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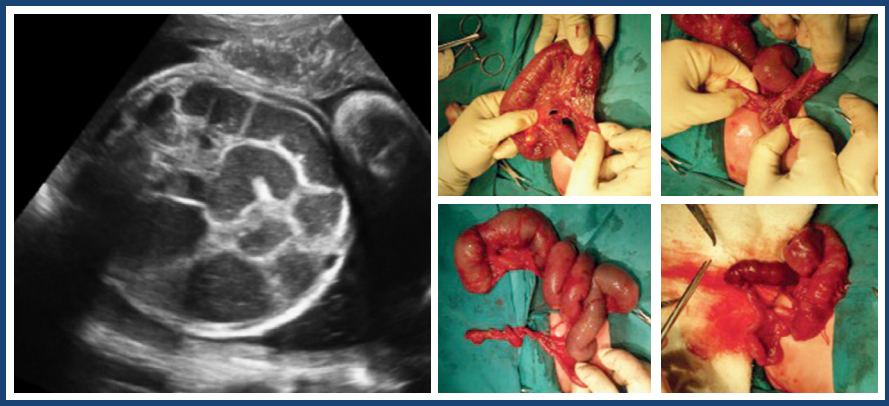

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▶ **Kandidal vulvovajinit**,

Gardnerella vaginalis ve anaerob bakterilerin oluşturduğu
▶ **Bakteriyel vajinozis**,

Trichomonas vaginalis'in oluşturduğu
▶ **Trikomonal vajinit**,

▶ **Mikst vajinal enfeksiyonların**

ampirik tedavisinde tek form ile etkilidir.*

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ÜRÜN ADI: TRİVAG 300 mg/200 mg/100 mg ovül FORMÜLÜ: Her bir ovül 300 mg tinidazol, 200 mg tiokonazol, 100 mg lidokain içerir. TERAPÖTİK ENDİKASYONLAR: Candida albicans'ın oluşturduğu kandidal vulvovajinit; Gardnerella vaginalis ve anaerob bakterilerin oluşturduğu bakteriyel vajinoz ve Trichomonas vaginalis'in oluşturduğu trikomonal vajinit ile mikst vajinal enfeksiyonların tedavisinde kullanılır. KULLANIM ŞEKLİ VE DOZU: Gece yatmadan önce bir ovül, 3 gün süreyle uygulanır. TRİVAG sırtüstü yatar pozisyonda, paketin içindeki parmaklıkların yardımı ile vajen derinliğine uygulanmalıdır. İSTENMEYEN ETKİLER: Güçsüzlük, bitkinlik, halsizlik, baş ağrısı, baş dönmesi, ağızda metalik/acı tat, mide bulantısı, anoreksi, iştahsızlık, midede gaz toplanması, dispepsi, abdominal kramp, epigastrik rahatsızlık, kusma, konstipasyon, idrar renginde koyulaşma. GEBELİK VE LAKTASYON: Gebelik kategorisi C'dir. Tinidazol anne sütüne geçtiğinden emzirme döneminde tedavi sırasında bebek sütten kesilmelidir, tedavi bittikten 72 saat sonra emzirmeye devam edilmelidir. DİĞER TIBBİ ÜRÜNLERLE ETKİLEŞİMLER VE DİĞER ETKİLEŞİM ŞEKİLLERİ: Birlikte kullanıldığında tinidazolün emilmesine bağlı olarak etkileşim görülebilir; asenokumarol, anisindion, dikumarol, fenindion, fenprokumon, varfarin, kolestiramin, simetidin, siklosporin, disülfiram, fluorourasil, fosfenitoin, ketokonazol, lityum, fenobarbital, fenitoin, rifampin, takrolimus, CYP3A4 indükleyicileri/inhibitörleri. Tiokonazolün emilmesine bağlı olarak etkileşim görülebilir; oksikodon. Lidokainin emilmesine bağlı olarak etkileşim görülebilir; propranolol, simetidin, antiaritmik ürünler, fenitoin veya barbitüratlar. KONTRENDİKASYONLARI: Bileşimindeki etkin maddelere veya bunların türevlerine karşı aşırı duyarlılığı bulunanlarda, gebeliğin ilk üç ayında, emzirme döneminde, organik nörolojik bozukluğu bulunanlarda, kan diskrazisi tablosu veya geçmişi bulunan hastalarda. ÖZEL KULLANIM UYARILARI VE ÖNLEMLERİ: Vajinal yoldan kullanılmalıdır. Geçici lökopeni ve nötropeni gelişebilir. Tedavi süresince ve tedavi bittikten 3 gün sonrasına kadar alkol alınmamalıdır. Cinsel olgunluğa erişmemiş kız çocuklarında ve bakirelerde kullanılmamalıdır. Kardiyovasküler hastalıkları olanlarda dikkatli kullanılmalıdır. Kontraseptif diyafram ve prezervatifle temas etmemelidir. Lidokain özellikle yüksek dozda ve geniş deri yüzeylerine, bilhassa da oklüzyon altında uygulandığında kalp ritm bozuklukları, nefes alma zorluğu, koma ve hatta ölüme yol açabilmektedir. Spermisidler, vajinal duşlar veya vajinal yoldan uygulanan diğer ürünlerle birlikte kullanılmamalıdır. Trikomonal vajinit vakalarında eş tedavisi de gereklidir. TİCARİ TAKDİM ŞEKLİ VE FİYATI: Trivag ovül (Ruhsat tarihi ve no: 29.09.2017-2017/742) 16,53 TL. (Fiyat Tarihi: Mayıs 2018) Ruhsat Sahibi: Bilim İlaç San. ve Tic. A.Ş. Son Güncelleme: Mayıs 2018. Reçeteli satılır. Daha geniş bilgi için "BİLİM İLAÇ SAN. ve TİC A.Ş. 34440 Beyoğlu-İSTANBUL" adresine başvurunuz. Ürünlerimiz ile ilgili advers olayları PHARMACOVIGILANCE@bilimilac.com adresine e-posta göndererek veya 0 212 365 1717 iletişim numarasını arayarak ürün güvenliliği sorumlusuna bildirebilirsiniz.

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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

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Journal of the
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Contents

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Journal of the
Turkish-German Gynecological Association

MOST CITED AND MOST VIEWED ARTICLES

Dear Readers,
We would like to present our most cited and most viewed articles on different platforms, like our website, Web of Science, Scopus and PubMed.

Website

Prognostic effect of isolated paraaortic nodal spread in endometrial cancer by Osman Türkmen, Derman Başaran, Alper Karalok, Günsu Cömert Kimyon, Tolga Taşçı, Işın Üreyen, Gökhan Tulunay, Taner Turan

Web of Science

Impact of obesity on infertility in women by Zeynep Özcan Dağ, Berna Dilbaz

Scopus

Impact of obesity on infertility in women by Zeynep Özcan Dağ, Berna Dilbaz

PubMed

Placental location and pregnancy outcome by Shumaila Zia

http://www.jtgga.org/

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Editorial

Dear Colleagues,

I am delighted to introduce the fourth issue of the "Journal of the Turkish German Gynecological Association (*J Turk Ger Gynecol Assoc*)" in the publishing year of 2018.

There are fake and LOW-QUALITY journals in the field of obstetrics and gynecology. Despite their appearances on the internet, they are not real medical journals. These "fake," "predatory," or "pseudo" journals misrepresent their peer-review and publication processes. These journals accept and publish almost all submissions, are not transparent about article processing (or publication) fees, often mimic the names and formats of legitimate journals to mislead authors and readers, and may collect fees but never publish the accepted work.

Last years have showed that the appearance of journals from mainstream publishers are based entirely on pseudoscience. On the surface, these publications look and act just like real scientific journals, but it's all just pretend. The publishers of these journals presumably care more about their bottom line than about scientific integrity.

