Mueller BA, Simon MS, Deapen D, Kamineni A, Malone KE, Daling JR. Childbearing and survival after breast carcinoma in young women. Cancer 2003; 98: 1131-40. [CrossRef]
91. Velentgas P, Daling JR, Malone KE, Weiss NS, Williams MA, Self SG, et al. Pregnancy after breast carcinoma: outcomes and influence on mortality. Cancer 1999; 85: 2424-32. [CrossRef]
92. Blakely LJ, Buzdar AU, Lozada JA, Shullaih SA, Hoy E, Smith TL, et al. Effects of pregnancy after treatment for breast carcinoma on survival and risk of recurrence. Cancer 2004; 100: 465-9. [CrossRef]
93. Malamos NA, Stathopoulos GP, Keramopoulos A, Papadiamantis J, Vassilaros S. Pregnancy and offspring after the appearance of breast cancer. Oncology 1996; 53: 471-5. [CrossRef]
94. Lethaby AE, O'Neill MA, Mason BH, Holdaway IM, Harvey VJ. Overall survival from breast cancer in women pregnant or lactating at or after diagnosis. Auckland Breast Cancer Study Group. Int J Cancer 1996; 67: 751-5. [CrossRef]
95. Gelber S, Coates AS, Goldhirsch A, Castiglione-Gertsch M, Marini G, Lindtner J, et al. International Breast Cancer Study Group. Effect of pregnancy on overall survival after the diagnosis of early-stage breast cancer. J Clin Oncol 2001; 19: 1671-5.
96. Janerich DT, Thompson WD, Mineau GP. Maternal pattern of reproduction and risk of breast cancer in daughters: results from the Utah Population Database. J Natl Cancer Inst 1994; 86: 1634-9. [CrossRef]
97. Averette HE, Mirhashemi R, Moffat FL. Pregnancy after breast carcinoma: the ultimate medical challenge. Cancer 1999; 85: 2301-4. [CrossRef]
98. Guzman RC, Yang J, Rajkumar L, Thordarson G, Chen X, Nandi S. Hormonal prevention of breast cancer: mimicking the protective effect of pregnancy. Proc Natl Acad Sci U.S.A 1999; 96: 2520-5. [CrossRef]
99. Petrek JA. Pregnancy safety after breast cancer. Cancer 1994; 74: 528-31. [CrossRef]
100. Clark RM, Chua T. Breast cancer and pregnancy: the ultimate challenge. Clin Oncol (R Coll Radiol) 1989; 1: 11-8. [CrossRef]
101. Mignot L, Morvan F, Berdah J, Querleu D, Laurent JC, Verhaeghe M, et al. Pregnancy after treated breast cancer. Results of a case-control study. Presse Med 1986; 15: 1961-4.

Development of bladder stone after tension free vaginal tape procedure: a case report

Gerilimsiz vajinal bant işleminden sonra mesane taşı gelişimi: Bir olgu raporu

Arman Özdemir[1], Seda Çakır[2], Hamdullah Sözen[2], Ahmed Namazov[2], Yeşim Akdemir[2], Ateş Karateke[2]

[1]Department of Urology, Zeynep Kamil Training and Research Hospital, İstanbul, Turkey

[2]Department of Urogynecology, Zeynep Kamil Training and Research Hospital, İstanbul, Turkey

Abstract

We present a case of a bladder stone which formed on the intravesical portion of tension free vaginal tape material secondary to bladder perforation. 8 years previously, a tension free vaginal tape (TVT) operation was performed. The patient was referred to hospital with persistent urinary infection and urinary incontinence. An intravesical stone that had formed on the TVT sling material was detected by cystoscopy and it was removed with the sling material by a supra pubic cystostomy approach. When evaulating patients presenting with urinary symptoms after a TVT procedure, bladder complications should be kept in mind.
(J Turkish-German Gynecol Assoc 2011; 12: 256-8)

Key words: Bladder stone, tension -free vaginal tape, perforation, urinary incontinence

Received: 18 August, 2010 **Accepted:** 20 September, 2010

Özet

Mesane perforasyonuna ikincil gerilimsiz vajinal bant materyalinin intravezikal kısmı üzerinde oluşan bir mesane taşı olgusunu sunuyoruz. Gerilimsiz vajinal bant (GVB) operasyonu 8 yıl önce yapılmıştı. Hasta sebat eden üriner enfeksiyonu ve idrar tutamama şikayeti ile hastaneye sevk edilmişti. GVB askı materyali üzerinde oluşan mesane içi taş, sistoskopi ile saptandı ve suprapubik sistostomi yaklaşımıyla askı materyaliyle çıkarıldı. Bir GVB işleminden sonra üriner semptomlarla gelen hastalar değerlendirilirken mesane komplikasyonları akılda tutulmalıdır.
(J Turkish-German Gynecol Assoc 2011; 12: 256-8)

Anahtar kelimeler: Mesane taşı, gerilimsiz vajinal bant, perforasyon, idrar tutamama

Geliş Tarihi: 18 Ağustos 2010 **Kabul Tarihi:** 20 Eylül 2010

Introduction

Urinary stress incontinence is a voiding dysfunction characterized by involuntary leakage of urine on effort, exertion, sneezing or caughing (1).

In 1995 Ulmsten et al proposed a surgical procedure during which synthetic polypropylene mesh is inserted under the mid urethra to restore continence (2). Studies on tension free vaginal tape surgery have demonstrated a 5 year success rate ranging from 80% to 90% in the general population (3, 4).

Owing to the anatomic relationships several urological complications can be seen in patients who have undergone the tension free vaginal tape (TVT) procedure. Bladder perforation is the most frequent intraoperative complication of TVT. Tamussino et al. reported perforation rates of 4.4% in TVT cases with previous surgery and 2% in the absence of any previous surgery (5).

In this article we present the case of a 56 year-old female who underwent the TVT procedure eight years previously. During the eight years a vesical calculus was formed on the polipropylene mesh.

Case Report

A fifty six year-old woman was referred to our hospital with persistant urinary infection, stone and urinary incontinence. Her history revealed that a TVT surgical procedure was performed eight years previously. Two years after the operation she attended various hospitals due to dysuria and voiding pain. Various antibiotherapies were prescribed to treat the urinary system infection. It was learnt from the patient`s history that the patient had been treated five to six times a year by antibiotherapies owing to urinary system infection.

Two weeks earlier her urine examination demonstrated pyuria, microscopic hematuria and oxalate crystals and she was referred to our clinic with urinary stone. Urinary ultrasound was performed and a vesical stone of 5x2 cm in dimension was seen in the right corner of bladder.

Cystoscopy performed on the patient showed a 5 cm portion of the tape passing across on the right side of bladder. On the polypropylene mesh there was a 2x5 cm bladder stone. In addition on pelvic examination kelloid formation was found within the right inguinal region. She had right inguinal pain with deep abdominal palpation.

With these findings, the surgical procedure of cystostomy was decided on. The synthetic material and vesical stone were removed and BURCH colposuspension procedure was performed for the symptoms of urinary incontinence. A Foley`s catheter was left for five days to allow healing of bladder mucosa (Figure 1, 2)

Address for Correspondence: *Ahmed Namazov, Zeynep Kamil Mahallesi, Üsküdar, İstanbul, Turkey*
Phone: +90 216 495 83 82 e.mail: enamazov@gmail.com

doi:10.5152/jtgga.2011.59

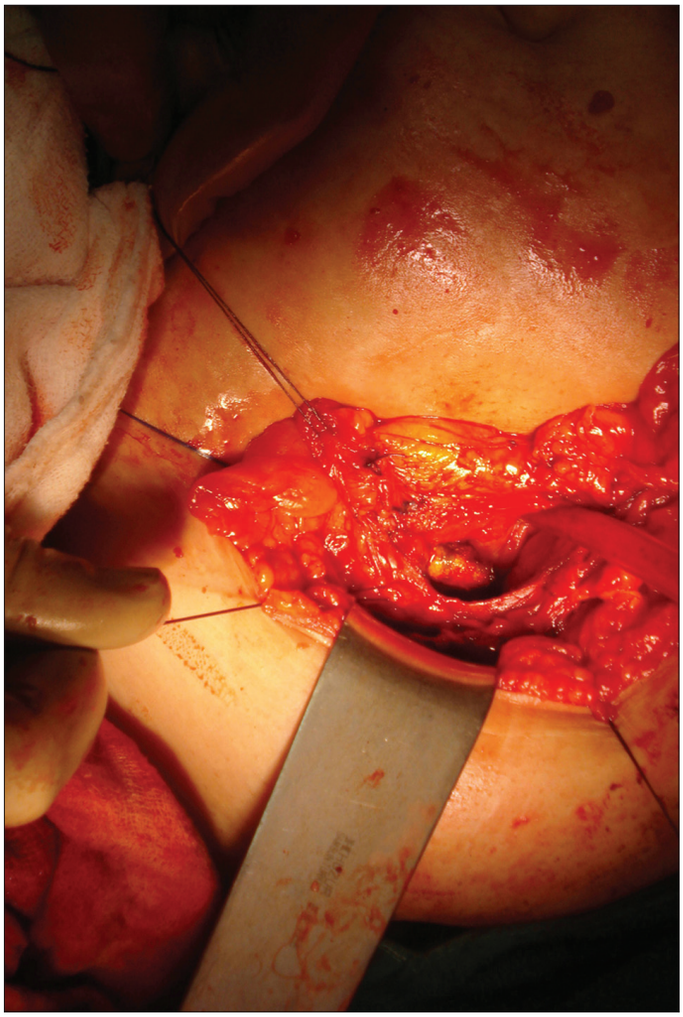


Figure 1. Cystostomy was performed

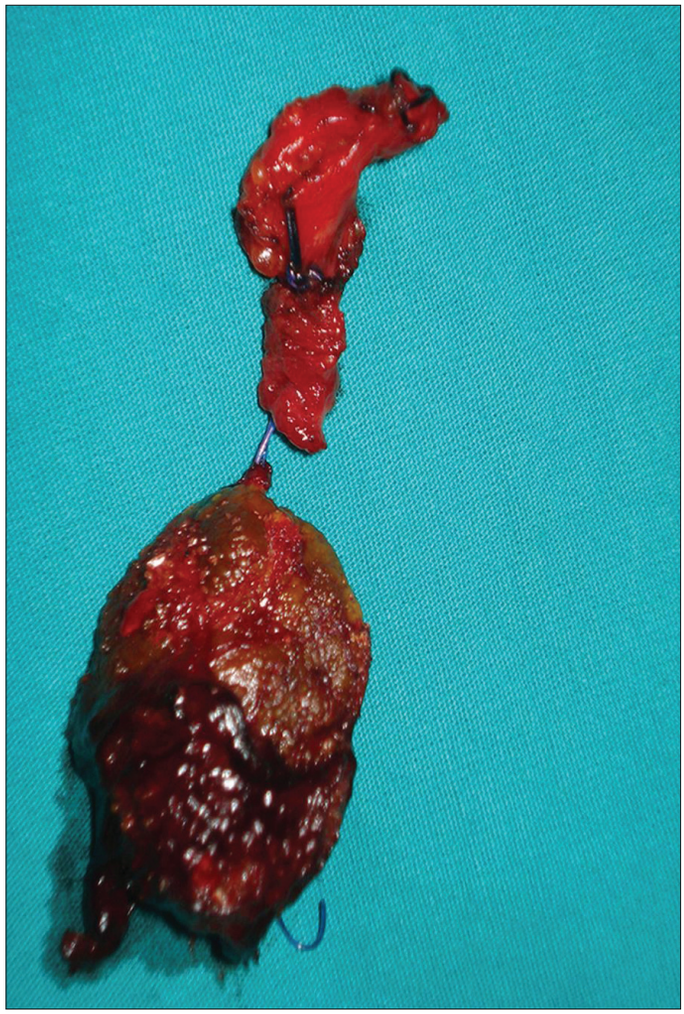


Figure 2. Polypropylene mesh and bladder stone were removed

Discussion

Various urogynecological clinics worldwide have been performing tension free vaginal tape (TVT) operation since its original introduction by Ulmsten in 1996 (2). Even though its safety and low number of complications were emphasised by the first description, a series of complications develop from this procedure. Bladder perforation, postoperative urinary retention, bruises, urinary urgency, dyspareunia and long term catheterization were reported by different clinics. Bladder perforation and stone formation on the TVT material have been described rarely (3-9).

After the TVT operation, bladder perforation and vesical stone formation were investigated in the literature and it was learnt from the literature that patients generally presented with painful urination, urinary sediments and pyuria. and different surgical approaches were performed. Irer et al. performed bladder endoscopic lithotripsy and simultaneously removed the synthetic material (7). Tzortis et al. performed suprapubic cystostomy and removed the lithiasis and synthetic material (8). Mustafa et al performed endoscopic pneumatic lithotripsy and transurethral mesh resection (9).

In our case it was decided to remove the mesh and stone in the bladder by cystostomy because of the ineffectiveness of lithotripsy in breaking the 2x5 cm stone, existence of kelloid structure in the right inguinal ragion caused by polypropylene mesh and continuation of urinary incontinence complaints. After this procedure, a Burch colposuspension operation was applied due to urinary stress incontinence.

Within the 6 months postoperative period, the urinary system infections which had previously recurred 5 to 6 times each year after the TVT operation disappeared.

Although the TVT operation still preserves its popularity due to its high success rates, various complications may appear due to failure of management. In our study stemming from the bladder perforation during the management of a TVT operation, a bladder stone formed in the bladder.

In recurring urinary system infections after the TVT operation and observation of crystalloids on urinary examination, bladder perforation should be kept in mind and cystoscopy should be performed. In order to remove the stone the procedure should be adapted taking into account the specifics of each patient and cystostomy and endoscopic lithotropsy must be considered as important.

Conflict of interest

No conflict of interest was declared by the authors.