Statistical analyses have verified that those who publish in predatory journals are, for the most part, young and inexperienced researchers from developing countries. Although most of the submissions in all of these journals have been from Third World scholars taking advantage of the low fees and the low standards for publication.

In fact, these journals are harmful on several counts. The first reason is that the "journals" are nothing of the sort. There is no telling how long they will be in existence. The second is that they take money under false pretenses; they are a fraud and they are polluting the field of science. Lastly, the reputation of such "journals" has become so bad that an impression has formed in some persons that all Open Access journals are of lowera than traditional journals. Worse, along these same lines, contributors may actually harm their professional standing and that of their institutions.

So the question remains, how can a scientist avoid the pitfalls of predatory journals? The answer, appropriately enough, is to do research before submission. Seeking the assistance of scientific mentors, senior colleagues, and others with many years of scholarly publishing experience may also be helpful. When choosing a journal, you want to keep in mind two factors: review times and policies on multiple submissions.

Journal of the
Turkish-German
Gynecological Association

Editorial

Dear Friends, Dear Researchers,

Unfortunately, there remains no validated mechanism to reliably define or identify fake, predatory, or pseudo journals. Researchers and readers must be aware of the existence of fake, predatory, or pseudo journals and avoid submitting research to them for publication or citing their content. Authors have a responsibility to evaluate the integrity, history, practices, and reputation of the journals to which they submit manuscripts.

The pressure to 'publish or perish' was another factor influencing many scholars' decisions to publish in these fast-turnaround journals. When trust is diminished, the scientific enterprise itself is threatened. We are proud to say that *J Turk Ger Gynecol Assoc* is now more popular than it's used to be.

In this issue, we are dealing with very interesting research articles. We worked hard to deliver you the journal with the best manuscripts in time. In this issue, you will read several good papers from all over the world. Please also enjoy solving a challenging quiz.

From now on we are going to accept video article in our journal. Video articles should include a brief introduction on case, surgery technique or a content of the video material. The main text should not exceed 500 words. References are welcomed and should not be more than 5. Along the main document, video material and 3 images should be uploaded during submission. Video format must be .mp4 and its size should not exceed 100 MB and be up to 10 minutes. Author should select 3 images, as highlights of the video, and provide them with appropriate explanations. Video and images must be cited within main text."

I would like to wish you a happy new year in 2019 and we are looking forward to receiving your valuable submissions, thank you in advance for your contributions.

Sincerely,

Prof. Cihat Ünlü, M.D.
Editor in Chief of *J Turk Ger Gynecol Assoc*
President of TGGF

The relationship between maternal age, body mass index, and the rate of preterm birth

Joachim W. Dudenhausen[1,7], Mirjam Kunze[2], Ursula Wittwer-Backofen[3], Hans Peter Hagenah[4], Alexander Strauss[5], Veronika Günther[6], İbrahim Alkatout[6], Amos Grunebaum[7], Manfred Voigt[3,8]

[1]Department of Obstetrics, Charité University School of Medicine, Berlin, Germany
[2]Department of Gynecology and Obstetrics, University of Freiburg School of Medicine, Freiburg, Germany
[3]Center of Medicine and Society, University of Freiburg, Freiburg, Germany
[4]Department of Obstetrics, Agaplesion Diakonieklinikum Rotenburg, Rotenburg, Germany
[5]University of Kiel, Kiel, Germany
[6]Department of Gynecology and Obstetrics, University Hospitals Schleswig-Holstein, Kiel, Germany
[7]Department of Gynecology and Obstetrics, Weill Cornell Medicine, New York, USA
[8]University of Freiburg School of Medicine, Freiburg, Germany

Abstract

Objective: The aim of the present study was to assess the influence of maternal age and maternal body mass index of early pregnancy on the risk of preterm delivery.

Material and Methods: The study included 2.1 million liveborn single newborns with documented data at perinatal surveys. Statistical analyses were performed using the SPSS statistics program.

Results: The risk of preterm births increased in obese women and in women with advanced age.

Conclusion: Strategies should be developed to reduce preconceptional body mass index, and guidelines are required to help advise women who postponed childbearing. (J Turk Ger Gynecol Assoc 2018; 19: 182-6)

Keywords: Maternal age, obesity, body mass index, premature birth, Germany

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Introduction

Over the past decades, there has been a significant increase in the average age among primipara women, and a rise in the body mass index (BMI) among pregnancies in high-income countries.

In 2011, the average age of the mother at the birth of her first child in Germany was 29.1 years, and overall, the average age of women at childbirth was 30.6 years.

Figure 1 shows the development of the age distribution of nulliparous women in the Federal Republic of Germany, Schleswig-Holstein and the German Democratic Republic, during the various years. Regional differences can be interpreted by social, political, and medical developments.

In the United States, according to the National Center for Health Statistics (1), the birth rate of 40 to 44-year-olds has doubled between 1981 and 2003. Many publications have shown that late maternity is associated with various risks to the mother and various risks to perinatal outcomes, such as preterm delivery (PTD), and chromosomal aberrations (2-4). Women are generally well informed about age-related decreasing fertility rates and the increasing risk of trisomy 21, but they are

Address for Correspondence: *İbrahim Alkatout*
e.mail: ibrahim.alkatout@uksh.de ORCID ID: orcid.org/0000-0002-7194-6034

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not as well informed about pregnancy-related risks based on increased maternal age (5). Controlled clinical studies and evidence-based guidelines for advising women who postpone childbearing are necessary (6).

In addition to an increasing maternal age, an increase in maternal weight could also be observed (7). The most commonly used measurement for defining obesity is BMI, which refers to an individual's weight in kilograms divided by the square of their height in meters (kg/m^2).

The consequences of the increase in maternal weight are significant for all health systems. For example, it is important to assess the impact of the mother's age-related weight increase on prematurity rates and fetal and neonatal outcomes.

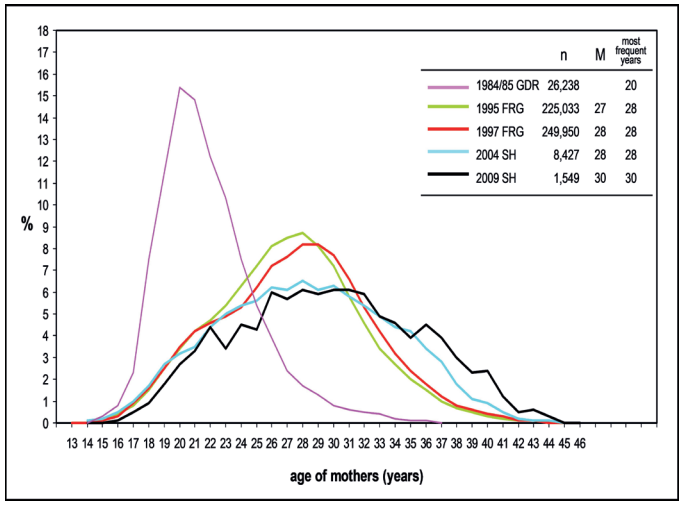

Figure 1. Distribution (%) of maternal age at the time of first pregnancy in Germany
GDR: German Democratic Republic; FRG: Federal Republic of Germany; SH: Schleswig-Holstein

Material and Methods

The World Health Organization and the Institute of Medicine define a BMI of under 18.5 kg/m^2 as underweight, from 18.5 to 24.9 kg/m^2 as a normal and healthy weight, from 25.0 to 29.9 kg/m^2 as overweight, and above 29.9 kg/m^2 as obesity. Within the obesity category, a further division into three can be made: a BMI from 30.0 to 34.9 kg/m^2 can be defined as obesity grade I, from 35.0 to 39.9 kg/m^2 as obesity grade II, and a BMI ≥40.0 kg/m^2 as obesity grade III (8).

This was a retrospective study that included singleton women who delivered in Germany. The data analyzed in the paper were obtained from the routine data collection undertaken by the German Perinatal Survey, a mandatory survey conducted throughout Germany. The data included singleton pregnancies from 1984/85 to 2008/2009 for Germany and the federal state Schleswig-Holstein. From 1995 to 1997, the state of Baden-Württemberg was excluded; from 1998 to 2000 the states of Baden-Württemberg, Berlin, Hesse, North Rhine-Westphalia, Rhineland-Palatine, Saarland, Schleswig-Holstein, were excluded. The inclusion and exclusion criteria are based on whether the individual federal states submitted their data with regard to the perinatal survey. The exclusion of a federal state is therefore due to non-existent data by the federal state itself. The study population consisted of a total of 2,130,594 pregnancies with liveborn infants.

The women's BMI was classified following the recommendations of the Institute of Medicine of the United States. PTD was defined as those <37 gestational weeks.

The data were analyzed using descriptive statistics. The data center of the University of Rostock performed the statistical analysis using the SPSS computer program, version 22.0.

Results

Between 1992 and 2009, there was a decrease in the rate normal pre-pregnancy BMI from 65.6% (362,419/552,026) to 57.8% (10,184/17,621), as well as an increase of obesity I-III from 7.9% (43,440/552,026) to 14.9% (2,719/17,621) (Figure 2). For 1992 to 1997, the data from all over Germany are shown, for the period 2001 to 2009, the data from Schleswig-Holstein were used as an example.

The PTD rate in those with a normal BMI was lower (6.8%; 99,918/1,468,286) as compared with 8.6% (1,423/16,461) in the group of obese women (III) (Figure 3). On the other hand, mothers with BMI ≤18.49 kg/m^2 had the highest risk of premature birth (9.6%).

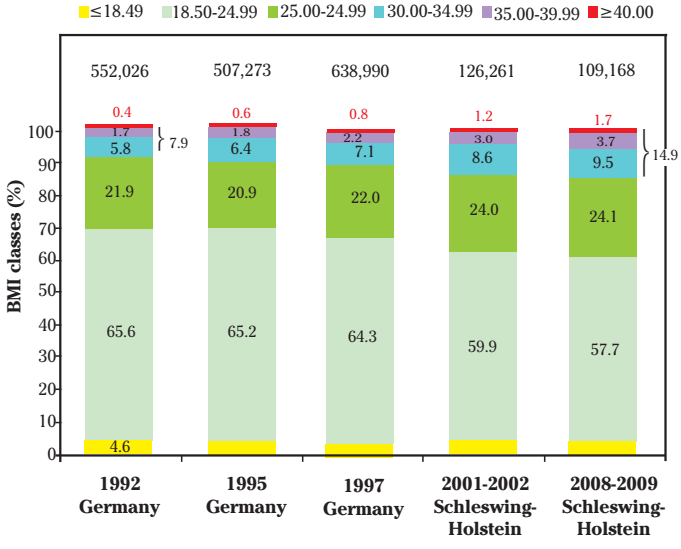

Figure 2. Trends in body mass index of mothers who delivered in Germany (between 1992 and 1997) and Schleswig-Holstein (between 2001 and 2009)
BMI: Body mass index

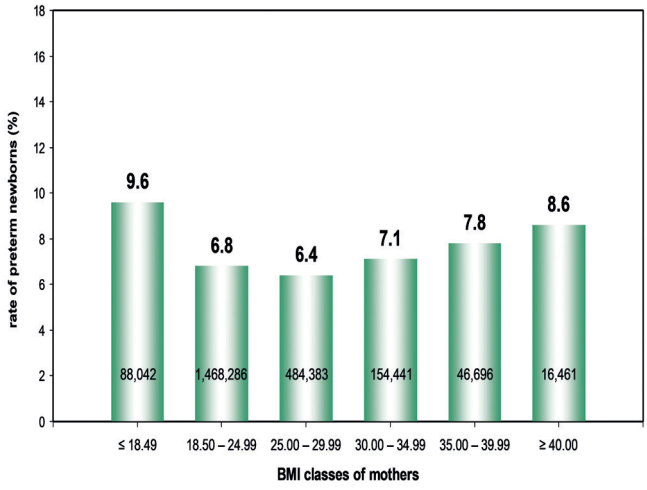

Figure 3. Rate of preterm newborns (%) in relation to the maternal body mass index (Germany, 1995-2000, n=2,258,309)
BMI: Body mass index

Mothers with a BMI between 25.00 and 29.99 kg/m^2 had the lowest risk of premature birth (6.4%). Subsequently, the risk of premature birth increased gradually with increasing BMI and reached its maximum in mothers of obesity group III with 8.6%. There were clear differences of the PTD rates related to maternal age and parity (Figure 4). Whether it was the first, second, third or fourth child - all curves showed a U-shaped course, which shows that the risk of premature birth was increased in both younger and older mothers. The lowest rates of premature birth can be observed in second-born children. At a maternal age of 16 years, the premature birth rate was 10%, then dropped to a low of 5% at a maternal age of 29 years, and then rose again. At a maternal age of 45 years, the premature birth rate was 11%. After second-born children, the third-born had the lowest rates of premature birth. Individual outliers of the curve can be explained by the low number of cases (e.g. third child at the maternal age of 19 years). This curve runs approximately parallel to the first one.

The premature birth rate in first-born children showed a steeper increase than the three other curves from a maternal age of 33 years.

The premature birth rate in the fourth child and subsequent children was relatively constant at 8.5% and only increased from a maternal age of 39 years (maximum at 44 years: premature birth rate of 13%).

Figure 5 shows the distribution of the BMI groups in the various years of childbirth in Germany in 1992 versus in Schleswig-Holstein in 2008/9. The proportion of 23-year-old women with normal BMI in early pregnancy was 63% in 1992 and 54.9% in 2008/9.

Discussion

Our data show an increase in obesity I-III, as well as a decrease in the rate of normal pre-pregnancy BMI between 1992 and 2009. The risk of PTD increases with the maternal BMI and shows a risk of 8.6% in obesity group III. Our results confirm the effect a mother's age has on the PTD rates. Especially as it relates to parity, this relationship is biphasic: the premature delivery rate is high at both ends of a woman's age, for young women (under 20 years) and also for older women. Many authors have demonstrated the increased risk of premature birth in younger people (under 18 years) and especially in older women (9,10). The lowest prematurity rates are found in the following age groups, depending on the parity: for the first child, the premature birth rates are lowest in the age range of 21 to 24 years, for the second child between the ages of 25 and 31 years, for the third child between 28 and 34 years, and from the 4th child on between 29 and 35 years (Figure 4). Therefore, the optimal age for a pregnancy with regard to the prematurity rate can only be determined from the parity aspect: for a woman with her 1st child, this 'optimal' age section is earlier than for women who have their 2nd, 3rd or 4th child.