References

1. Abrams P, Cardozo L, Fall M, Griffiths D, Rosier P, Ulmsten U, et al. The standardisation of terminology of lower urinary tract function: report from the standardisation sub-committee of the International Continence Society. Neurourol Urodyn 2002; 21: 167-78. [CrossRef]
2. Ulmsten U, Henriksson L, Johnson P, Varhos G. An ambulatory surgical procedure under local anesthesia for treatment of female urinary incontinence. Int Urogynecol J Pelvic Floor Dysfunct 1996; 7: 81-5. [CrossRef]
3. Boustead GB. The tension-free vaginal tape for treating female stress urinary incontinence. BJU Int 2002; 89: 687-93. [CrossRef]
4. Nilsson CG, Kuuva N, Falconer C, Rezapour M, Ulmsten U. Long-term results of the tension-free vaginal tape (TVT) procedure for surgical treatment of female stress urinary incontinence. Int Urogynecol J Pelvic Floor Dysfunct 2001; 12: 5-8. [CrossRef]
5. Tamussino K, Hanzal E, Kölle D, Ralph G, Riss P. The Austrian tension-free vaginal tape registry. Int Urogynecol J Pelvic Floor Dysfunct 2001; 12: 28-9. [CrossRef]
6. Peyromaure M, Dayma T, Zerbib M. Development of bladder stone following a tension-free vaginal tape intervention. J Urol 2004; 171: 337. [CrossRef]
7. Irer B, Aslan G, Cimen S, Bozkurt O, Celebi I. Development of vesical calculi following tension-free vaginal tape procedure. Int Urogynecol J Pelvic Floor Dysfunct 2005; 16: 245-6. [CrossRef]
8. Tzortzis V, Mitsogiannis IC, Moutzouris G, Aravantinos E, Anagnostou T, Gravas S, et al. Bladder stone formation after a tension-free vaginal tape procedure: report on two cases. Urol Int 2007; 79: 181-2. [CrossRef]
9. Mustafa M, Wadie BS. Bladder erosion of tension-free vaginal tape presented as vesical stone; management and review of literature. Int Urol Nephrol 2007; 39: 453-5. [CrossRef]

Prenatal early diagnosis of dacryocystocele, a case report and review of literature

Dakriyosistoselin prenatal erken tanısı, vaka sunumu ve literatür taraması

Banu Bingöl, Alin Başgül, Nilgün Güdücü, Herman İşci, İlkkan Dünder

Department of Obstetrics and Gynecology, Faculty of Medicine, Bilim University, İstanbul, Turkey

Abstract

Dacryocystocele (mucocele, amniocele) is a relatively rare variant of nasolacrimal duct obstruction which refers to the cystic dilatation of lacrimal pathway above and below the lacrimal sac. It is a benign pathology and can be treated successfully after birth, but its prenatal detection is important, because it may be seen in numerous syndromes and may serve as their marker. Bilateral cysts have the possibility for intranasal extension and an obstruction to the nasal passages may result in neonatal respiratory distress requiring surgical intervention Unilateral cases are important for the differential diagnosis with serious facial abnormalities. We present a case of early prenatal detection of a 28 year-old G: 1 P: 0 pregnant woman with bilateral dacryocystocele. She presented a live, normally developed singleton fetus on sonographic examination at 12, 16 and 22 weeks. At 25th weeks, we diagnosed a hypoechogenic mass, that was situated inferomedially to the eyes in the fetal face with 2 and 3-D ultrasound. A 3850-g live female infant was delivered by Cesarean section due to breech presentation at 39 weeks following preterm rupture of membranes. We report the case with intranasal components studied during fetal life by 2 and 3-D ultrasound and magnetic resonance (MR) imaging.

(J Turkish-German Gynecol Assoc 2011; 12: 259-62)

Key words: Dacryocystocele, mucocele, amniocele, magnetic resonance imaging, nasolacrimal duct cyst

Received: 24 August, 2010 **Accepted:** 24 September, 2010

Özet

Dakriyosistosel (mukosel, amniosel), lakrimal drenaj sisteminin distalde ve proksimalde oklüze olarak, mukus ve amniotik sıvının birikmesi ile kistik dilatasyonudur. Dakriyosistosel; benign bir patolojidir ve doğum sonrası başarılı bir şekilde tedavi edilebilir. Dakriyosistoselin konjenital anomaliler ve sendromlarla birlikteliği, prenatal tanısının önemini göstermektedir. Bilateral dakriyosistosel, neonatal nasal obstrüksiyona neden olabileceği ve resüsitasyon gerektirebileceği için doğum sırasında dikkat edilmelidir. Unilateral vakalar, bazı ciddi fasiyal anomalilerle ayrıcı tanı açısından önemlidir. Prenatal dakriyosistosel nadir görülen bir patoloji olup, literatürde prenatal tanı alan vaka sayısı son dececе azdır. Bu olgu sunumunda 28 yaşında, G: 1 P: 0 bir gebede 25. haftada tespit edilen bilateral dakriyosistoselin, 2-D ve 3-D ultrasonografik ve magnetik rezonans incelemesi (MRI) görüntüleri sunulmuştur. Gebeliğin takibindeki 12., 16., 22. haftalardaki ultrasonografik incelemelerinde herhangi bir patoloji saptanmamıştır, 25. haftada fetal yüzde orbitaların inferomedialinde bilateral hipoekojen kistik yapılar tespit edilmiştir. 39. haftada membran rüptürünü takiben makat prezentasyonu nedeniyle sezaryen ile doğum gerçekleştirilmiş ve 3850 gr kız bebek doğurtulmuştur. Tedavi postnatal dönemde aralıklı lakrimal kanal masajları ile gerçekleştirilmiştir. Literatüre bakıldığında en erken tanı alan ve en küçük çapta tespit edilen olgudur.

(J Turkish-German Gynecol Assoc 2011; 12: 259-62)

Anahtar kelimeler: Dakriyosistosel, mukosel, amniosel, manyetik rezonans inceleme, nasolakrimal kanal kisti

Geliş Tarihi: 24 Ağustos 2010 **Kabul Tarihi:** 24 Eylül 2010

Introduction

Dacryocystocele is the occlusion of lacrimal drainage system distally and proximally during the fetal life; it dilates accumulating mucus and amniotic fluid (1). The causes of dacryocystocele include congenital deformities, trauma, primary and recurrent tumors affecting the nasolacrimal duct, idiopathic blockage of the nasolacrimal duct and iatrogenic causes including treatment of head and neck cancer in the sinonasal region (2, 3). Prenatal diagnosis is nevertheless important, because bilateral dacryocystocele, extending intranasally is one of the possible causes of neonatal nasal obstruction (4, 5). Moreover unilateral cases make the prenatal differential diagnosis of serious facial anomalies more difficult. Besides, it may be part of some syndromes, which makes the prenatal early diagnosis more important (6-8).

Case Report

A 28 year-old G:1 P:0 pregnant woman admitted to our clinic for antenatal follow up. A first trimester nuchal translucency measurement of 1.7 mm was associated with an adjusted risk for trisomy 21 of 1 in 730. Normal anatomy of the fetus including face and central nervous system were demonstrated at the 22-week examination. In 25th week, she admitted for a control and oral glucose loading test. The ultrasound examination demonstrated a breech presentation. We noticed bilateral cystic lesions measuring 0.5×0.6 mm and 0.5×0.4 mm inferomedial to the orbits. The facial profile seemed normal and the intraocular anatomy was also normal with synchronous movement of the eyes. The detailed examination of the brain appeared normal. The genetic sonogram revealed no evidence of any other associated abnormality. We referred

Address for Correspondence: *Banu Bingöl, Burhanettin Üstünel Cad., No: 20, Üsküdar 34668 İstanbul, Turkey*
Phone: +90 212 266 66 46 e.mail: banubingol1975@yahoo.com

doi:10.5152/jtgga.2011.60

the patient for a fetal magnetic resonance (MR) imaging examination. The abnormality was shown with 2D -3D ultrasound and MR as shown in Figure 1-3. A repeat scan 2 weeks later revealed the cyst volumes to be slightly increased (0.7×0.8 mm and 0.8×0.9) with no additional findings. We examined the patient every 2 weeks and followed the dimensions of the cysts and other possible abnormal findings. We diagnosed the case as bilateral dacryocystocele and the parents were counseled about the pathology. It is explained that dacryocystocele is usually an isolated pathology however rarely; it might be part of a syndrome that may not be identified antenatally. A 3850-g live female infant was delivered by Cesarean section due to breech presentation at 39 weeks following preterm rupture of membranes. The dimension of cysts at right and left sides were 1.2×1.3 and 1.1×1.4 respectively. There were no clinical signs of respiratory compromise and the infant revealed a blue swelling on the medial borders of both orbits confirming the diagnosis of dacryocystocele. Postnatally, the infant was consulted with Ophthalmology Department and the dacryocystocele was treated with gentle massage in 2 weeks. The infant seemed normal after the treatment and no further treatment was necessary. She completed 6 months of life and there is no evidence of any recurrence.

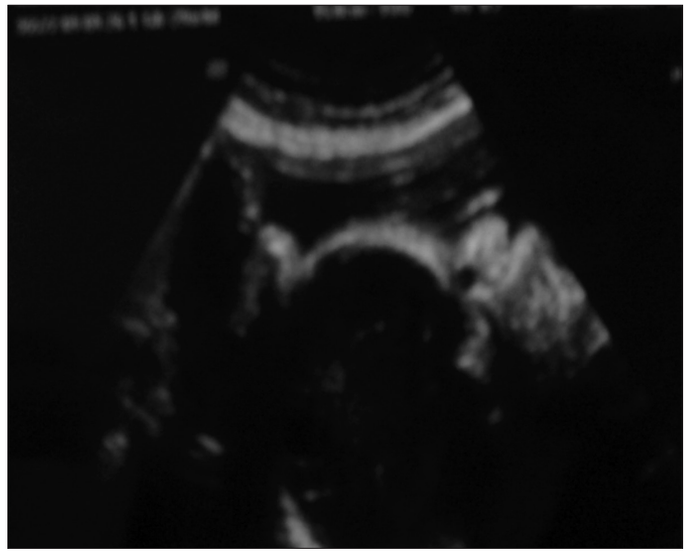


Figure 1. 2-D view of dacryosystocele

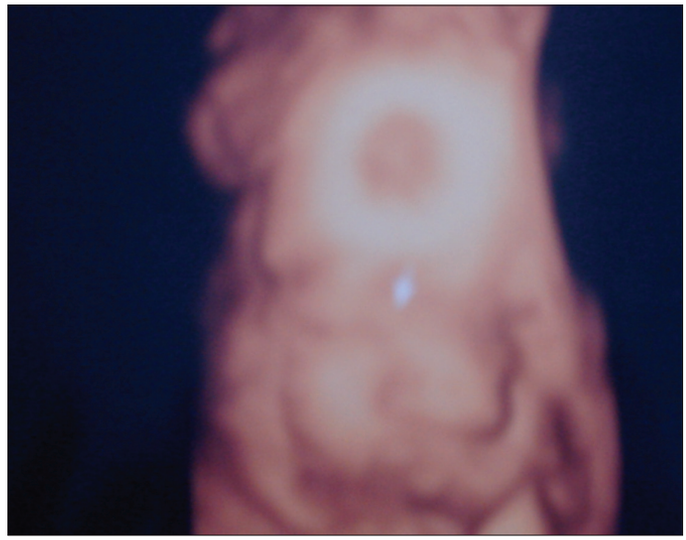


Figure 2. 3-D view of dacryosystocele

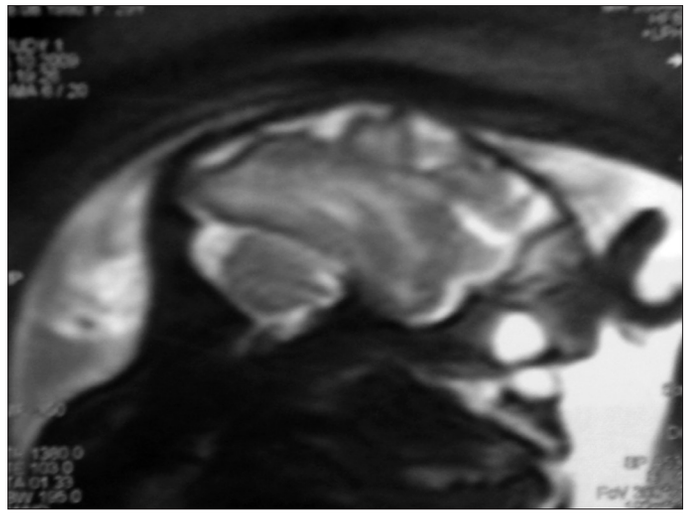


Figure 3. MRI view of dacryosystocele

Discussion

Dacryocystocele or lacrimal duct cyst is a very rare condition caused by obstruction of the lacrimal duct, usually due to a thin membrane remaining at its distal end. Rupture of the cyst is normally spontaneous during the first month of life (9). Prenatal of congenital dacryocystocele has been described but there are a few reports in the literature, and the earliest diagnosis was made in 27th gestational weeks (10).

The diagnosis is potentially important because bilateral cysts have the possibility for intranasal extension and an obstruction to the nasal passages may result in neonatal respiratory distress requiring surgical intervention (11). Because neonates obligate nasal breathers, the risk of acute respiratory distress in the early neonatal period should be considered and a pediatrician should be present for delivery (6).

The differential diagnosis of perinatal masses includes dacryocystocele, cystic teratomas, dermoid cysts, hemangiomas, encephalocele, nasal glioma and rhabdomyosarcoma (7). The differential diagnosis between dacryocystocele and other less benign periorbital masses is often not possible only with ultrasound, especially when the lesion is unilateral. Prenatal MR imaging better defines the location of the various components of lacrimal system dilatation and their relation to the nasal cavity. This information may provide the possible postnatal respiratory distress (12).

The lacrimal drainage system begins to develop around the 6th week of pregnancy. As the surface ectoderm in the naso-optic fissure thickens, an epithelial cord detaches from it and buries itself between the lateral nasal and maxillary processes. Cephalic and caudal growth of this epithelial cord will give rise to the lacrimal canaliculi, sac and duct (8). Canalization of the nasolacrimal pathway begins at about 12 weeks of gestation

and is complete from as early as 24 weeks. However, the nasal (distal) end may perforate only at birth or even later (10). In our case, the first antenatal diagnosis was made at 25th gestational week. To our knowledge, it is the earliest case diagnosed prenatally in the literature.

Atresia of the Hasner valve, at the distal end of the nasolacrimal duct, is the main cause of simple congenital dacryostenosis (13). Dacryocystocele is a much less common disorder, is already symptomatic at birth. In this condition, additional functional obstruction at the orbital end of the lacrimal sac is present together with the atresia of the distal end of the nasolacrimal duct. The sac fills with amniotic fluid and intrinsic mukoid secretion and becomes distended, causing the canaliculi to kink. These then act as a one-way valve, permitting fluid only to enter into the sac (14).

There are a few numbers of prenatal diagnoses of dacryocystocele reported in the literature. In two reports of Rand and Walsh, the diagnoses were made at 30-36 weeks of gestation (15, 16). Sharony et al. who reported the earliest diagnosis was at 27th gestational weeks; desribed 6 cases of dacryocystocele; accompanying some syndromes and other pathologies as Canavan disease, pyelectasis, dysplastic kidney and maternal diabetes (10). Westbrook et al., reported recurrent bilateral dacryocystoceles in Wegener's granulomatosis which is defined as a chronic disease with peak onset between the ages of 20-40 years (17). The classic Wegener's triad includes; necrotizing granulomas of the upper and lower respiratory tract, vasculitis, and glomerulonephritis (18). In our case there was no other pathology neither defined on prenatal scan nor after birth.

Dacryocystocele is seen as a hypoechogenic mass locating inferiomedially to the orbit that may be seen in the coronal or parasagittal plane including the nose and medial angle of the orbits. Hemangioma is cutaneous in origin and it is located in the head or neck, septated or solid (19). It is differentiated form the dacryocystocele with its typical Doppler patterns (20). A dermoid cyst has a complex appearance and hyperechogenic situated superolaterally to the globe on ultrasound with areas of calcification usually present. Anterior cephalocele is a mid-line defect accompanied by a calvarial defect and usually hydrocephalus. The other orbital masses are neurofibromatosis, lymphangioma and rhabdomyosarcoma, but these are solid in origin and extremely rare. Nasolacrimal mucocele is also very rare and difficult to be diagnosed prenatally (21).

The sonographic appearance of dacryocystocele allows the differential diagnosis of this pathology, revealing the location, size, time of appearance, echogenicity and Doppler flow characteristics (22). MRI is helpful in ruling out a potential intracranial connection (23).

The treatment of dacryocystocele is controversial (24-26). Most of the physicians have advocated conservative treatment with antibiotics and massage (27, 28). Lucarelli et al. reported a case of corneal ectasia associated with massage of dacryocystocele (29). In our patient, we used gentle massage and treated the pathology successfully with no complication. Some physicians have recommended early surgical intervention if there is not a rapid response to conservative therapy or recommended prompt surgical therapy (21, 23).