Frederiksen et al. (11) investigated the relationship between advanced maternal age and unfavorable outcomes of pregnancy. Approximately 370,000 single pregnancies were included between 2008 and 2014. Pregnant women of advanced age were divided into two groups: 35-39 years and 40 years and older. The comparison group was formed by pregnant women aged 20-34 years. Pregnancies were followed from the end of the first trimester until birth. The primary endpoint was the occurrence of an unfavorable outcome, such as chromosomal abnormalities, congenital

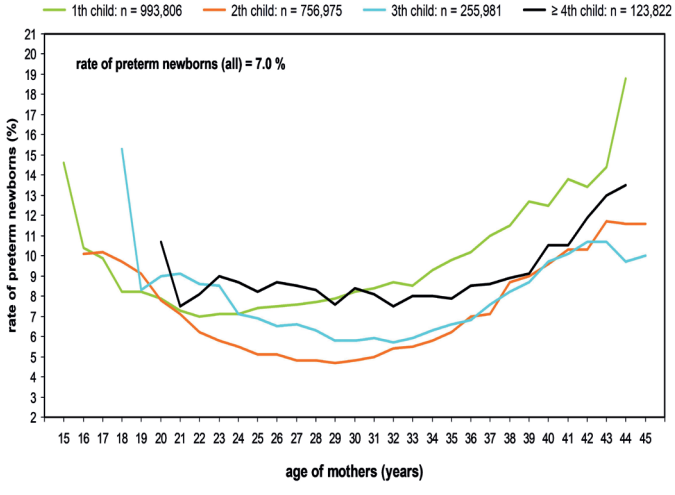

Figure 4. Rate of preterm birth as a function of maternal age and parity (Germany, 1995-2000, n=2,130,584)

malformations, stillbirth and premature birth before the 34th SSW. The researchers concluded that pregnant women aged over 40 years had a significantly higher risk of chromosomal abnormalities (increased 7.4 times), miscarriages (increased 3.1 times), and premature births before the 34th SSW (increased 1.7 times) compared with women aged 20-34 years. These results must be taken into account during prenatal care and this risk group must be monitored more closely. These results from Denmark are congruent to the results regarding the maternal age in our study.

A study from Sweden (12) investigated the relationship between maternal obesity and the risk of premature birth. A total of 1,599,551 deliveries were examined between 1992 and 2010. Preterm deliveries were divided into three groups: (extremely premature, 22-27 weeks; very premature, 28-31 weeks and moderately premature, 32-36 weeks). Risks of extremely, very, and moderately preterm deliveries increased with BMI and the overweight and obesity-related risks were highest for extremely preterm deliveries. Among normal-weight women (BMI 18.5 to <25 kg/m^2), the rate of extremely PTD was 0.17%. As compared with normal-weight women, rates (%) and adjusted odds ratios [ORs (95% CI)] of extremely PTD were as follows: BMI 25 to <30 <(0.21%; OR, 1.26; 95% CI: 1.15-1.37), BMI 30 to <35 (0.27%; OR, 1.58; 95% CI: 1.39-1.79), BMI 35 to <40 (0.35%; OR, 2.01; 95% CI: 1.66-2.45), and BMI of >40 or greater (0.52%; OR, 2.99; 95% CI: 2.28-3.92). Risk of spontaneous extremely PTD increased with BMI among obese women (BMI ≥30 kg/m^2).

In Sweden, as well as in our study, maternal overweight and obesity during pregnancy were associated with increased risks of PTD, especially extremely PTD.

Cleary-Goldman et al. (13) confirmed the influence of maternal age on the rate of preterm deliveries in around 36,000 single deliveries, that increased age of the mother was an independent risk factor for pregnancy outcomes such as gestational diabetes and macrosomia. This was confirmed by Abu Hamad et al. (14), by Kenny et al. (15) for the influence of the socio-economic status, and by Baer et al. (16) regarding the influence of ethnicity.

This development is partly due to the use of artificial reproductive medicine (17), but also by the extension of the education period, the later entry into the working life, later marriages and longer phases of the partner search, the argument of the loss of work or the threat to the career of the employed woman, which all lead to delayed childbirth and family planning with increasingly shifted higher age in many women. Whether 'social freezing' has had a statistical impact in recent years is unclear. In the future, however, one would expect a further shift in the gestational age, which will then be associated with significantly higher premature infants and possibly other risks for maternal and fetal outcome.

According to the Federal Statistical Office, 52% of the adult population in Germany was overweight in 2013 (62% of men and 43% of women). In comparison to 1999, the proportion of overweight adults has risen (48%, 56% of men and 40% of women). A total of 16% were obese in Germany (17% of men and 14% of women) (18).

In perinatal data from 1989 to 2000 in Germany, Briese et al. (19) showed a significantly increased 3.3-fold rate of severe hypertrophic neonates among morbidly obese women when compared with normal weight women. The rate of complications such as preeclampsia, gestational diabetes, and neonatal infections and hyperbilirubinemia were significantly more frequent. The rate of caesarean delivery among women with a BMI over 45 kg/m^2 was 38% as compared with normal weight women with 18%.

Future increasing maternal age and increasing pre-pregnancy BMI suggest a further future rise in PTD rates in high-income countries. Premature delivery is often associated with fetal risks, such as respiratory adaptation disorders, temperature regulation or aggravated food intake. Preventive preconception strategies for reductions of pre-pregnancy BMI of overweight and obese women and guidelines for counselling women

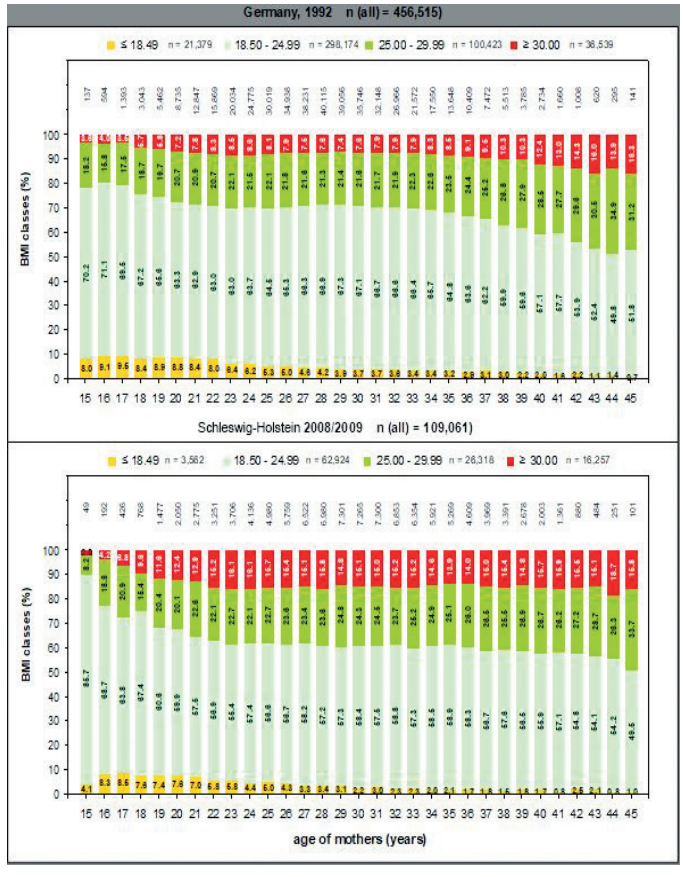

Figure 5. Distribution of body mass index at maternal age in Germany 1992 and Schleswig-Holstein 2008/2009
BMI: Body mass index

who plan to postpone their wishes for children to later life are urgently needed.

Ethics Committee Approval: *Retrospective study.*

Informed Consent: *Retrospective study.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Concept - J.W.D., M.V., İ.A., A.G., M.K., U.W.B., H.P.H., A.S., V.G.; Design - J.W.D., M.V., İ.A., A.G., M.K., U.W.B., H.P.H., A.S., V.G.; Supervision - J.W.D., M.V., İ.A., A.G., M.K., U.W.B., H.P.H., A.S., V.G.; Materials - J.W.D., M.V., İ.A., A.G., M.K., U.W.B., H.P.H., A.S., V.G.; Data Collection and/or Processing - M.K., U.W.B., H.P.H., A.S.; Analysis and/or Interpretation - J.W.D., M.V., İ.A., A.G.; Writer - J.W.D.*

Conflict of Interest: *No conflict of interest is declared by the authors.*

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References

1. National Center for Health Statistics. Birth right for woman aged 40-44 years rose in 2003. New Reports finds. http://www.cdc.gov/nchs/pressroom/04facts/birthrates.htm
2. Nohr EA, Bech BH, Vaeth M, Rasmussen KM, Henriksen TB, Olsen J. Obesity, gestational weight gain and preterm birth: a study within the Danish National Birth Cohort. Paediatr Perinat Epidemiol 2007; 21: 5-14.
3. Ritzinger P, Dudenhausen JW, Holzgreve W. Späte Mutterschaft und deren Risiken. J Endokrinol Reprod 2011; 8: 112-22.
4. Maier JT, Schalinski E, Gauger U, Hellmeyer L. Antenatal body mass index (BMI) and weight gain in pregnancy - its association with pregnancy and birthing complications. J Perinat Med 2016; 44: 397-404.
5. Niessen K, Werner-Bierwisch T, Metzing S, Zu Sayn-Wittgenstein F. Mutterschaft ab 35 Jahren: Das Altersrisiko in der Wahrnehmung von Frauen - eine Literaturstudie. Z Geburtsh Neonatol 2017; 221: 111-2.
6. Dudenhausen JW, Grunebaum A, Staudinger UM. Optimization of women's health before conception when pregnancy has been postponed. J Perinat Med 2013; 41: 23-5.
7. World Health Organization. Obesity: Pregnancv and managing the global epidemic. Geneva, WHO, 2000.
8. Rooney BL, Schauberger CW. Excess pregnancy weight gain and long-term obesity: one decade later. Obstet Gynecol 2002; 100: 245-52.
9. Carolan M. Maternal age ≥45 years and maternal and perinatal outcomes: a review of the evidence. Midwifery 2013; 29: 479-89.
10. Waldenström U, Cnattingius S, Vixner L, Norman M. Advanced maternal age increases the risk of very preterm birth, irrespective of parity: a population-based register study. BJOG 2017; 124: 1235-44.
11. Frederiksen LE, Ernst A, Brix N, Braskhøj Lauridsen LL, Roos L, Ramlau-Hansen CH, et al. Risk of Adverse Pregnancy Outcomes at Advanced Maternal Age. Obstet Gynecol 2018; 131: 457-63.
12. Cnattingius S, Villamor E, Johansson S, Edstedt Bonamy AK, Persson M, Wikström AK, et al. Maternal obesity and risk of preterm delivery. JAMA 2013; 309: 2362-70.
13. Cleary-Goldman J, Malone FD, Vidaver J, Ball RH, Nyberg DA, Comstock CH, et al. Impact of maternal age on obstetric outcome. Obstet Gynecol 2005; 105(5 Pt 1): 983-90.
14. Abu Hamad Kh, Abed Y, Abu Hamad B. Risk factors associated with preterm birth in the Gaza Strip: hospital-based case-control study. East Mediterr Health J 2007; 13: 1132-41.
15. Kenny LC, Lavender T, McNamee R, O'Neill SM, Mills T, Khashan AS. Advanced maternal age and adverse pregnancy outcome: evidence from a large contemporary cohort. PLoS One 2013; 8: e56583.
16. Baer RJ, Yang J, Berghella V, Chambers CD, Coker TR, Kuppermann M, et al. Risk of preterm birth by maternal age at first and second pregnancy and race/ethnicity. J Perinat Med 2018; 46: 539-46.
17. Ooki S. Effect of Maternal Age and Fertility Treatment on the Increase in Multiple Births in Japan: Vital Statistics, 1974-2009. J Epidemiol 2011; 21: 507-11.
18. Scholz R. Demographischer Wandel und Einfluss auf mütterliche Risikofaktoren. Plattform Ernährung und Bewegung, Berlin, 13.10.2016.
19. Briese V, Voigt M, Hermanussen M, Wittwer-Backofen U. Morbid obesity: pregnancy risks, birth risks and status of the newborn. Homo 2010; 61: 64-72.

Psychometric properties of the Persian language version of the Female Sexual Function index among postmenopausal women

Masaudeh Babakhanian[1], Masumeh Ghazanfarpour[2], Mona Najaf Najafi[3], Fatemeh Rajab Dizavandi[4], Talat Khadivzadeh[2], Minoo Safaei[2], Mehdi Jabbari Nooghabi[5]

[1]Social Determinants of Health Research Center, Semnan University of Medical Sciences, Semnan, Iran
[2]Department of Midwifery, Kerman University of Medical Sciences School of Nursing and Midwifery, Kerman, Iran
[3]Department of Community Medicine, Imam Reza Clinical Research Units, Faculty of Medicine Mashhad University of Medical Sciences, Mashhad, Iran
[4]Department of Community Health and Psychiatric Nursing, School of Nursing and Midwifery, Mashhad University of Medical Sciences, Mashhad, Iran
[5]Department Professor of Statistics, Ferdowsi University of Mashhad, Mashhad, Iran

Abstract

Objective: The present research aimed to evaluate the psychometric properties of the Persian language version of the Female Sexual Function Index (FSFI) among postmenopausal women.

Material and Methods: This secondary analysis examined 402 healthy postmenopausal Iranian women presenting to healthcare centers across Iran. The sampling method was convenience sampling. The translation of the FSFI and its cross-cultural adaptation were conducted under the guidelines proposed by Beaton. The reliability (Cronbach's alpha coefficient and test-rest reliability) and construct validity confirmatory factor analysis) were assessed. Model fitting index [such as the root mean square error of approximation (RMSEA), the Goodness of Fit Index (GFI) and the Comparative Fit Index (CFI)] was calculated.

Results: The mean age of the study participants was 53.63±7.8 years. Test-retest reliability was high for both the entire scale ($r=0.964$; $p<0.001$) and its six dimensions (0.76-0.94; $p<0.001$). The Cronbach's alpha of the entire scale and its dimensions was greater than 0.80. The original six-factor was used, which showed a relatively poor fit ($\chi2=667.054$; $p<0.001$; $\chi2/df=4.86$; GFI=0.92; RMSEA=0.098; GFI=0.85). After adding three correlated error terms to the six-factor model, an acceptable fit was obtained ($\chi2=470.542$; $p<0.001$; $\chi2/df=3.51$; CFI=0.95; RMSEA=0.079; GFI=0.89).

Conclusion: According to our results, the FSFI tool indicated a satisfactory fit for a six-factor model, as similar to the original English version, for use in clinical practice and research regarding healthy postmenopausal Iranian women. More research needs to be conducted on this scale to assess all of its psychometric properties. (J Turk Ger Gynecol Assoc 2018; 19: 187-92)

Keywords: Female Sexual Function index, psychometric properties, confirmatory factor analysis

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Introduction

Human sexuality is an integral role in everyone's life (1,2). A growing body of literature has examined sexual issues in older adults. The World Association for Sexual Health changed its slogan to "Sexual Health for All" (2). Menopause negatively affects nearly all the dimensions of sexual function, including lubrication, pain, and orgasm (3). Almost 50% of postmenopausal women in the United States of America are estimated to have sexual dysfunction (4). Sexual problems

Address for Correspondence: *Masumeh Ghazanfarpour*
e-mail: masumeh.ghazanfarpour@yahoo.com ORCID ID: orcid.org/0000-0003-4639-3711

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negatively affect quality of life and personal relationships (5) and can even lead to divorce (6).

Based on recent rapid advances, valid instruments are required for diagnosing and treating female sexual dysfunction (FSD) (7). Among these, Rosen et al. (8) developed the Female Sexual Function index (FSFI) as a multidimensional self-report tool to detect the dimensions of female sexual function. The original English version of this instrument exhibited an excellent internal consistency (α=0.82 or higher) and a proper test-retest reliability during a two-week interval (r=0.79-0.88). The tool is well capable of discriminating between healthy women and women with sexual dysfunction, suggesting its good discriminant validity. The psychometric properties of this instrument have been assessed in different languages and cultures (9-12).