In conclusion, when the diagnosis is contemplated prenatally, it is important to be aware of the risk of acute respiratory distress in the early neonatal period especially if the pathology is bilateral (30). Besides, other possible causes of duct obstruction or cystic dilatation must be excluded. The possibility of associations with syndromes must be kept in mind and other structural abnormalities should be controlled carefully.

Conflict of interest

No conflict of interest was declared by the authors.

References

1. Ogawa GS, Gonnering RS. Congenital nasolacrimal duct obstruction. J Pediatr 1991; 119: 12-7. [CrossRef]
2. Shashy RG, Durairaj VD, Holmes JM, Hohberger GG, Thompson DM, Kasperbauer JL. Congenital dacryocystocele associated with intranasal cysts: diagnosis and management. Laryngoscope 2003; 113: 37-40. [CrossRef]
3. Bhaya M, Meehan R, Har-El G. Dacryocystocele in an adult: endoscopic management. Am J Otolaryngol 1997; 18: 131-4. [CrossRef]
4. Castillo M, Merten DF, Weissler MC. Bilateral nasolacrimal duct mucocele, a rare cause of respiratory distress: CT findings in two newborns. Am J Neuroradiol 1993; 14: 1011-3.
5. Hepler KM, Woodson GE, Kearns DB. Respiratory distress in the neonate. Sequela of a congenital dacryocystocele. Arch Otolaryngol Head Neck Surg 1995; 121: 1423-5.
6. Alper CM, Chan KH, Hill LM, Chenevey P. Antenatal diagnosis of a congenital nasolacrimal duct cyst by ultrasonography: a case report. Prenat Diagn 1994; 14: 623-6. [CrossRef]
7. Shipp TD, Bromley B, Benacerraf B. The ultrasonographic appearance and outcome for fetuses with masses distorting the fetal face. J Ultrasound Med 1995; 14: 673-8.
8. Sevel D. Development and congenital abnormalities of the nasolacrimal apparatus. J Pediatr Ophthalmol Strabismus 1981; 18: 13-9.
9. Salvetat ML, D'Ottavio G, Pensiero S, Vinciguerra A, Perissutti P. Prenatal sonographic detection of a bilateral dacryocystocele. J Pediatr Ophthalmol Strabismus 1999; 36: 295-7.
10. Sharony R, Raz J, Aviram R, Cohen I, Beyth Y, Tepper R. Prenatal diagnosis of dacryocystocele: a possible marker for syndromes. Ultrasound Obstet Gynecol 1999; 14: 71-3. [CrossRef]
11. Right PD, Hubbell RN, Lawrol PP Jr. Respiratory distress associated with bilateral nasolacrimal duct cysts. Int J Pediatr Otorhinolaryngol 1993; 26: 199-203. [CrossRef]
12. Bianchini E, Zirpoli S, Righini A, Rustico M, Parazzini C, Triulzi F. Magnetic resonance imaging in prenatal diagnosis of dacryocystocele: report of 3 cases. J Comput Assist Tomogr 2004; 28: 422-7. [CrossRef]
13. Cassady JV. Developmental anatomy of nasolacrimal duct. AMA Arch Ophthalmol 1952; 47: 141-58.
14. Rand PK, Ball WS Jr, Kulwin DR. Congenital nasolacrimal mucoceles: CT evaluation. Radiology 1989; 173: 691-4.
15. Walsh G, Dubbins PA. Antenatal sonographic diagnosis of a dacryocystocele. J Clin Ultrasound 1994; 22: 457-60. [CrossRef]
16. Davis WK, Mahony BS, Carroll BA, Bowie JD. Antenatal sonographic detection of benign dacrocystoceles (lacrimal duct cysts). J Ultrasound Med 1987; 6: 461-5.
17. Westbrook BJ, Scurry WC Jr, Hudak DT, McGinn J, Stack BC Jr. Recurrent bilateral dacryocystoceles in Wegener's granulomatosis: a rhinologic perspective. Am J Otolaryngol 2006; 27: 409-12. [CrossRef]
18. Gubbels SP, Barkhuizen A, Hwang PH. Head and neck manifestations of Wegener's granulomatosis. Otolaryngol Clin North Am 2003; 36: 685-705. [CrossRef]

19. Meizner I, Bar-Ziv J, Holcberg G, Katz M. In utero prenatal diagnosis of fetal facial tumor-hemangioma. J Clin Ultrasound 1985; 13: 435-7. [CrossRef]
20. Pennell RG, Baltarowich OH. Prenatal sonographic diagnosis of a fetal facial hemangioma. J Ultrasound Med 1986; 5: 525-8.
21. Mansour AM, Barber JC, Reinecke RD, Wang FM. Ocular choristomas. Surv Ophthalmol 1989; 33: 339-58. [CrossRef]
22. Sepulveda W, Wojakowski AB, Elias D, Otaño L, Gutierrez J. Congenital dacryocystocele: prenatal 2- and 3-dimensional sonographic findings. J Ultrasound Med 2005; 24: 225-30.
23. Brugger PC, Weber M, Prayer D. Magnetic resonance imaging of the fetal efferent lacrimal pathways. Eur Radiol 2010; 20: 1965-73. [CrossRef]
24. Harris GJ, DiClementi D. Congenital dacryocystocele. Arch Ophthalmol 1982; 100: 1763-5.
25. Schnall BM, Christian CJ. Conservative treatment of congenital dacryocele. J Pediatr Ophthalmol Strabismus 1996; 33: 219-22.
26. Paysse EA, Coats DK, Bernstein JM, Go C, de Jong AL. Management and complications of congenital dacryocele with concurrent intranasal mucocele. J AAPOS 2000; 4: 46-53. [CrossRef]
27. Levy NS. Conservative management of congenital amniotocele of the nasolacriminal sac. J Pediatr Ophthalmol Strabismus 1979; 16: 254-6.
28. Grin TR, Mertz JS, Stass-Isern M. Congenital nasolacrimal duct cysts in dacryocystocele. Ophthalmology 1991; 98: 1238-42.
29. Lucarelli MJ, DeBry P. Corneal ectasia associated with massage of dacryocystoceles. Cornea 2002; 21: 419-20. [CrossRef]
30. Tsai YS, Huang JK. Neonatal nasal obstruction caused by bilateral dacryocystoceles. Pediatr Radiol 2006; 36: 1221. [CrossRef]

Spontaneous adrenal hemorrhage during pregnancy: review of literature and case report of successful conservative management

Gebelik sırasında spontan adrenal kanama: Literatürün gözden geçirilmesi ve başarılı bir konservatif tedavi olgu raporu

Mohammad Saleem Wani[1], Zahoor Ahmed Naikoo[2], Munfat Ahmed Malik[2], Arif Hamid Bhat[1], Muneer Ahmed Wani[1], Syed Arshid Qadri[2]

[1]Department of Urology, Sher-i- Kashmir Institute of Medical Sciences, Soura Srinagar, Kashmir, J and K, India

[2]Department of General Surgery, Sher-i- Kashmir Institute of Medical Sciences, Soura Srinagar, Kashmir, J and K, India

Abstract

Spontaneous adrenal hemorrhage is an acute hemorrhage from the adrenal gland which occurs in the absence of trauma. The incidence of this condition during pregnancy is unknown. We describe a patient with massive unilateral adrenal hemorrhage which occurred during labor. The patient was successfully managed conservatively with complete resolution of the hematoma. A review of the literature of this rare condition is also presented.

(J Turkish-German Gynecol Assoc 2011; 12: 263-5)

Key words: Adrenal hemorrhage, post partum, retroperitoneal bleed

Received: 5 September, 2010 **Accepted:** 20 October, 2010

Özet

Spontan adrenal kanama, adrenal bezden travma olmaksızın oluşan akut kanamadır. Bu durumun gebelik sırasındaki insidansı bilinmemektedir. Biz doğum sırasında ortaya çıkan ağır tek taraflı adrenal kanamalı bir hasta olgusunu tarif ediyoruz. Hasta başarılı bir şekilde konservatif olarak tedavi edildi ve hematom tamamen ortadan kalktı. Ayrıca bu ender durumun literatür derlemesi de sunulmaktadır.

(J Turkish-German Gynecol Assoc 2011; 12: 263-5)

Anahtar kelimeler: Adrenal kanama, doğum sonrası, retroperitoneal kanama

Geliş Tarihi: 05 Eylül 2010 **Kabul Tarihi:** 20 Ekim 2010

Introduction

Adrenal hemorrhage during delivery is a rare cause of massive retroperitoneal hematoma and must be differentiated from hemorrhage caused by trauma, primary adrenal or metastatic tumors (1). The main symptoms are hemorrhagic shock, flank pain and fever in some cases. Adrenal hemorrhage has been reported in 0.3% -1.8% of undetected cases in autopsy studies, although extensive bilateral adrenal hemorrhage may be present in 15% of individuals who die of shock (2). Unilateral adrenal hemorrhage most frequently is caused by blunt abdominal trauma (traumatic adrenal rupture), but it also has occurred in liver transplant recipients and patients with primary adrenal or metastatic tumors (3). Unilateral adrenal hemorrhage is infrequently associated with otherwise uncomplicated pregnancy, neurofibromatosis, or long term non steroidal anti-inflammatory drug use (4). During pregnancy, idiopathic spontaneous, unilateral adrenal hemorrhage has been reported rarely (5).

We present a case of idiopathic spontaneous unilateral massive adrenal hemorrhage occurring during labor, which was managed conservatively.

Case Report

A 35 year-old female in the ninth month of a twin pregnancy with complaints of pregnancy induced hypertension was admitted for delivery. On examination the patient was hypertensive and had pitting edema in the lower limbs and puffiness of her face. Ultrasonography showed a live twin pregnancy of 38 weeks with one cephalic and one breech presentation, adequate liquor and a fundal placenta. Haemogram and serum chemistry of the patient were within normal limits.

The patient was induced and delivered of live twins. The placenta was removed spontaneously and completely. The patient developed atonic post partum hemorrhage (PPH), which was managed conservatively with Inj. Syntocinon 20 IU in 5% dextrose and Inj. Prostadin (PGF2a) and Tab Misoprostol (PGE1) 400 mcg per vaginum. Twelve hours after the delivery, the patient developed breathlessness and a dry cough. Her pulse rate was 126 beats/ min and BP 90/60mmHg, the chest. X-ray and ECG were normal. The patient was resuscitated with O2 inhalation, blood transfusion and inotropic support with Inj. Dopamine 5mcg/min. She developed severe pain in

Address for Correspondence: *Zahoor Ahmed Naikoo, Department of General Surgery, Sher-i- Kashmir Institute of Medical Sciences, Soura Srinagar, Kashmir, J and K, India Phone: 0194-2401353 e.mail: drahmedzahoor@gmail.com*

doi:10.5152/jtgga.2011.61

her right flank and ultrasonography revealed a right peri-renal hematoma. Contrast CT scan of the abdomen revealed an extensive abdomino-pelvic retroperitoneal hematoma extending from the dome of the diaphragm to the pelvis (Figure 1, 2). The patient's vital signs stabilized on intra-venous fluids, antibiotics and blood transfusion. The retroperitoneal hematoma self- tamponaded and did not require surgical management. Upon conservative therapy, the clinical condition improved and parameters of inflammation normalized. Contrast CT carried out after 4 weeks revealed a resolving retroperitoneal hematoma with a thick walled adrenal cyst around the adrenal gland. There was no evidence of a hormone producing adrenal tumor, adrenal insufficiency caused by adrenal hemorrhage or a coagulopathy. A contrast CT 8 weeks later revealed a thick walled adrenal cyst with complete resolution of the retroperitoneal hematoma (Figure 3).

Discussion

Adrenal hemorrhage is a relatively uncommon condition with a variable and non- specific presentation that may lead to acute adrenal crisis, shock and death unless it is recognized promptly and treated appropriately. Several risk factors have been associated with it based on case reports.

Although the precise mechanisms leading to adrenal hemorrhage are unclear; in non-traumatic cases available evidence has implicated Adreno-Corticotropic Hormone (ACTH), adrenal vein spasm and thrombosis and normally limited venous drainage of adrenal in the pathogenesis of the condition (2). Unilateral adrenal hemorrhage occurs in 2% of patients with penetrating trauma (1), whereas right adrenal hemorrhage is reported in 2% of liver transplant patients. Hemorrhagic tumor infarction due to primary or metastatic tumors can cause unilateral adrenal hemorrhage (3, 6). Isolated case reports in association with long term non-steroidal anti-inflammatory drug use in uncomplicated pregnancy and neurofibromatosis have been reported. Idiopathic unilateral adrenal hemorrhage is a rare entity that may either have an acute presentation, such as massive retroperitoneal bleeding or an adrenal mass (2).

The main symptoms of the condition are hemorrhagic shock, flank pain and fever. Signs of acute abdomen including guarding, rigidity or rebound tenderness have been reported in 15%-20% because of the retroperitoneal location of adrenals (2). Obstetric causes of bilateral adrenal hemorrhage include

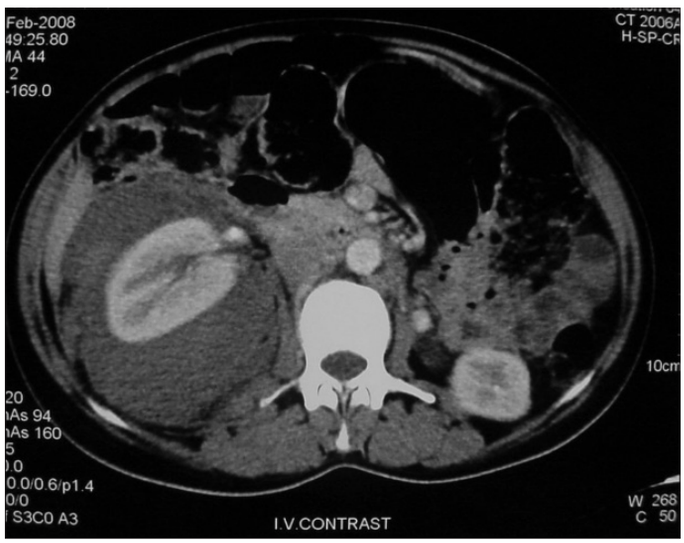


Figure 1. Contrast Enhanced Computerized Tomogram of abdomen at time of admission

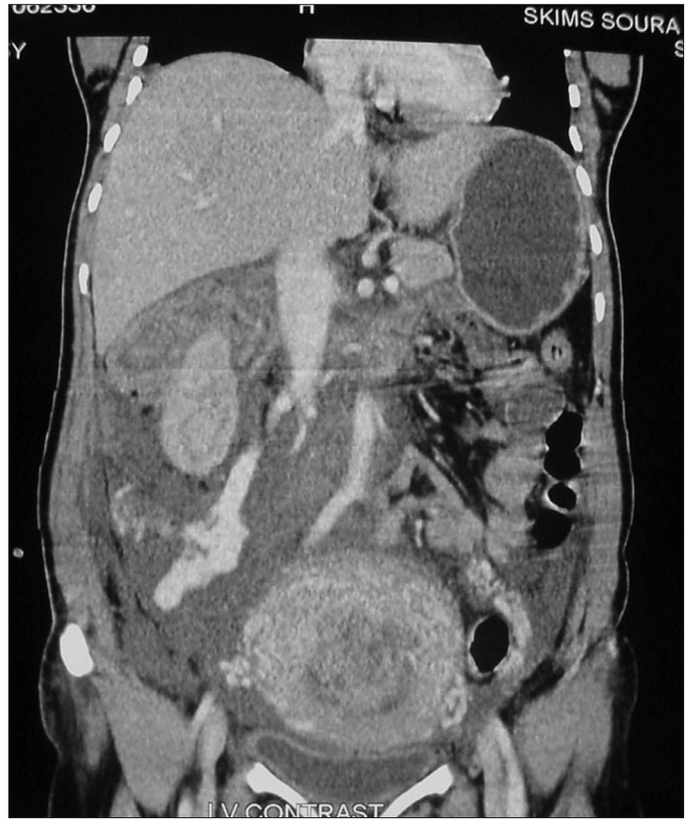


Figure 2. Reconstructed coronal view showing the extent of hematoma

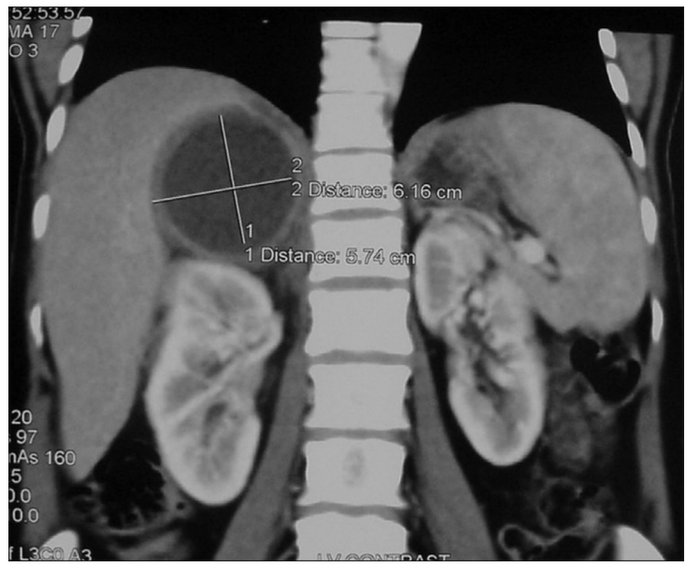


Figure 3. Adrenal Cyst on follow up scan

toxemia of pregnancy, spontaneous abortion, postpartum hemorrhage, twisted ovarian cyst in pregnancy and, more recently described, antiphospholipid antibody syndrome (7). Spontaneous adrenal hemorrhage during pregnancy has rarely been described. The present case also had pregnancy induced hypertension.

Ultrasonography examination of the adrenals, including Doppler study, provides useful information regarding hemorrhage into the adrenal gland. Several weeks after the acute event, as the hematoma becomes cystic, the central echogenicity associated with adrenal hemorrhage decreases.

Contrast CT scan of the adrenals is the study of choice in demonstrating the adrenal hemorrhage. The findings suggestive of hemorrhage into the adrenal are adrenal echogenicity with contrast enhancement, streaky appearance of peri-renal fat, perinephric hematoma, and retroperitoneal hematoma in a massive bleed. Several weeks after the acute hemorrhage, the CT scan shows a gradual decrease in size and attenuation. In addition, the adrenal may have a cystic appearance as was seen with the present case. Spontaneous adrenal hemorrhage may occur in pregnancy in the absence of trauma or sepsis. Thus, adrenal hemorrhage should be considered in the differential diagnosis of abdominal or flank pain with retroperitoneal hematoma in patients after delivery.

Conclusion

Spontaneous adrenal hemorrhage during pregnancy is a rare condition. The diagnosis is confirmed by MRI and CT scan. Adrenal hemorrhage should be considered in the differential diagnosis of a massive retroperitoneal hematoma in pregnant women after delivery.

Conflict of interest

No conflict of interest was declared by the authors.

References

1. Gavrilova-Jordan L, Edmister WB, Farrell MA, Watson WJ. Spontaneous adrenal hemorrhage during pregnancy: a Review of literature and case report of successful conservative management. Obstet Gynecol Surv March 2005; 60: 191-5. [CrossRef]
2. Kovacs KA, Lam YM, Pater JL. Bilateral massive adrenal hemorrhage. Assessment of putative risk factors by case controlled method. Medicine (Baltimore) 2001; 80: 45-53. [CrossRef]
3. Hiroi N, Yanagisawa R, Yoshida-Hiroi M, Endo T, Kawase T, Tsuchida Y, et al. Retroperitoneal hemorrhage due to bilateral adrenal metastasis from lung adenocarcinoma. J Endocrinol Invest 2006; 29: 551-4.
4. Merkle W. Spontaneous adrenal gland hemorrhage in adults. Urologe A 1986; 25: 343-6.
5. Kazarians B, Kausch I, Gellissen J, Doehn C, Jocham D. Spontaneous hemorrhage of adrenal gland during pregnancy. Aktuelle Urol 2007; 38: 403-5. [CrossRef]
6. Shah HR, Love LL, Williamson MR, Buckner BC, Feriss EJ. Hemorrhagic adrenal metastasis: CT findings. J Comput Assist Tomogr 1989; 13: 77-81. [CrossRef]
7. Espinosa G, Santos E, Cervera R, Piette JC, de la Red G, Gil V, et al. Adrenal involvement in antiphospholipid syndrome: clinical and immunological characteristic of 86 patients. Medicine (Baltimore) 2003; 82: 106-18. [CrossRef]

VACTERL-H syndrome: first trimester diagnosis

İlk trimester tanısı konan VACTERL-H Sendromu

Banu Dane[1], Zeynep Kayaoğlu[1], Cem Dane[1], Figen Aksoy[2]
[1]Department of Gynecology and Obstetrics, Haseki Training and Research Hospital, İstanbul, Turkey
[2]Department of Pathology, Cerrahpaşa Faculty of Medicine, İstanbul University, İstanbul, Turkey

Abstract

We present two consecutive female fetuses with identical upper limb anomalies. The first of the cases was found to have ventriculomegaly, atrial septal defect, anal atresia, narrowing of the duodenal lumen and unilateral renal agenesis at the end of the second trimester. These abnormalities were characteristic of autosomal recessive VACTERL-H syndrome. The second case was diagnosed to have absent radii and thumbs at 11 weeks. Detailed examination of fetal limbs in the first trimester screening in cases with high risk is useful for early detection of this malformation.

(J Turkish-German Gynecol Assoc 2011; 12: 266-9)

Key words: VACTERL-H syndrome, radial aplasia, first trimester diagnosis

Received: 12 July, 2010 **Accepted:** 3 September, 2010

Özet

Bu sunumda benzer üst ekstremite anomalisi saptanan iki kardeş fetüs takdim edilmektedir. İlk vakada ventrikülomegali, atriyal septal defekt, anal atrezi, duedonum lümeninde daralma ve unilateral renal agenezi tanısı ikinci trimester sonunda konmuştur. Bu anomaliler otozomal resesif geçişli VACTERL-H sendromunun karekteristik belirtilerindendir. İkinci vakada 11. haftada radius ve baş parmak yokluğu ultrasonografi ile tanı konmuştur. İlk trimester taramasında fetal ekstremitelerin detaylı incelemesi bu nadir malformasyonun erken tanısının konmasını sağlayabilir.

(J Turkish-German Gynecol Assoc 2011; 12: 266-9)

Anahtar kelimeler: VACTERL-H sendromu, radial aplazi, ilk trimester tanı

Geliş Tarihi: 12 Temmuz 2010 **Kabul Tarihi:** 03 Eylül 2010

Introduction

VACTERL association is defined as a combination of vertebral, anal, cardiac, tracheoesophageal, renal and limb anomalies. VACTERL cases are defined as having 2 or more of these anomalies and the majority (>90%) of these patients have two or three (1). The combination of VACTERL anomalies with hydrocephalus (VACTERL-H) was suggested to be a specific entity (2, 3). Patients with VACTERL-H syndrome often have bilateral and symmetrical radial ray abnormalities especially radial aplasia, imperforate anus and genital anomalies (4). X-linked (5-7) sporadic (8), and autosomal recessive (9) inheritance of this association were described in previous reports. There is only one case of VACTERL syndrome detected in the first trimester in the literature, which presented with megalourethra and hyperechogenic kidney (10).

In this case report we aimed to point out the importance of first trimester screening in cases with high risk of the familial form of this malformation.

Case Reports

Case 1

It was the first pregnancy of the mother (26-years), who was married to her cousin. First trimester screening was performed at 12 weeks of gestation and the patient was included in the high risk group (nuchal translucency: 2.1mm, PAPP-A: 0.3 MoM, Free Beta HCG 0.29 MoM). The lateral ventricle of the fetus was 11mm and double bubble sign was remarkable in the second trimester (at 19 weeks of gestation). Karyotype analysis from the fetal blood at 22 weeks was normal (46, XX), no chromosome breakage was found. At 23 weeks, ventriculomegaly was more prominent (14 mm). The parents opted for termination. At birth (24 weeks), the female fetus weighed 420 g, length was 35 cm (Figure 1). Autopsy findings included ventriculomegaly, unilateral radial aplasia, contralateral hypoplasia, bilateral absence of the thumbs (Figure 2), anorectal atresia, narrowing of the duodenal lumen, absence of the right kidney and pelvic location of the left one and atrial septal defect.

Case 2

In the second pregnancy the mother was admitted to our department at 11 weeks of gestation (CRL: 40.9 mm). At a detailed sonographic examination, bilateral absence of the radii and thumbs were detected (Figure 3a, 3b). After two weeks, the nuchal translucency was 1.6 mm, anomalies of upper limbs were more prominent and the fetus did not have any associated malformation at the sonographic examination (Figure 4). The parents opted for termination at 13 weeks of gestation. Autopsy findings were only bilateral radial aplasia and bilateral aplasia of the thumbs (Figure 5). The karyotype of the fetus was normal (46, XX). Analysis for chromosome breakage was not performed. The parents were informed about the 25% risk of recurrence and the availability of prenatal diagnosis.

Address for Correspondence: *Banu Dane, Emlak Bankası Konutları, B1 Blok, Daire: 12, Fatih, İstanbul, Turkey*
Phone: +90 212 621 75 21 e.mail: banudane@yahoo.com

doi:10.5152/jtgga.2011.62

Discussion

Phenotypic manifestations of the autosomal recessive form of VACTERL-H syndrome and the X linked recessive form have been reported to be almost identical, and Lurie et al. suggested that the only exception might be the absence of cardiovascular malformations in cases with X linked inheritance (11). Atrial septal defect was one of the associated anomalies of our first case, as in other cases with the autosomal recessive form.

Lurie et al. reported also that patients with autosomal recessive inheritance and sporadic cases of VACTERL-H association showed two differences: Firstly, radial bone involvement occurred in all familial but only in some sporadic cases. Secondly, cardiovascular malformations were more severe in patients with autosomal recessive inheritance (11). As proposed by the authors, both of our cases had radial aplasia as in other familial cases.

Kovacs et al. presented two consecutive mid trimester fetuses in a family, which had ventriculomegaly with radial and renal defects (12). Progressive ventriculomegaly served the basis for prenatal diagnosis in their cases, as in our first case. Since this was not a prominent finding before 19 weeks of gestation, it could not be useful in early detection in the second case.

In a review of 12 cases, Harris et al. reported that the dilatation of the colon in anal atresia may be related to gestational age and is more likely to be present beyond 27 weeks (13). However, Lam et al. reported a case of anorectal malformation (ARM) which illustrated that a dilated colon may be present in association with anal atresia as early as 12 weeks (14). Even in multiple associated anomalies, prenatal detection of ARM by fetal ultrasonographic examination was found to have a low sensitivity (36%) (15). The presence of anorectal atresia was not detected prenatally in the first case because of the absence of dilated colon at 23 weeks.

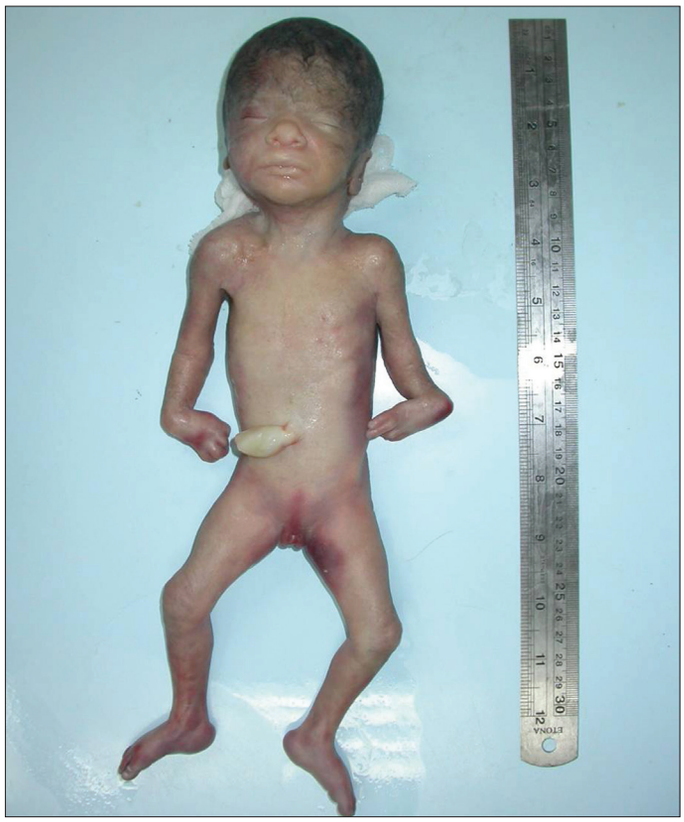


Figure 1. Macroscopic appearance of the first case

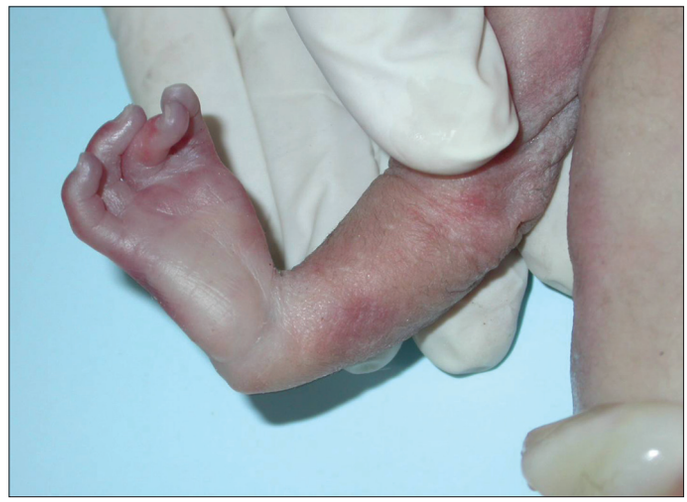


Figure 2. Absence of the thumb

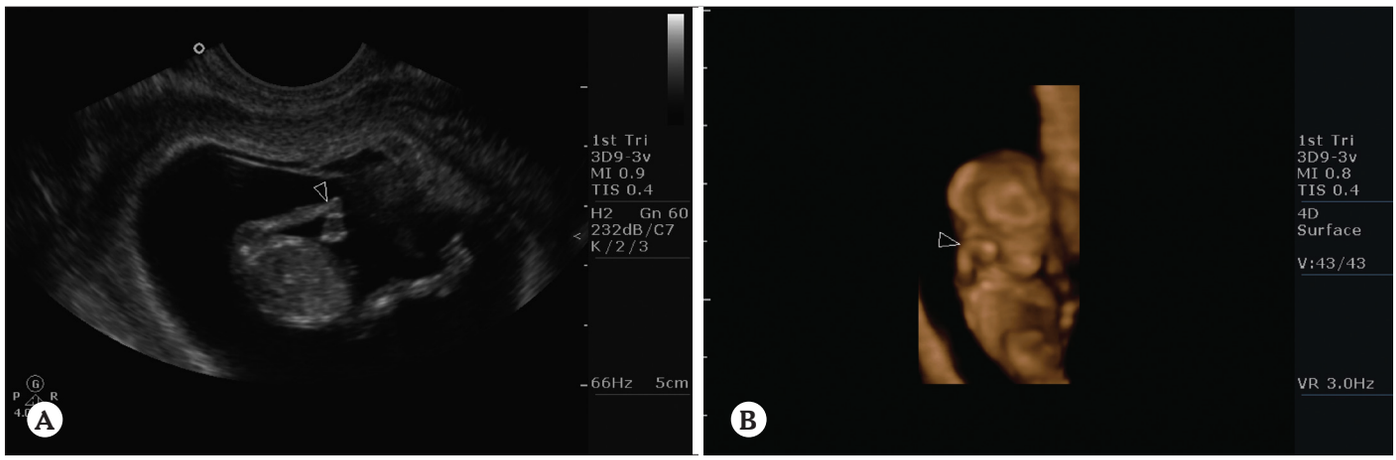


Figure 3. A) Absence of radius and thumb at 11 weeks (CRL 40.9 mm), B) 3D sonographic appearance of the hand (note the position of the fingers)