Almost all tools have been designed and developed to measure the different dimensions of sexual function in younger people. Research on sex and sexuality in older women may encounter difficulties due to the sensitive nature of the subject (13), especially in Iran, because talking about sexual relations is a taboo, in particular among older women, so there has been limited research on sexuality in the elderly population (13). Changes are induced with aging and menopause such as changes in sexual response, the female genitalia, orgasmic function, and sexual hormones (11).

A review of the literature in national and international databases yielded only two studies assessing the psychometric of the FSFI in Iran (14,15). The psychometric properties of this instrument have never been assessed in postmenopausal women who are mostly at risk of FSD as compared with their younger counterparts (16). The objective of the current study was to evaluate the psychometric properties of the FSFI in Iranian postmenopausal women.

Material and Methods

This secondary analysis combined the data collected in two previous cross-sectional studies conducted at different times in Iran. One study was conducted in the Semnan province in northern-central Iran (sample 2=202) and the other one in the city of Torbat-e Heydarieh, in Mashhad province (sample 1=200). The ethics committee of Torbat-e Heydarieh University of Medical Science and Semnan University of Medical Science approved the two previous studies. The patients completed informed consent forms voluntarily. Menopause was defined as being older than 45 and having had amenorrhea for at least one year. The subjects with severe medical diseases or psychiatric disorders were excluded from the study.

Translation and cross-cultural adaptation

The translation of the FSFI and its cross-cultural adaptation were conducted under the guidelines proposed by Beaton et al. (17). A team of two bilingual translators whose mother tongue was Persian and who were fluent in English translated the English scale into Persian. Two native English speakers back-translated the Persian version into the English scale. The translators and the researcher synthesized the two translations into a single version and wrote a report about the synthesis process. An expert committee of translators, two health professionals, and one expert in psychometrics consolidated all the translations into the pre-final version, which was tested on 40 women.

Assessment of content validity

The quantitative content validity of the Persian scale was assessed via the Content Validity index (CVI) and the Content Validity Ratio (CVR). An expert panel of eight sexual and reproductive health specialists and gynecologists assessed the content validity of the scale, which was reported to be excellent based on the CVR and the CVI.

The Female Sexual Function index

This is a brief, multidimensional, self-reporting index used to assess sexual dysfunction, consisting of 19 items within six dimensions rated on a Likert scale from 0 to 5 or from 1 to 5, including desire (items 1-2), subjective arousal (items 3-6), lubrication (items 7-10), orgasm (items 11-13), satisfaction (items 14-16), and pain (items 17-19). Zero scores belong to those reporting no sexual intercourse within the past four weeks. Higher scores (in total, for the items or for the dimensions) indicate less sexual dysfunction (8).

Reliability and validity assessment

Cronbach's alpha coefficient was used to calculate the internal consistency of the entire FSFI and relevant dimensions, including fair consistency if reported as 0.7, moderate if reported as 0.7 to 0.8, and excellent if reported as 0.9 and over (18). Pearson's r coefficient was applied to evaluate the test-retest reliability, which was fulfilled within a two-week interval on a sub-sample of 40 women.

The FSFI factor structure was also evaluated using the confirmatory factor analysis (CFA), conducted on a sample of 402 postmenopausal women. The inter-correlation between the dimensions of the scale and the correlation between the entire scale and its dimensions were estimated using Pearson's correlation coefficient.

Statistical analysis

The CFA was performed in AMOS-18 (http://www3.ibm.com/software/products/en/spss-amos) using the maximum-likelihood method for parameter estimation. The root mean square error of approximation (RMSEA), the goodness of fit index (GFI) and the comparative fit index (CFI) determined the modified eight-factor data model. Values above 0.9 were

recommended for CFI and GFI, and below 0.08 for RMSEA (19,20). The ratio of chi-square to the degree of freedom ($\chi 2$/df<5) was found to be acceptable by Marsh and Hocevar (21).

Results

Table 1 presents the demographic characteristics of the study participants, including 402 postmenopausal women with a mean age of 53.63±7.8 years.

More than half of the subjects were illiterate or had primary school education and only 6% had university education. A total of 78.6% had more than two children. There were no missing data because questions were immediately checked after they were returned by the participants.

Confirmatory factor analysis

The original English six-factor [Rosen et al. (8)] was used, which showed a relatively poor fit ($\chi 2$=667.054; p<0.001; $\chi 2$/df=4.86; GFI=0.92; RMSEA=0.098; GFI=0.85). After adding three correlated error terms to the six-factor model based on the largest modification indices provided by AMOS, an acceptable fit was obtained ($\chi 2$=470.542; p<0.001; $\chi 2$/df=3.51; CFI=0.95; RMSEA=0.079; GFI=0.89) (Table 2). The correlated errors were between item 10 and 12, between 15 and 16, and between 9 and 11. The factor loading of these items was in a range of 0.46 to 0.94 (Figure 1). The chi-square value, however, remained significant, which could be attributed to the large sample size (Table 2). The strongest correlated error was observed between item 15 and item 16.

Although an acceptable fit with the data was found for the six-factor model, we also tested other models suggested in other studies (10,14,22). Initially, the first-order, one-factor model was tested to evaluate if of 19-item FSFI could be included into a single factor. This model revealed poor fit with the data ($\chi 2$=1832.362; p<0.001; $\chi 2$/df=12.05; GFI=0.57; RMSEA=0.166; CFI=0.75). The five-factor model was tested and showed a poor fit to the data (p<0.001; $\chi 2$/df=5.67; CFI=0.9; GFI=0.823; RMSEA=0.1) (Table 2).

Table 1. Participants' characteristics

Variable	Samples n=402
Age*	53.63±7.8
Women's education**	
Illiterate	99 (24.6%)
Primary school	133 (33.1%)
Junior high school	76 (18.9%)
High school	68 (16.9%)
University	24 (6%)
Missing data	2 (0.5%)
Spouse's education**	
Illiterate	1 (0.2%)
Primary school	104 (25.9%)
Junior high school	94 (23.4%)
High school	92 (22.9%)
University	111 (27.7%)
Number of children*	
More than two	316 (78.6%)
Less than two	86 (21.4%)
*: Mean ± standard deviation, **: Number (%)	

Reliability

The analysis was performed using the data obtained from sample 1. A total of 40 subjects were asked to visit again to complete the FSFI. The degree of agreement between the two assessments was measured within a two-week interval and was found to be high for both the entire scale (r=0.964; p<0.001) and its six dimensions (0.76-0.94; p<0.001) (Table 3).