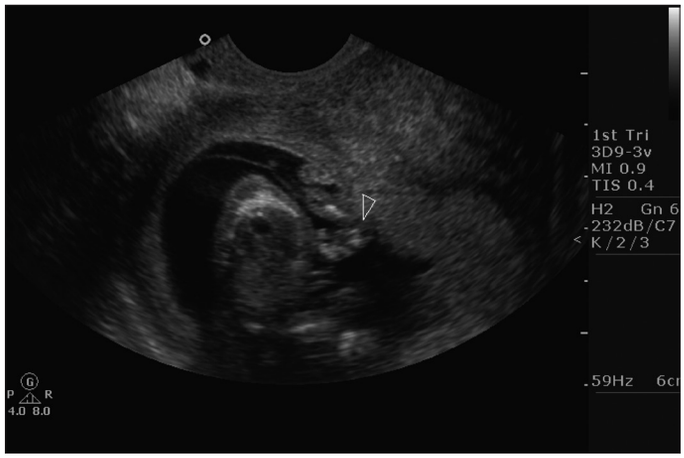


Figure 4. Absence of the radius and thumb at 12 weeks 4 days (CRL 65 mm)

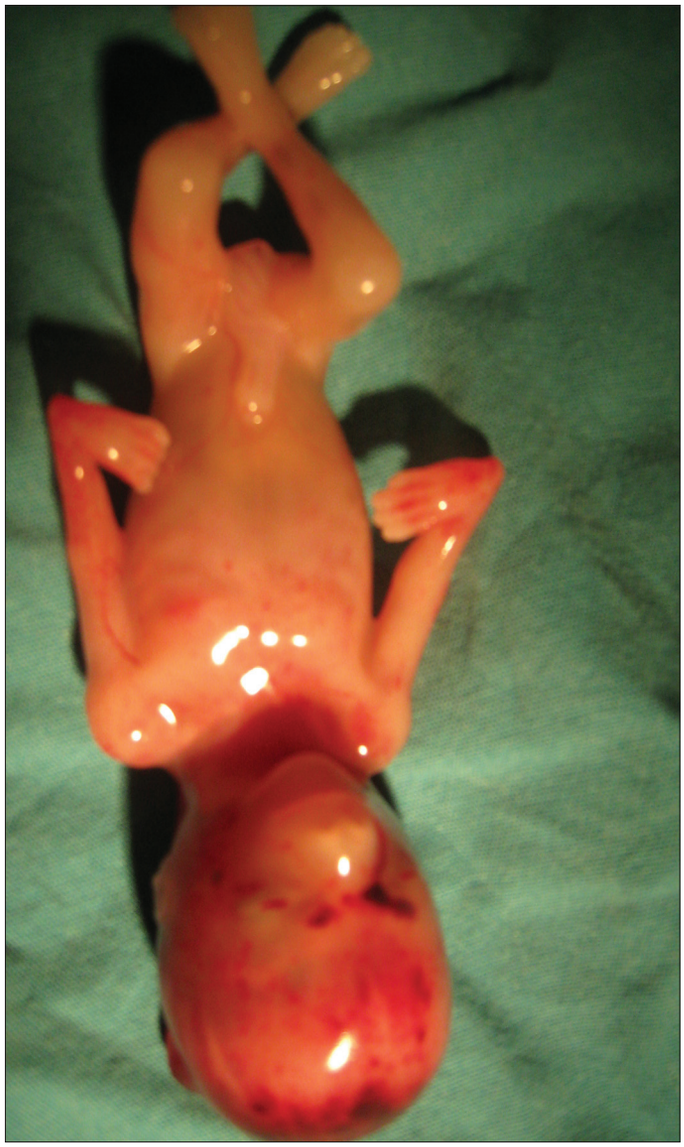


Figure 5. Macroscopic appearance of the second case

The presence of duodenal atresia in cases of VACTERL syndrome have been reported (16, 17). Fujishiro et al. proposed that, in the absence of limb anomalies, signs of esophageal atresia and renal anomalies, the diagnosis might be very difficult. The detection of characteristic findings of duodenal atresia (double bubble: enlarged stomach and duodenum) was suggested to be useful for the prenatal diagnosis of such cases (17). Although in the first case the presence of double bubble at 19 weeks was a prominent sign, it could only be detected during the late second trimester.

The VACTERL- H phenotype is recognized to be a severe manifestation of autosomal recessive Fanconi anaemia. Although Lomas et al. did not show any increased chromosome breakage in amniocytes from affected cases when challenged with mitomycin C (18), Wang et al. found that chromosomes from males with VACTERL-H syndrome had increased spontaneous breakages and sensitivity to mitomycin C (19). Mutations in FANCB gene are reported to be the cause of X linked VACTERL-H syndrome (19, 20). Both of our cases were female and in the first case we did not find any chromosome breakage. Analysis of the parents and their relatives might reveal new forms of mutations.

In a review of 44 VACTERL association cases, Kolon et al reported a 100% survival rate (21). The prognosis of VACTERL-H syndrome is reported to be poor because of the severity of the malformations (5, 22-24). However the survivors require significant surgical treatment and care (25, 26). Early detection of this anomaly provides the option of termination. Unfortunately some of the major components could not be detected until the end of the second trimester with ultrasonography. Detailed examination of upper limbs in the first trimester screening may be a useful sign in the detection of familial cases.

Conflict of interest

No conflict of interest was declared by the authors.

References

1. Rittler M, Paz JE, Castilla EE. VACTERL association, epidemiologic definition and delineation. Am J Med Genet 1996; 63: 529-36. [CrossRef]
2. Evans EJ, Stranc LC, Kaplan P, Hunter AGW. VACTERL with hydrocephalus: Further delineation of the syndrome(s). Am J Med Genet 1989; 34: 177-82. [CrossRef]
3. Reardon W, Zhou XP, Eng C. A novel germline mutation of the PTEN gene in a patient with macrocephaly, ventricular dilatation and features of VATER association. J Med Genet 2001; 38: 820-3. [CrossRef]
4. Lomas FE, Dahlstrom JE, Ford JH. VACTERL with hydrocephalus: family with x-linked VACTERL-H. Am J Med Genet 1998; 76: 74-8. [CrossRef]
5. Froster UG, Wallner SJ, Reusche E, Schwinger E, Rehder H. VACTERL with hydrocephalus and branchial arch defects: Prenatal, clinical and autopsy findings in two brothers. Am J Med Genet 1996; 62: 169-72. [CrossRef]
6. Genuardi M, Chiurazzi P, Capelli A, Neri G. X-linked VACTERL with hydrocephalus: The VACTERL-H syndrome. Birth Defects Orig Artic Ser 1993; 29: 235-41.
7. Kunze J, Huber-Schumacher S, Vogel M. VACTERL plus hydrocephalus: a monogenic lethal condition. Eur J Pediatr 1992; 151: 467-8. [CrossRef]

8. Porteous MEM, Cross I, Burn J. VACTERL with hydrocephalus: One end of the Fanconi anemia spectrum of anomalies? Am J Med Genet 1992; 43: 1032-4. [CrossRef]
9. Corsello G, Giuffre L. VACTERL with hydrocephalus: A further case with probable autosomal recessive inheritance. Am J Med Genet 1994; 49: 137-8. [CrossRef]
10. Krapp M, Geipel A, Germer U, Krokowski M, Gembruch U. First-trimester sonographic diagnosis of distal urethral atresia with megalourethra in VACTERL association. Prenat Diagn 2002; 22: 422-4. [CrossRef]
11. Lurie IW, Ferencz C. VACTERL- hydrocephaly, DK- phocomelia, and cerebro-cardio-radio-reno-rectal community. Am J Med Genet 1997; 70: 144-9. [CrossRef]
12. Kovács T, Csécsei K, Szabó M, Tóth Z, Veress L, Papp Z. Ventriculomegaly with radial and renal defects: Prenatal diagnosis in two consecutive sibs. Am J Med Genet 1997; 73: 259-62. [CrossRef]
13. Harris RD, Nyberg DA, Mack LA, Weinberger E. Anorectal atresia: prenatal sonographic diagnosis. AJR Am J Roentgenol 1987; 149: 395-400.
14. Lam YH, Shek T, Tang HY. Sonographic features of anal atresia at 12 weeks. Ultrasound Obstet Gynecol 2002; 19: 523-4. [CrossRef]
15. Stoll C, Alembik Y, Dott B, Roth MP. Associated malformations in patients with anorectal anomalies. Eur J Med Genet 2007; 50: 281-90. [CrossRef]
16. Choudhry MS, Rahman N, Boyd P, Lakhoo K. Duodenal atresia: associated anomalies, prenatal diagnosis and outcome. Pediatr Surg Int 2009; 8: 727-30. [CrossRef]
17. Fujishiro E, Suzuki Y, Sato T, Kondo S, Miyachi M, Suzumori K. Characteristic findings for diagnosis of baby complicated with both VACTERL association and duodenal atresia. Fetal Diagn Ther 2004; 19: 134-7. [CrossRef]
18. Lomas FE, Dahlstrom JE, Ford JH. VACTERL with hydrocephalus: family with X-linked VACTERL-H. Am J Med Genet 1998; 76: 74-8. [CrossRef]
19. Wang H, Hunter AG, Clifford B, Mclaughlin M, Thompson D. VACTERL with hydrocephalus: spontaneous chromosome breakage and rearrangement in a family showing apparent sex-linked recessive inheritance. Am J Med Genet 1993; 47: 114-7. [CrossRef]
20. Holden ST, Cox JJ, Kesterton I, Thomas NS, Carr C, Woods CG. Fanconi anaemia complementation group B presenting as X linked VACTERL with hydrocephalus syndrome. J Med Genet 2006; 43: 750-4. [CrossRef]
21. Kolon TF, Gray CL, Sutherland RW, Roth DR, Gonzales ET Jr. Upper urinary tract manifestations of the VACTERL association. J Urol 2000; 163: 1949-51. [CrossRef]
22. Balci S, Senocak M, Derbent M. Triphalangeal thumb in a case of VACTERL-hydrocephalus association. Genetic Counselling 2003; 14: 257-8.
23. Vatansever U, Başaran UN, Güzel A, Acunaş B, Balci S. VACTERL-H with triphalangeal thumb and hypothyroidism in a female patient. Clin Dysmorphol 2004; 13: 29-30. [CrossRef]
24. Aliefendioglu D, Bademci G, Keskil S, Somuncu S, Misirlioglu E, Cakmak AM. VACTERL-H associated with central hypothyroidism: a case report. Genet Couns 2007; 18: 331-5.
25. Herman TE, Siegel MJ. VACTERL-H Syndrome. J Perinatol 2002; 22: 496-8. [CrossRef]
26. Miller OF, Kolon TF. Prenatal diagnosis of VACTERL association. J Urol 2001; 166: 2389-91. [CrossRef]

There is no neo-oogenesis in the adult mammalian ovary

Erişkin memeli overde neo-oogenezi yoktur

Alain Gougeon[1], Elena Notarianni[2]
[1]Inserm U1052, ANIPATH U.F.R. de médecine Lyon, Lyon, France
[2]Department of Biological and Biomedical Sciences, Durham University, Durham, UK

Introduction

In 2004 and 2005, Johnson et al. published two very provocative studies (1, 2), in which they claimed that in the adult mouse ovary, neo-oogenesis takes place and originates either from the ovarian surface epithelium (OSE) (1) or from the bone marrow (BM) via circulating blood cells (2). These studies were provocative since they challenged the long-held view that mammals are born with a finite number of eggs that declines with ageing. Consequently, an intensive discussion has developed among experts in the field, some of whom are proponents of neo-oogenesis, while others are opponents (Table 1).

Neo-oogenesis is a very complex issue that has led to many questions. The aim of our lecture is to explain why we believe that spontaneous neo-oogenesis does not take place in the adult ovary by addressing three of these questions.

Is there any evidence for spontaneous neo-oogenesis in adult rodent and human ovaries?

Johnson et al. (1) claimed that, in mice, atresia in the immature follicle pool (i.e. including follicles from the primordial to the preantral stage) is so high that complete exhaustion of the pool would be predicted for young adults. Consequently, according to Johnson et al., only the renewal of oocytes as generated by neo-oogenesis can explain the fact that mice are still fertile after the advanced age of 1 year.

To estimate the rate of this atresia, Johnson et al. (1) scored as atretic those immature follicles exhibiting a “condensed, involuted or fragmented oocyte”, and consequently counted around 2700 healthy follicles and 200 to 400 atretic immature follicles at postnatal day 30 PN in C57/Bl6 mice. Meanwhile, Bykov et al. (3) counted between 1810 and 3280 healthy and 235-480 atretic follicles, i.e. similar numbers to the data of Johnson et al. (1). However, except for 3 atretic primordial follicles, all immature (primordial, primary and preantral) follicles were healthy, which is in agreement with previous published data showing that atresia of immature follicles is very low in rodents and human ovaries. Among the atretic follicles, antral atretic follicles exhibited a healthy looking oocyte, whereas degenerated and fragmented oocytes were only observed in atretic follicles at a late stage of atresia and which were previously antral follicles. Thus, it emerges that Johnson et al. (1), misattributed as atretic immature follicles those 200 to 400 atretic follicles that were already present at least 8 days earlier, as shown by their BrdU labeling. How can we explain such a misinterpretation? Returning to the criteria used to categorize atresia above, whereas condensation of oocytes (Figure 1) constitutes the normal fate of atretic resting follicles, and oocyte degeneration the normal fate of early growing follicles, fragmented oocytes are only seen in antral follicles at a late stage of atresia (Figure 2). When antral follicles undergo atresia, they progressively lose their antrum and shrink to the size of preantral follicles. This is the likely reason why Johnson et al. (1) mistook these follicles as being “immature”.

Consequently, it cannot be deduced (1) that between postnatal days 30 and 42, 10% to 33% of the immature pool is atretic. These percentages apply to antral follicles that degenerate instead of becoming preovulatory, with the oocyte being one of the last of the constituent cells to disappear. This issue is crucial since the rate of follicle depletion in the postnatal mouse ovary provided by Johnson et al. (1), which was deduced from the percentages of immature atretic follicles at different ages, was calculated based on a follicular clearance rate of between 3 and 18h rather than the more appropriate estimate for antral follicles of more than 8 days. Consequently, the ovarian reserve would not be completely exhausted by young adulthood and adult female mice would not need neo-oogenesis for maintaining a normal ovarian function.