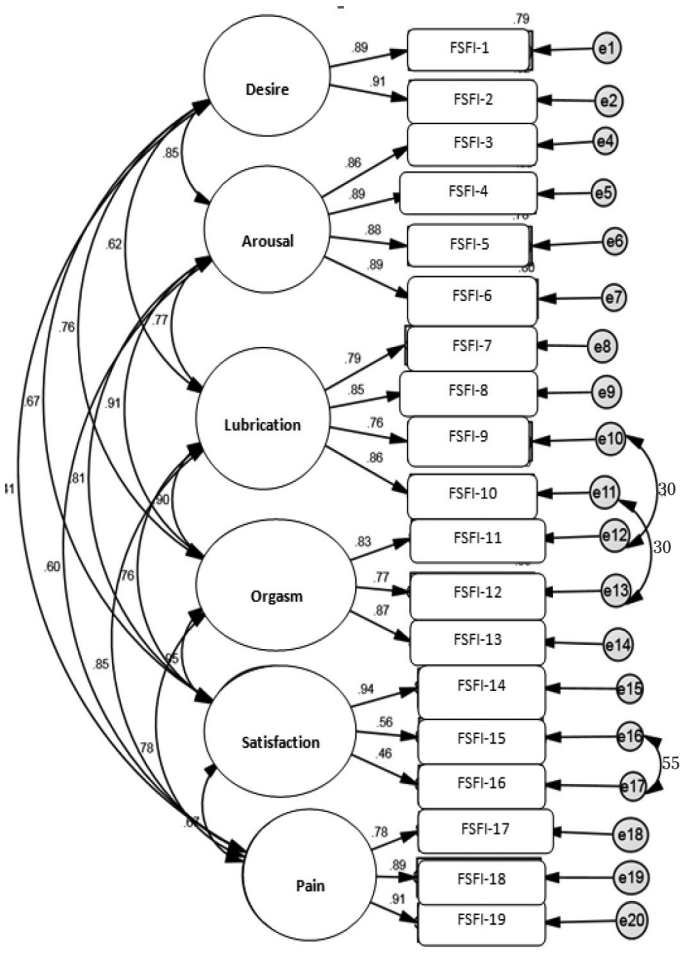

Figure 1. Six covariated factor model
p<0.05; FSFI: Female Sexual Function index

The internal consistency of the scale was determined using the Cronbach's alpha coefficient. As can be seen in Table 3, the Cronbach's alpha values of the FSFI and its dimensions, which were greater than 0.80 for almost all the dimensions and for the entire scale, revealed excellent internal consistency reliability (Table 3).

Inter-correlations

There were slightly high significant correlations between the different dimensions of the FSFI, among which the strongest correlation between orgasm and satisfaction (0.938), and the weakest between pain and desire (p=0.409). The p values became significant for all the dimensions. The correlations were significant and positive and greater than 0.4 between all the items (Table 4).

Discussion

The objective of the current study was to evaluate the psychometric properties of the FSFI in Iranian postmenopausal women. The main conclusion of this study was that six-factor models with three correlated error terms were a good fit to the data, but other models (five-factor model, second-order, six-factor model, and one-factor model) showed a poor fit to the data.

Table 2. The fit indices of the original model and the five other models

Model	$\chi 2$	df	$\chi 2$/df	p value	CFI	GFI	RMSEA
Six-factor model (original model)	667.054	137	4.86	p<0.001	0.92	0.85	0.98
Six-factor model (original model with two correlated errors) Between item 9 and item 11 Between item 10 and item 12 Between item 15 and item 16	470.542	134	3.51	p<0.001	0.95	0.89	0.79
Five-factor model	805.412	141	5.67	p<0.001	0.9	0.823	0.10
Second-order six-factor model	903.171	146	6.18	p<0.001	0.899	0.78	0.11
First-order, one-factor model	1832.362	152	12.055	p<0.001	0.75	0.57	0.16
$\chi 2$: chi-square, df: The degree of freedom, $\chi 2$/df: The ratio of chi-square to the degree of freedom, CFI: Comparative Fit index, GFI: The Goodness of Fit index, RMSEA: The root mean square error of approximation							

Table 3. The test-retest and Cronbach's alpha of the Persian version of the FSFI

FSFI dimension	Test-retest reliability* (n=40)	Level of significance	Cronbach's alpha (n=402)
Desire (1-2)	0.80	<0.001	0.89
Subjective arousal (3-6)	0.92	<0.001	0.93
Lubrication (7-10)	0.94	<0.001	0.88
Orgasm (11-13)	0.90	<0.001	0.85
Satisfaction (14-16)	0.76	<0.001	0.74
Pain (17-19)	0.92	<0.001	0.80
Total score	0.96	<0.001	0.95
Pearson's r coefficient, FSFI: Female Sexual Function index			

Table 4. The correlation between the FSFI dimensions

	*FSFI dimension	1	2	3	4	5	6
11	Desire						
22	Subjective arousal	0.853*					
33	Lubrication	0.645*	0.791*				
44	Orgasm	0.751*	0.914*	0.932*			
55	Satisfaction	0.682*	0.830*	0.794*	0.938*		
66	Pain	0.409*	0.598*	0.840*	0.783*	0.505*	
77	Total score	0.401*	0.890*	0.897*	0.936*	0.782*	0.787*
*FSFI: Female Sexual Function index							

Menopause is an important stage during the lifetime of all women (11). All domains of sexual function (orgasm, lubrication, desire and sexual pain) are likely to be negatively affected by menopause (23). Sexual problems have been frequently reported in Asian countries, such as Korea and Iran, due to the conservative nature of sex and sexuality in such countries (11,23). Despite the importance of sexuality for adults in Korea, only 2% of women and men consult health providers (11). According to a qualitative study, many Iranian postmenopausal women do not discuss their sexual problems with healthcare providers for a variety of reasons, including traditional, cultural, and religious beliefs (13).

The FSFI instrument has been developed for both clinical practice and research. It has been translated into various languages and also validated in different samples of women. Nevertheless, it has never been validated for use in postmenopausal women despite its extensive use in research. In terms of reliability, the FSFI shows excellent internal consistency. As for construct validity (CFA), six-factor models indicated a good fit with the data. This result is consistent with the findings of Rosen et al. (8) and Opperman et al. (24), who also found that a six-factor model of the FSFI with 19 items appropriately fitted with data.

Of significant correlation found between the dimensions of the FSFI, the strongest correlation was reported between orgasm and satisfaction (r=0.938). Takahashi et al. (10) in Japan and Nowosielski et al. (25) in Poland observed the strongest correlation between lubrication and arousal. In a study by Vallejo-Medina et al. (26) in Spain, the strongest correlation existed between satisfaction and arousal. Fakhri et al. (14) in Iran also found the strongest correlation between lubrication and desire. The difference between the present study and the other studies cited may be due to the study population, i.e. postmenopausal women, who often have some degree of vaginal atrophy and vaginal dryness (27).

The present study found excellent internal consistency for the entire scale and a good internal consistency for all its dimensions. These findings are in line with previous studies, in which the internal consistency of the scale was high to excellent with Cronbach's alpha ranging from 0.84 to 0.95 in the Japanese (10), 0.83-0.94 in Chinese (9), 0.85 to 0.94 in Arabic (28), and 0.88 to 0.96 in Iranian versions regarding women of reproductive ages (14).

The two-week test-retest reliability was found to be high in the entire scale and associated dimensions, which is consistent with the findings of other studies. For example, Fakhri et al. (14) in Iran, Takahashi et al. (10) in Japan, and the assessment of the original English version (8) showed good or excellent test-retest reliabilities for the scale. In the study by Nowosielski et al. (25), good internal consistency and test-retest reliabilities of the scale were exhibited among both healthy subjects and women with sexual problems (29).

According to the present model, the best fit was observed in the six-factor model, although some studies have found the five-factor model to have better psychometric properties (26).

Study limitations

This study had several limitations. First, it assessed only healthy menopausal women with no serious diseases who were selected through convenience sampling; the generalization of the results for all Iranian women in different subgroups (including reproductive ages or sexual problems or diseases) should therefore be pursued with caution. Second, we assessed neither the discriminant validity of the study scale nor the measurement invariance of menopause status. Similar studies are required to assess the other psychometric properties of this scale.

According to our results, the FSFI tool indicated a satisfactory fit for six factor model, similar to the original English version, for use in clinical practice and research regarding healthy postmenopausal Iranian women. More research needs to be conducted on this scale to assess all of its psychometric properties.

Ethics Committee Approval: *The ethics committee of Torbat-e Heydarieh University of Medical Science and Semnan University of Medical Science approved the two previous studies.*

Informed Consent: *The patients completed informed consent forms voluntarily.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Concept - M.G., M.B.; Data Collection or Processing - F.R.D., T.K., M.S.; Analysis or Interpretation - M.J.N., M.G., M.N.N.; Writer - M.G., M.B.*

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References

1. Saunamäki N, Andersson M, Engström M. Discussing sexuality with patients: nurses' attitudes and beliefs. J Adv Nurs 2010; 66: 1308-16.
2. Ghazanfarpour M, Kaviani M, Abdolahian S, Bonakchi H, Najmabadi Khadijeh M, Naghavi M, et al. The relationship between women's attitude towards menopause and menopausal symptoms among postmenopausal women. Gynecol Endocrinol 2015; 31: 860-5.