In the rat, meanwhile, Meredith et al. (4) have shown, using BrdU incorporation, that approximately 60% of resting follicles present at a given time are still present 5 months later. Also, in this species, Zhang et al. (5), failed to detect early meiosis-specific proteins at the transcriptional (SCP1, SCP3, SPO11) or translational (SCP1, STRA8) levels in the post natal rat ovary. Together, the long half-life of the resting follicles and the absence of cells in early meiosis argue against the existence of spontaneous neo-oogenesis in the adult rat ovary.

If, as required by the neo-oogenesis concept, oogonia exists in the adult ovary as intermediates between stem cells and

Address for Correspondence: *Alain Gougeon, INSERM U1052 7, rue Guillaume Paradin 69372 Lyon cedex 08 France*
Phone: 33 (0)6 77 95 02 62 e.mail: alain.gougeon@inserm.fr

doi:10.5152/jtgga.2011.63

Table 1. Some articles written by proponents and opponents of neo-oogenesis in the adult ovary

Experimental studies supporting neo-oogenesis
Johnson et al., 2004. Nature 425: 145-50.
Bukovsky et al., 2004. Reprod Biol Endocrinol 2: 20.
Johnson et al., 2005. Cell 122: 303-15.
Bukovsky et al., 2005. Reprod Biol Endocrinol 3: 17.
Bukovsky et al., 2005. Reprod Biol Endocrinol 3: 36.
Kerr et al., 2006. Reproduction 132: 95-109.
Lee et al., 2007. Cell Cycle 6: 2678-84.
Lee et al., 2007. J Clin Oncol 25: 3198-204.
Bukovsky et al., 2008. Cell Cycle 7: 683-6.
Virant-Klun et al., 2008. Differentiation 76: 843-56.
Virant-Klun et al., 2009. Stem Cells Dev 18: 137-49.
Niikura et al., 2009. Aging 1: 971-8.
Zou et al., 2009. Nature Cell Biol 11: 631-6.
Pacchiarotti et al., 2010. Differentiation 79: 159-70.
Parte et al., 2010. Stem Cells Dev 20: 1451-64.
Celik et al., 2009. Int J Gynaecol Obstet 106: 218-22.
Reviews, opinions and comments supporting neo-oogenesis
Johnson et al., 2005. Cell Cycle 4: e36-e42.
Tilly and Johnson 2007. Cell Cycle 6: 879-83.
Skaznik-Wikiel etal., 2007. Differentiation 75: 93-9.
Abban & Johnson, 2009. Hum Reprod 24: 2974-8.
Bukovsky et al., 2009. Birth Defects Res C Embryo Today 87:64-89.
Tilly et al., 2009. Biol Reprod 80: 2-12.
De Felici 2010. Mol Hum Reprod 16: 632-6.
Virant-Klun et al., 2010. Aging 2: 3-6.
Virant-Klun et al., 2011. Histol Histopathol. 26: 1071-82.
Experimental studies concluding that "there is no neo-oogenesis"
Telfer, 2004. Reprod Biol Endocrinol 2: 24.
Byskov et al., 2005. Differentiation 73: 438-46.
Eggan et al., 2006. Nature 441: 1109-14.
Bristol-Gould et al., 2006. Dev Biol 298: 149-54.
Liu et al., 2007. Dev Biol 306: 112-20.
Veitia et al. Stem Cells. 25: 1334-5.
Begum et al., 2008. Hum Reprod 10: 2326-30.
Faddy and Gosden 2009. Biol Reprod 81: 231-2.
Zhang et al., 2010. Reprod Dom Anim 45: e447-e53.
Byskov et al., 2011. Hum Reprod 26: 2129-39.
Reviews, opinions and comments concluding that there is no neo-oogenesis
Albertini, 2004. Reproduction 127: 513-4.
Gosden 2004. Hum Reprod Update 10: 193-5.
Greenfeld and Flaws 2004. BioEssays 26: 829-32.
Ainsworth, 2005. Nature 436: 609.
Hutt and Albertini 2006. J Exp Clin Ass Reprod 3: 6.
Notarianni 2011. J Ov Res 4: 1.

oocytes, they will enter meiosis at various times and progress through leptotene, pachytene and zygotene stages to reach diplotene (dictyate oocyte) stage, at which meiosis is blocked. If Johnson's calculation for follicle renewal were correct (1), around 60 oocytes would be in transit through meiosis every day. Figure 3 shows that it is very easy to discriminate between oocytes in the intermediate (pre-dictyate) stages of meiosis and those that are arrested (dictyate). Despite the hundreds of thousands of primordial follicles that have been analysed for quantification and quality assessment purposes, pre-dictyate-stage meiotic oocytes have never been observed in either primates (at least 250 human and adult macaca ovaries examined by A. Gougeon) or rodent ovaries. Also, Liu et al. (6), failed to observe early meiotic oocytes and proliferating germ cells, or to detect mRNA for early meiosis-specific or oogenesis-associated genes (SPO11, PRDM9, SCP1, TERT and NOBOX), in adult human ovaries. In addition, Byskov et al. (7) observed that, in 82 human ovaries (from the embryonic stage to the age of 32 years), almost all oogonia stained exclusively for SSEA4, NANOG, OCT4 and c-kit, whereas only a small fraction stained for oogonia-specific MAGE-A4. These few oogonia disappeared from the ovary before 2 years of age, leaving only dictyate oocytes that stained for c-kit.

Consequently, it can be concluded that oogonia, the bona fide female germline stem cells, do not persist to support spontaneous neo-oogenesis in the adult human ovary.

What is the true effect of busulfan on folliculogenesis?

In the study of Johnson et al. (2), strain CB57/Bl6 female mice were treated with a mixture of cyclophosphamide and busufan (Cy/Bu). Two months after the treatment, no follicles were detectable within the ovaries. According to the authors, the spontaneous depletion of the ovarian reserve by atresia of the immature pool was no longer counteracted by neo-oogenesis, owing to the destruction by Bu of premeiotic germ cells present in the ovary at the time of treatment, leading to a complete exhaustion of the ovarian reserve within 3 weeks.

Although logical, this conclusion assumes that Bu is not cytotoxic to the resting and growing follicles already present at the time of treatment. However, Bu treatment disrupts the whole process of folliculogenesis by inducing both atresia and abnormal follicular growth (8); and, as shown by Generoso et al. (9), one injection of Bu has a dose-dependent detrimental effect on fertility via attrition of the oocyte pool. Interestingly, Bu is cytotoxic to follicles, via suppression of c-kit/SCF signaling (10), this system being crucial for activation of resting follicles and for survival of resting and growing follicles.

It can therefore be concluded that, in the study of Johnson et al. (1), Bu would not have inhibited neo-oogenesis, but rather would have destroyed growing follicles and strongly depleted the ovarian reserve. Consequently, all studies using Bu and claiming the existence of neo-oogenesis must be considered with caution.

In addition to the claim contested here that the combination treatment with Cy/Bu depletes the ovary via destruction only of oocytes generated by neo-oogenesis prior to meiosis, Johnson et al. (2) reported that bone marrow transfer (BMT) performed

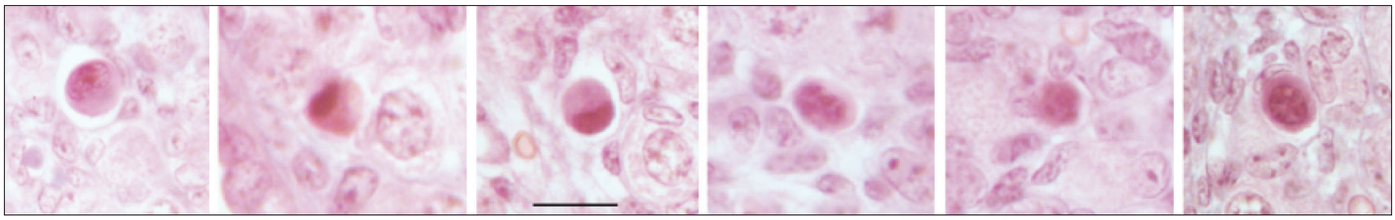


Figure 1. Some examples of atretic resting follicles exhibiting a condensed oocyte in the mouse ovary (bar=20 μm)

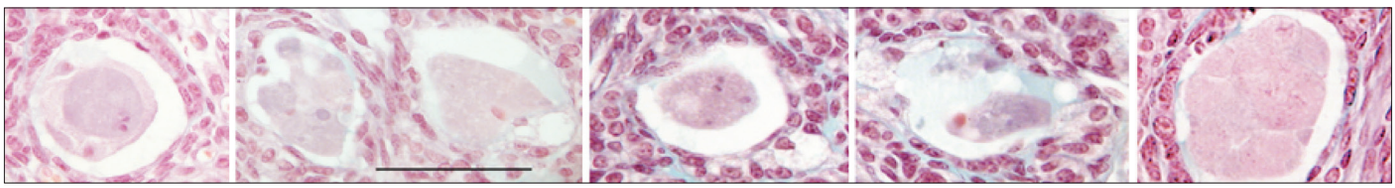


Figure 2. Some examples of involuted and fragmented oocytes from atretic antral follicles at a late stage of atresia in the mouse ovary (bar=80 μm)

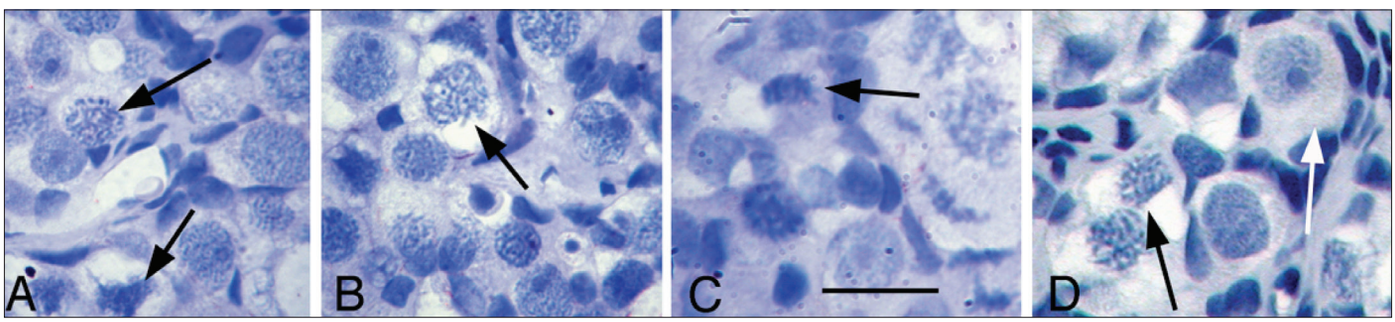


Figure 3. Leptotene, pachytene, zygotene (black arrows) and diplotene (white arrow) stages of meiosis in the foetal monkey (Macaca fascicularis). Notice the difference between the primordial follicle at the diplotene (dictyate) stage and the intermediate stages (Leptotene, pachytene, zygotene) of meiosis (Bar=30 μm)

7 days after the Cy/Bu treatment was able to refurbish the ovary with follicles at all stages of development. They subsequently concluded that neo-oogenesis occurred from germline stem cells present in the bone marrow. However this conclusion was challenged by three studies. Eggan et al. (11) have used parabiosis between a wild-type (WT) mouse, either treated or untreated with Cy/Bu, and a GFP (green fluorescent protein)-transgenic mouse. Six to eight months later the two parabionts were treated with PMSG for superovulation and oocytes harvested. Despite a strong BM chimaerism in both parabionts, the GFP-transgenic mice ovulated only GFP oocytes , whereas the WT-mice only ovulated WT-oocytes. Hence, no transmission of blood-borne oocyte precursors had occurred between parabionts. In another study by Lee et al. (12), using a similar experimental design to that of Johnson et al. (2), mice were treated with Cy/Bu followed by BMT from a GFP transgenic mouse one week later and immediate mating, and none of the offspring produced were GFP positive. Some GFP-positive cells were observed and termed “oocytes”, but were very small in number (1.4%) and apparently only located in small follicles. In a third study, performed by Begum et al. (13), irradiated (and therefore sterilized) WT ovaries were grafted under the kidney capsule of GFP-transgenic recipient mice. One month later, none of the resulting 48 growing oocytes were GFP positive. A similar experiment, consisting of grafting untreated WT-ovaries under the kidney capsule of GFP-transgenic recipient mice, has shown that none of the resulting 819 oocytes, either resting or growing, were GFP positive, 2, 4 and 8 weeks after the grafting procedure. Taken together, the results of these studies failed to show that neo-oogenesis occurs from putative germline stem cells (GSC) that are present in and circulated from the bone marrow.

Are de novo oocytes really generated by the ovarian surface epithelium?

The most recent papers supporting the concept of neo-oogenesis in the postnatal ovary postulate that GSC originate in the ovarian surface epithelium (OSE). Many alternative explanations concerning the presence of putative GSC within the OSE have been recently reported (15), and some of them are presented below.

Johnson et al. (1), as well as Zou et al. (14), reported the presence of cells positively stained for both BrdU and mouse vasa homolog (Mvh) in the OSE. They concluded that replicative GSC are present in the OSE. However, on the one hand BrdU may stain the mitochondrial DNA during its process of replication and/or DNA repair (15); and on the other hand Mvh, which is a germinal cell marker, can stain primordial follicles that are known to be located in the OSE. Consequently, the BrdU/Mvh stained cells present in the OSE are not GSC, but most likely

primordial oocytes during their physiologic extrusion process. Virant-Klun et al. (16), described small round cells, above and below the postmenopausal OSE, which they considered to be GSC. However, they may correspond instead to the small immune cells previously described in this location by Motta et al. (17). Zou et al., (14) claimed to have successfully isolated and purified GSC from disaggregated postnatal and adult mouse ovaries via Mvh-specific binding to an anti-Mvh antibody. However, they did not consider the possibility that their lines come from quiescent oogonia, which are known to be present in the postnatal mouse ovary up to day 7 (15). In addition, the presence of mitotic oogonia in the adult ovary is difficult to reconcile with the absence of the stage specific embryonic antigen-1, as oogonia proliferating in vivo are demonstrably positive for this marker.

Conclusion

This review highlights crucial issues in the debate over the existence, or otherwise, of female germline stem cells in the mammalian ovary. Further experimentation is needed to fully disprove the concepts that the mammalian ovary contains cells with stem cell-like characteristics that can be provoked to enter a differentiation process to oocytes, at least in vitro; and that in vitro conditions may allow the conversion of OSE cells into a multipotent stem cell-like phenotype. However, we claim that, in normal in vivo conditions, neo-oogenesis does not take place in the adult mammalian ovary.

References

1. Johnson J, Canning J, Kaneko T, Pru JK, Tilly JL. Germline stem cells and follicular renewal in the postnatal mammalian ovary. Nature 2004; 428: 145-50. [CrossRef]
2. Johnson J, Bagley J, Skaznik-Wikiel M, Lee HJ, Adams GB, Niikura Y, et al. Oocyte generation in adult mammalian ovaries by putative germ cells in bone marrow and peripheral blood. Cell 2005; 122: 303-15. [CrossRef]
3. Byskov AG, Faddy MJ, Lemmen JG, Andersen CY. Eggs forever? Differentiation 2005; 73: 438-46. [CrossRef]
4. Meredith S, Dudenhoeffer G, Jackson K. Classification of small type B/C follicles as primordial follicles in mature rats. J Reprod Fertil 2000; 119: 43-8. [CrossRef]
5. Zhang P, Lv LX, Xing WJ. Early meiotic-specific protein expression in post-natal rat ovaries. Reprod Domest Anim 2010; 45: 447-53. [CrossRef]
6. Liu Y, Wu C, Lyu Q, Yang D, Albertini DF, Keefe DL, et al. Germline stem cells and neo-oogenesis in the adult human ovary. Dev Biol 2007; 306: 112-20. [CrossRef]
7. Byskov AG, Høyer PE, Yding Andersen C, Kristensen SG, Jespersen A, Møllgård K. No evidence for the presence of oogonia in the human ovary after their final clearance during the first two years of life. Hum Reprod 2011; 26: 2129-39. [CrossRef]
8. Burkl W, Schiechl H. The growth of follicles in the rat ovary under the influence of busulphan and endoxan. Cell Tissue Res 1978; 186: 351-9. [CrossRef]
9. Generoso WM, Stout SK, Huff SW. Effects of alkylating chemicals on reproductive capacity of adult female mice. Mutat Res 1971; 13: 171-84. [CrossRef]
10. Choi YJ, Ok DW, Kwon DN, Chung JI, Kim HC, Yeo SM, et al. Murine male germ cell apoptosis induced by busulfan treatment correlates with loss of c-kit-expression in a Fas/FasL- and p53- independent manner. FEBS Lett 2004; 575: 41-51. [CrossRef]
11. Eggan K, Jurga S, Gosden R, Min IM, Wagers AJ. Ovulated oocytes in adult mice derive from non-circulating germ cells. Nature 2006; 441: 1109-14. [CrossRef]
12. Lee H-J, Selesniemi K, Niikura Y, Niikura T, Klein R, Dombkowski DM, et al. Bone marrow transplantation generates immature oocytes and rescues long-term fertility in a preclinical mouse model of chemotherapy-induced premature ovarian failure. J Clin Oncol 2007; 25: 3198-204. [CrossRef]
13. Begum S, Papaioannou VE, Gosden RG. The oocyte population is not renewed in transplanted or irradiated adult ovaries. Hum Reprod 2008; 23: 2326-30. [CrossRef]
14. Zou K, Yuan Z, Luo H, Sun K, Zhou L, Xiang J, et al. Production of offspring from a germinal stem cell line derived from neonatal ovaries. Nat Cell Biol 2009; 11: 631-6. [CrossRef]
15. Notarianni E. Reinterpretation of evidence advanced for neo-oogenesis in mammals, in terms of a finite oocyte reserve. J Ovarian Res 2011; 4: 1. [CrossRef]
16. Virant-Klun I, Rozman P, Cvjeticanin B, Vrtacnik-Bokal E, Novakovic S, Rülicke T, et al. Parthenogenetic embryo-like structures in the human ovarian surface epithelium cell culture in postmenopausal women with no naturally present follicles and oocytes. Stem Cells Dev 2009; 18: 137-49. [CrossRef]
17. Motta PM, Heyn R, Makabe S. Three-dimensional microanatomical dynamics of the ovary in postreproductive aged women. Fertil Steril 2002; 78: 360-70. [CrossRef]

Neo-oogenesis: Has its existence been proven?

Neo-oogenezi: Varlığı kanıtlandı mı ?

Önder Çelik, Ebru Çelik, Ilgın Türkçüoğlu
Department of Obstetrics and Gynecology, Faculty of Medicine, İnönü University, Malatya, Turkey

Introduction

It was long believed that mammals are born with a fixed number of primordial follicles, each of which encloses an oocyte arrested at the diplotene step of meiotic prophase. Arising at or after birth, small numbers of primordial follicles become activated each day and enter a folliculogenic growth phase during which follicles either degenerate by atresia or complete maturation, resulting in ovulation (1-7). The reserve of follicles is claimed to continue depletion by the process of atresia throughout adulthood to the point of exhaustion around menopausal age, driving ovarian failure (8, 9).
Recently, the work of Johnson et al challenged the half century old dogma that the primordial follicle pool cannot be replenished after birth (10). Since then, the concept of neo-oogenesis has been a subject of vigorous experimentation and debate. Existing and challenging developments in female reproductive biology have led to a growing body of evidence that the ovarian follicle pool may be replenished during adult life.

Evidence of Neo-ogenesis:
The idea of a follicle arising from the surface epithelial cells of a postnatal mouse ovary was suggested as early as 1917 by Kingery (11). Recently, Kerr et al. reported a finding similar to the previous study showing that, consequent to a marked depletion of follicles and oocytes during the first postnatal week, the mean primordial follicle numbers per ovary, measured by using unbiased and assumption free stereological methods, remained constant and stable in the subsequent 13 weeks up to day 100 of age in C57BL/6 female mice, and their recruitment into the population of mature follicles was associated with no significant decay in the total numbers of all healthy follicles over the same period (12). The first demonstration of the isolation of germ-line stem cells (GSC) carrying a green fluorescent protein reporter gene from adult mouse ovaries that produce spontaneously component oocyte has challenged the classical belief (13). In 2004, an innovative study showed that the mouse ovary has an ability to produce new oocytes and follicles from GSC present in the ovarian surface epithelium after their destruction (10). Additionally, they reported the presence of presumptive mitotically active GSC, expressing the germ cell specific VASA protein, in or proximal to the OSE of juvenile and adult mice. However, some discordance in the rate of follicle loss versus follicle atresia in the neonatal ovaries and adult ovaries make it unlikely, in the opinion of the authors themselves that these cells can represent adult GCS. Based on the previous study, the same group also proposed the possibility of an extra gonadal source of germ cells in adult mice. The existence of ovarian primordial cells was depleted, and subsequently OSE cells were revoked in favor of bone marrow and peripheral blood cells as the derivation of newly formed oocyte-containing primordial follicles (14). In turn, a subsequent study demonstrated that, in a parabiotic model, no developmentally component oocytes derived from blood born progenitor germ were produced after bone marrow transplantation (15). Some concerns were expresed in this study that each used only two mice and a total of seven ovulated eggs or fewer were retrieved and examined. Indeed, a subsequent study has provided surprising insight into the presence of germ stem cells in the OSE of postmenopausal women. In this study, the isolation of rare putative stem cells with germ-line characteristics in the OSE of post-menopausal human ovaries devoid of oocytes was documented. Further, these cells have the ability to spontaneously form oocytes or oocyte-like cells and also could undergo pathogenetic development to generate blastocyst-like structures in vitro under certain conditions (16). In 2009, we demonstrated successfully developed ovarian cells and possible follicular structures by using porcine intestinal submucosa to reconstruct an ovarian defect. In addition, noticeable PCNA staining was found in oocytes arrested in the meitotic stage (17).
A recent case report somewhat contradicts the claim that precursors in peripheral blood may produce new functional germ cells; genetic analysis of a single offspring conceived by a female patient with Fanconi anemia who received bone marrow transplantation from an unrelated donor after a chemotherapy protocol along with thoraco-abdominal irradiation. Analysis of polymorphic microsatellites revealed a genetic link between the daughter and mother, but not with the donor. Based on this finding, the authors stated that unaccomplished depletion of the ovarian follicle pool,not the

Address for Correspondence: *Önder Çelik, Department of Obstetrics and Gynecology, Faculty of Medicine, İnönü University, Turgut Özal Medical Center, Elazığ Road 8th, Malatya, Turkey Phone: +90 533 513 03 73 e.mail: oncelik@inonu.edu.tr*

doi:10.5152/jtgga.2011.64

transplantation of BM derived GSC, could be the reason for an improvement of fertility (18). Nonetheless, it is noteworthy that the capacity of the ovarian follicle reserve was not evaluated prior and subsequent to the BMT.

In parallel to continued studies into the regulation and function of germ stem cell in mouse ovaries, attempts are required to demonstrate the presence of a comparable population of GSC in human ovaries. However, the study aiming to demonstrate expression of SCP3 marker for meiosis in adult human ovarian tissue failed, in contrast to fetal ovary controls (19). The authors concluded, "If postnatal oogenesis is confirmed in mice, then this species would represent an exception to the rule that neo-oogenesis does not occur in adults". The inability of Liu et al. to detect the early oocyte marker, NOBOX, in their adult ovary samples is the major limitation despite being inconsistent with previously reported abundant expression of NOBOX in adult human ovaries. It is significant that SPC3 expressions also were detected in all of their adult human ovaries, even from a patient at 53 years of age. Moreover, Liu et al. themselves underscore the possible lack of functional conservation of genes claimed to be meiosis-specific from animal studies, concluding from their gene expression survey that MLH1, MSH5 and REC8 are not meiotic specific in humans. The authors did not determine either oogonia or oogonium-like cells expressing PCNA in the tunica albuginea and OSE among 231 sections from seven adult ovaries; in addition, their method for detection of oogonia or oogonium-like cells is ambiguous.

Of note, Niikura et al. demonstrated high expression of the premeiotic marker Stra8 and Daz1 in the ovary of aged female mice despite the absence of oocytes (20). Moreover, by grafting ovarian tissue harvested from an aged female germline specific GFP-expressing mice (PE-Oct4-Gfp or TgOG2 transgenic) into the ovarian bursal sac of young adult-type female recipients, a small number of GFP-positive germ cells, as immature follicles and co-expressing the primordial oocyte marker NOBOX, were detected. However, the number of immature follicles in the ovary of the young adult mouse, exposed directly to an aged systemic environment, was decreased. Further, this finding supports the concept that the rapid deterioration of follicles mostly involves impaired oocyte renewal rather than accelerated loss. In keeping with previous studies (21), Zou et al. established ovarian cells within the ovarian surface epithelium (OSE) that were double-positive for Mouse Vasa Homolog (MVH) and 5'-bromodeoxyuridine (BrDU) incorporation. High telomerase activity was detected in these cells when transplanted into females, giving rise to offspring that carried the signal. Recently, Pacchiarotti et al further illustrated the validation of cell lines as ovarian germline stem cells. The authors reported the isolation of cells containing the putative stem cells from the OSE of neonatal mice of the TgOG2. Likewise, these cells were presented from formation of "embryonic bodies" containing differentiated derivatives of the three germ layers and production of early stage of oocytes during culture (22).

Conclusion

The question of whether germ line stem cells exist in ovaries of humans should be actively addressed. This issue fascinates scientists involved in support offspring production and quality of life improvements in aged women. The view of neo-oogenesis in mammalian species is still controversial. There is growing evidence established in in vitro studies concerning the existence of putative GCS within adult mammalian ovaries, including humans. It may be possible that a small number of PGCs/oogonia or PGC-derived undifferentiated cells with GSC characteristics remain in the postnatal and adult ovary, and under certain conditions may resume mitosis, enter meiosis and give rise to oocytes. However, further studies to provide evidence favoring germ cell proliferation and oocyte renewal in adult human ovaries are needed.

References

1. Zuckerman S. The number of oocytes in the mature ovary. Rec Prog Horm Res 1951; 6: 63-109.
2. Peters H. The development of the mouse ovary from birth to maturity. Acta Endocrinol (Copenh) 1969; 62: 98-116.
3. Albertin DF. Micromanagement of the ovarian follicle reserve-do stem cells play into the ledger? Reproduction 2004; 127: 513-4. [CrossRef]
4. Gosden RG. Germline stem cells in the postnatal ovary: is the ovary more like a testis? Hum Reprod Update 2004; 10: 193-5. [CrossRef]
5. Greenfeld C, Flaws JA. Renewed debate over post- natal oogenesis in the mammalian ovary. Bioessays 2004; 26: 829-32. [CrossRef]
6. Telfer EE. Germline stem cells in the postnatal mammalian ovary: a phenomenon of prosimian primates and mice? Reprod Biol Endocrinol 2004; 2: 24. [CrossRef]
7. Telfer EE, Gosden RG, Byskov AG, Spears N, Albertini D, Andersen CY, et al. On regenerating the ovary and generating controversy. Cell 2005; 122: 821-2. [CrossRef]
8. Gosden RG, Laing SC, Felicio LS, Nelson JF, Finch CE. Imminent oocyte exhaustion and reduced follicular recruitment mark the transition to acyclicity in aging C57BL/6J mice. Biol Reprod 1983; 28: 255-60. [CrossRef]
9. Richardson SJ, Senikas V, Nelson JF. Follicular depletion during the menopausal transition: evidence for accelerated loss and ultimate exhaustion. J Clin Endocrinol Metab 1987; 65: 1231-7. [CrossRef]
10. Johnson J, Canning J, Kaneko T, Pru JK, Tilly JL. Germline stem cells and follicular renewal in the postnatal mammalian ovary. Nature 2004; 428: 145-50. [CrossRef]
11. Kingery HM. Oogenesis in the white mouse. J Morphol 1917; 30: 261-316. [CrossRef]
12. Kerr JB, Duckett R, Myers M, Britt KL, Mladenovska T, Findlay JK. Quantification of healthy follicles in the neonatal and adult mouse ovary: evidence for maintenance of primordial follicle supply. Reproduction 2006; 132: 95-109. [CrossRef]
13. Hübner K, Fuhrmann G, Christenson LK, Kehler J, Reinbold R, De La Fuente R. Derivation of oocytes from mouse embryonic stem cells. Science 2003; 300: 1251-6. [CrossRef]
14. Johnson J, Bagley J, Skaznik-Wikiel M, Lee HJ, Adams GB, Niikura Y, et al. Oocyte generation in adult mammalian ovaries by putative germ cells derived from bone marrow and peripheral blood. Cell 2005; 122: 303-15. [CrossRef]
15. Eggan K, Jurga S, Gosden R, Min IM, Wagers AJ. Ovulated oocytes in adult mice derive from non-circulating germ cells. Nature 2006; 441: 1109-14. [CrossRef]
16. Virant-Klun I, Rozman P, Cvjeticanin B, Vrtacnik-Bokal E, Novakovic S, Rülicke T. Parthenogenetic embryo-like structures in the human ovarian surface epithelium cell culture in postmenopausal women with no naturally present follicles and oocytes. Stem Cells Dev 2009; 18: 137-49. [CrossRef]

17. Celik O, Esrefoglu M, Hascalik S, Gul M, Tagluk ME, Elter K, et al. Use of porcine small intestinal submucosa to reconstruct an ovarian defect. Int J Gynecol Obstet 2009; 106: 218-22. [CrossRef]
18. Veitia RA, Gluckman E, Fellous M, Soulier J. Recovery of fertility after chemotherapy, irradiation and bone marrow allograft: further evidence against massive oocyte regeneration by bone marrow-derived germline stem cells. Stem Cells 2007; 25: 1334-5. [CrossRef]
19. Liu Y, Wu C, Lyu Q, Yang D, Albertini DF, Keefe DL, et al. Germline stem cells and neo-oogenesis in the adult human ovary. Dev Biol 2007; 306: 112-20. [CrossRef]
20. Niikura Y, Niikura T, Tilly JL. Aged mouse ovaries possess rare premeiotic germ cells that can generate oocytes following transplantation into a young host environment. Aging (Albany NY) 2009; 1: 971-8.
21. Zou K, Yuan Z, Yang Z, Luo H, Sun K, Zhou L, et al. Production of offspring from a germline stem cell line derived from neonatal ovaries. Nature Cell Biol 2009; 11: 631-6. [CrossRef]
22. Pacchiarotti J, Maki C, Ramos T, Marh J, Howerton K, Wong J, et al. Differentiation potential of germ line stem cells derived from the postnatal mouse ovary. Differentiation 2010; 79: 159-70. [CrossRef]

What is your diagnosis?

What is your diagnosis: A woman with a history of cesarean section and chronic cyclic abdominal pain in her history showed these ultrasound findings in the abdominal wall.

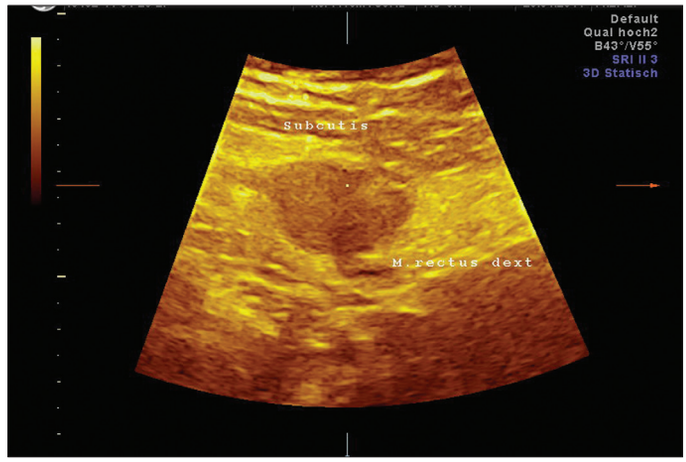


Figure 2. 2D imaging of abdominal wall mass

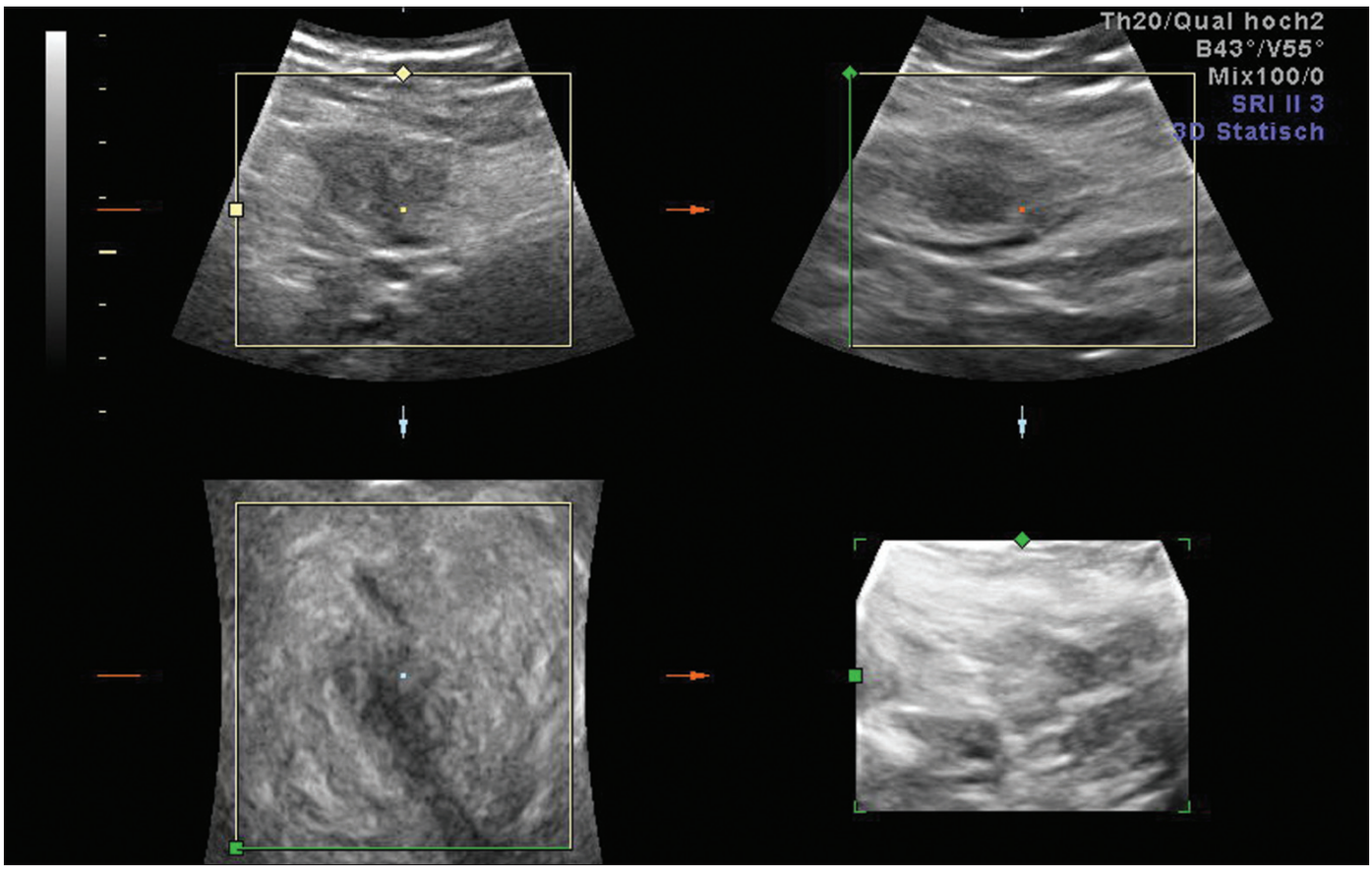


Figure 1. 3D multiplanar imaging of abdominal wall mass

Address for Correspondence: *Cemil Yaman, Department of Obstetrics and Gynecology, General Hospital of Linz, Karankenhausstr.9 4020 Linz, Akh-Linz, Austria*
Phone: 00436644219600 e.mail: cemil.yaman@akh.linz.at

doi:10.5152/jtgga.2011.65

Answer

Abdominal wall endometriosis

Abdominal wall scar endometriosis is a rare disease which is difficult to diagnose and should always be considered as a differential diagnosis of painful abdominal masses in women (1, 2). Any scar lesion that is involved in response to menstrual cycles should be considered as endometriosis, thus requiring wide surgical resection (1-3). The late onset of symptoms after surgery is the usual cause of misdiagnosis. Futhermore, malignant degeneration of extra pelvic endometriosis is described in the literature (3, 4).

The presented case has a 3x2 cm mass in the abdominal wall as shown with 3D multiplanar view in Figure 1 and with 2D view in Figure 2. The diagnosis is made only after excision and histopathology of the lesion. Preoperative differentials include hernia, lipoma, suture granuloma or abscess. Hence an awareness of the entity avoids delay in diagnosis, helps clinicians to a more tailored treatment and also avoids unnecessary referrals. The definitive diagnosis was established by histopathological studies.

Cemil Yaman, Dietmar Haas, Peter Oppelt
Department of Obstetrics and Gynecology,
General Hospital of Linz, Akh-Linz, Austria

References

1. Singh KK, Lessels AM, Adam DJ, Jordan C, Miles WF, Macintyre IM, et al. Presentation of endometriosis to general surgeons: a 10-year experience. Br J Surg 1995; 82: 1349-51. **[CrossRef]**
2. Dwivedi AJ, Agrawal SN, Silva YJ. Abdominal wall endometriomas. Dig Dis Sci 2002; 47: 456-61. **[CrossRef]**
3. Omranipour R, Najafi M. Papillary serous carcinoma arising in abdominal wall endometriosis treated with neoadjuvant chemotherapy and surgery. Fertil Steril 2010; 93: 1347. **[CrossRef]**
4. Da Ines D, Bourdel N, Charpy C, Montoriol PF,Petitcolin V, Canis M, Garcier JM. Mixed endometrioid and serous carcinoma developing in abdominal wall endometriosis following Cesarean section. Acta Radiol 2011; 52: 587-90. Epub 2011. **[CrossRef]**

Acknowledgements for the Year 2011-1

On behalf of the office staff and the editorial board of the *Journal of the Turkish-German Gynecological Association* (JTGGA), we would like to extend our thanks to all of our reviewers during the past year for your outstanding contributions.

We continue to see an increase in the number of submissions to JTGGA as well as the quality. JTGGA is clearly becoming the journal of choice for obstetrics and gynecology healthcare issues in our region. We can afford to be somewhat more selective, and our rejection rate of 33.3% (in 2011), (and 38.6% since the beginning-2000) approaches that of other major medical journals. The reviews submitted by you are among the best that we have seen among a number of major medical journals. The office regularly receives letters from authors thanking JTGGA for such thorough and helpful reviews, which enables them to produce much better manuscripts.

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

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

Best regards.

Cihat Ünlü,
Editors in Chief
İstanbul-Turkey

Peter Mallmann,
Editors in Chief
Köln-Germany

Klaus Vetter,
Former Editor
Berlin-Germany

Eray Çalışkan,
Editors
Kocaeli-Turkey

Gazi Yıldırım,
Editors
İstanbul-Turkey

A. Kubilay Ertan,
Associate Editors
Leverkusen-Germany

H. Taylan Öney,
Associate Editors
Bremen-Germany

Cenk Sayın,
Associate Editors
Edirne-Turkey

Hüseyin Mete Tanır,
Associate Editors
Eskişehir-Turkey

H. Alper Tanrıverdi,
Associate Editors
Aydın-Turkey

Cemil Yaman,
Associate Editors
Linz-Austria

Status	2000	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	Total
Saved	0	0	0	0	0	0	0	0	0	0	0	**0**
Waiting for Secretary	0	0	0	0	0	0	0	0	0	0	0	**0**
At Editor	0	0	0	0	0	0	0	0	0	0	4	**4**
On Review	0	0	0	0	0	0	0	0	0	0	13	**13**
Reviewed	0	0	0	0	0	0	0	0	0	0	0	**0**
Minor Revision	0	0	0	0	0	0	0	0	0	0	5	**5**
Major Revision	0	0	0	0	0	0	0	0	0	0	1	**1**
Rewriting Needed	0	0	0	0	0	0	0	0	0	0	1	**1**
Waiting for Final Decision	0	0	0	0	0	0	0	0	0	0	0	**0**
Accepted	0	0	0	0	0	0	0	0	0	6	24	**30**
Rejected	0	0	0	0	20	53	61	69	64	73	43	**383**
Refused	0	0	0	0	0	4	2	10	26	16	6	**64**
Withdrawn	0	0	0	0	0	12	35	8	5	8	4	**72**
Published	9	11	46	51	80	73	79	84	48	81	46	**608**
Total	**9**	**11**	**46**	**51**	**100**	**142**	**177**	**171**	**143**	**184**	**147**	**1181**

Acknowledgements for the Year 2011-2

Experts contributing at the review process in 2011(alphabetical order)

1-2 Manuscripts→Bronze, 3-5 Manuscripts→Silver, 6-10 Manuscripts→Gold

Eray Çalışkan (E)	6	Emek Doğer	2	Ercan Yılmaz	1
Cemil Yaman	5	Ender Yalçınkaya	2	Esra Bulgan Kılıçdağ	1
Ebru Tarim	5	Esra Esim Büyükbayrak	2	Fatma Ferda Verit	1
Gazi Yıldırım	5	Evin Nil Uğurlu	2	Fulya Kayıkçıoğlu	1
Olus Api	5	Harika Bodur Öztürk	2	Gökhan Yıldırım	1
Ali Gedikbaşı	4	Hüseyin Görkemli	2	Güler Şahin	1
Bahar Müezzinoğlu	4	Kemal Naci Kuşçu	2	Gürkan Bozdağ	1
M. Murat Naki	4	Korhan Kahraman	2	Gürkan Kiran	1
Mutlu Meydanlı	4	Mehmet Bilge Çetinkaya	2	Horu Gazi	1
Yaprak Engin Üstün	4	Mehmet Harma	2	İbrahim Gülhan	1
Ahmet Gul	3	Mehmet Tunc Canda	2	İsmet Gün	1
Arif Serhan Cevrioğlu	3	Mete Güngör	2	Jale Metindir	1
Aslıhan Polat	3	Metin İngeç	2	Jing Liu	1
Banu Dane	3	Murat Özekinci	2	Kayhan Yakın	1
Erhan Şimşek	3	Murat Ulukus	2	Levent Tütüncü	1
Evrim Erdemoğlu	3	Ömer Kandemir	2	Lütfü Önderoğlu	1
Hüseyin Mete Tanır	3	Özlem Özdeğirmenci	2	Mehmet Erdem	1
Kemal Güngördük	3	Petek Balkanlı Kaplan	2	Mehmet Osmanağaoğlu	1
Mekin Sezik	3	Selçuk Ayas	2	Münire Erman Akar	1
Nur Dokuzeylül	3	Tufan Öge	2	Narter Celalettin Yeşildağlar	1
Özlem Pata	3	Yiğit Cakiroglu	2	Osman Balcı	1
Sefa Kelekci	3	Yusuf Üstün	2	Pelin Coştur Bıyıksız	1
Serdar Filiz	3	Ahmet Yalınkaya	1	Rukset Attar	1
Aylin Pelin Cil	2	Aslı Somunkıran	1	S. Sinan Ozalp	1
Aysel Derbent	2	Aysun Karabulut	1	Sabiha Özdemir Özkan	1
Banu Kumbak Aygün	2	Banu Bingöl	1	Salih Taşkın	1
Barış Ata	2	Behiye Pınar Göksedef	1	Satish Kumar Adiga	1
Başak Baksu	2	Cem Atabekoğlu	1	Talat Umut Kutlu Dilek	1
Başar Tekin	2	Cem Dane	1	Taylan Hüseyin Öney (SE)	1
Berna Dilbaz	2	Cenk N. Sayın	1	Tevfik Yoldemir	1
Canan Aygün	2	Ebru Çelik	1	Yalçin Kimya	1
Candan İltemir Duvan	2	Ebru Öztürk	1	Yılmaz Güzel	1
Cem Çelik	2	Ekin Murat	1	Yılmaz Şahin	1
Deniz Cemgil	2	Enis Özkaya	1		
Cemgil Arıkan	2	Eray Çalışkan (R)	1		

We would like also to acknowledge the professionals contributing at the technical development process of the journal:

Kara, İbrahim (AVES)
Ardıç, Sevilay (AVES)
Özer, Ünal (AVES)
Çimen, Gökhan (AVES)
Yaman, Neslihan (AVES)
Özcengiz, Burak (AVES)
Şahin, Ali (AVES)
Ateş, Metin (Look-US)
Kınay, Rauf (TAJEV Asistant)

T

U

V

W

CONGRESS CALENDAR

1-2 December 2011	**3rd ANNUAL SEMINAR LAPAROSCOPIC & ROBOTIC Hysterectomy, and Intensive Hands-on Laparoscopic Suturing&Knot Tying** *New York, USA* www.nywomenshealth.com
8-11 December 2011	**15th World Congress on Controversies in Obstetrics, Gynecology & Infertility (COGI)** Hainan, CHINA http://www.congressmed.com/cogichina/
12-15 January 2012	**4. Minimal İnvaziv Jinekoloji Sempozyum ve Çalıştayı Prof. Dr. M. Mete Cengiz Kültür Merkezi** Bursa, Turkey www.uludagendoskopi.org
1-4 February 2012	**SLS-ASIAN-AMERICAN MULTISPECIALTY SUMMIT V. Laparoscopy and Minimally Invasive Surgery Hilton Hawaiian Village Beach Resort• Honolulu,** Hawaii, USA http://www.laparoscopy.blogs.com/asianamerican_summit
28-31 March 2012	**9th European Congress on Menopause and Andropause (EMAS)** Athen, Greece http://www2.kenes.com/emas
19-21 April 2012	**5. Ege Jinekolojik Endoskopi Sempozyumu Crowne Plaza** İzmir, Turkey http://www.ege2012.org
26-29 April 2012	**Comprehensive Colposcopy (American Society for Colposcopy and Cervical Pathology) Rhode** Island, USA http://www.asccp.org
9-12 May 2012	**ACOG 60th Annual Clinical Meeting (ACOG)** San Diego, CA USA http://www.acog.org/acm