3. Levine KB, Williams RE, Hartmann KE. Vulvovaginal atrophy is strongly associated with female sexual dysfunction among sexually active postmenopausal women. Menopause 2008; 15: 661-6.
4. Berman JR, Berman LA, Werbin TJ, Goldstein I. Female sexual dysfunction: anatomy, physiology, evaluation and treatment options. Curr Opin Urol 1999; 9: 563-8.
5. Liu H, Yu J, Chen Y, He P, Zhou L, Tang X, et al. Sexual function in cervical cancer patients: psychometric properties and performance of a Chinese version of the Female Sexual Function Index. Eur J Oncol Nurs 2016; 20: 24-30.
6. Sidi H, Abdullah N, Puteh SEW, Midin M. The female sexual function index (FSFI): Validation of the Malay version. J Sex Med 2007; 4: 1642-54.
7. ter Kuile MM, Brauer M, Laan E. The female sexual function index (FSFI) and the female sexual distress scale (FSDS): psychometric properties within a Dutch population. J Sex Marital Ther 2006; 32: 289-304.
8. Rosen R, Brown C, Heiman J, Leiblum S, Meston C, Shabsigh R, et al. The Female Sexual Function Index (FSFI): a multidimensional self-report instrument for the assessment of female sexual function. J Sex Marital Ther 2000; 26: 191-208.
9. Sun X, Li C, Jin L, Fan Y, Wang D. Development and validation of Chinese version of female sexual function index in a Chinese population - a pilot study. J Sex Med 2011; 8: 1101-11.
10. Takahashi M, Inokuchi T, Watanabe C, Saito T, Kai I. The female sexual function index (FSFI): Development of a Japanese version. J Sex Med 2011; 8: 2246-54.
11. Lee Y, Lim MC, Son Y, Joo J, Park K, Kim JS, et al. Development and evaluation of Korean version of Quality of Sexual Function (QSF-K) in healthy Korean women. J Korean Med Sci 2014; 29: 758-63.
12. Rehman KU, Asif Mahmood M, Sheikh SS, Sultan T, Khan MA. The Female Sexual Function Index (FSFI): Translation, Validation, and Cross-Cultural Adaptation of an Urdu Version "FSFI-U". Sex Med 2015; 3: 244-50.
13. Ghazanfarpour M, Khadivzadeh T, Latifnejad Roudsari R, Mehdi Hazavehei SM. Obstacles to the discussion of sexual problems in menopausal women: a qualitative study of healthcare providers. J Obstet Gynaecol 2017; 37: 660-6.
14. Fakhri A, Pakpour AH, Burri A, Morshedi H, Zeidi IM. The Female Sexual Function Index: translation and validation of an Iranian version. J Sex Med 2012; 9: 514-23.
15. Mohammadi KH, Heydari M, Faghihzadeh S. The female sexual function index (FSFI): validation of the Iranian version. Payesh 2008; 3: 269-78.
16. Esposito K, Ciotola M, Giugliano D. Prevalence and risk factors for female sexual dysfunction in Turkish women. J Urol 2006; 176: 840-1.
17. Beaton DE, Bombardier C, Guillemin F, Ferraz MB. Guidelines for the process of cross-cultural adaptation of self-report measures. Spine (Phila Pa) 2000; 25: 3186-91.
18. Cronbach LJ. Coefficient alpha and the internal structure of tests. Psychometrika 1951; 16: 297-334.
19. Byrn B. Structural equation modeling with AMOS: Basic concepts, applications, and programing. Translated by. In: Hosainzadeh H, Erfani H, Shamsi Koshki S, Mehdi Mohammadi S, editors. Shiraz Islamic Azad University Press; 2011.
20. MacCallum RC, Browne MW, Sugawara HM. Power analysis and determination of sample size for covariance structure modeling. Psychological Methods 1996; 1: 130.
21. Marsh H, Hocevar D. Application of confirmatory factor analysis to the study of self-concept: First-and higher order factor models and their invariance across groups. Psychol Bull 1985; 97: 562-82.
22. Wiegel M, Meston C, Rosen R. The female sexual function index (FSFI): cross-validation and development of clinical cutoff scores. J Sex Marital Ther 2005; 31: 1-20.
23. Ghazanfarpour M, Khadivzadeh T, Babakhanian M. Investigating the Relationship Between Sexual Function and Quality of Life in Menopausal Women. J Family Reprod Health 2016; 10: 191-7.
24. Opperman EA, Benson LE, Milhausen RR. Confirmatory factor analysis of the female sexual function index. J Sex Res 2013; 50: 29-36.
25. Nowosielski K, Wróbel B, Sioma-Markowska U, Poreba R. Development and validation of the Polish version of the Female Sexual Function Index in the Polish population of females. J Sex Med 2013; 10: 386-95.
26. Vallejo-Medina P, Pérez-Durán C, Saavedra-Roa A. Translation, Adaptation, and Preliminary Validation of the Female Sexual Function Index into Spanish (Colombia). Arch Sex Behav 2018; 47: 797-810.
27. Ghazanfarpour M, Shokrollahi P, Khadivzadeh T, Baharian Sharghi N, Mirzaii Najmabadi K, Babakhanian M, et al. Effect of Foeniculum vulgare (fennel) on vaginal atrophy in postmenopausal women: A double-blind, randomized, placebo-controlled trial. Post Reprod Health 2017; 23: 171-6.
28. Anis TH, Gheit SA, Saied HS, Al_kherbash SA. Arabic translation of Female Sexual Function Index and validation in an Egyptian population. J Sex Med 2011; 8: 3370-8.
29. Ryding EL, Blom C. Validation of the Swedish version of the Female Sexual Function Index (FSFI) in women with hypoactive sexual desire disorder. J Sex Med 2015; 12: 341-9.

Glutathione S-transferase omega gene polymorphism as a biomarker for human papilloma virus and cervical cancer in Iranian women

Sara Zamani[1], Amir Sohrabi[2], Marjan Rahnamaye-Farzami[3], Seyed Masoud Hosseini[1]

[1]Department of Microbiology and Microbial Biotechnology, Shahid Beheshti University Faculty of Life Sciences and Biotechnology, Tehran, Iran

[2]Department of Medical Epidemiology and Biostatistics, Karolinska Institute, Stockholm, Sweden

[3]Research Center of Health Reference Laboratory, Ministry of Health and Medical Education, Tehran, Iran

Abstract

Objective: Human papillomavirus (HPV) infection is an important sexually-transmitted infection worldwide. Persistent infections with different high-risk HPV genotypes may cause cervical intraepithelial neoplasia and cervical cancer. Single nucleotide polymorphisms of glutathione S-transferase omega (GSTO) 1 and 2 play an important role in cancer progression. To evaluate GSTO gene polymorphism influence on women's susceptibility to low-risk or high-risk HPV infections and also risk of cervical cancer development.

Material and Methods: We examined 50 patients with cervical cancer, 43 patients who were positive for HPV, and 43 healthy individuals as negative controls. We used polymerase chain reaction-restriction fragment length polymorphism to determine GSTO1 A140D and GSTO2 N142D variants in study participants.

Results: We found a significant association between the GSTO1 A140D gene polymorphism and HPV 6, 16, 18, 16/18 infections and cervical cancer in Iranian women. We noted a significant difference for the 140AD/142NN combination genotype between patients in the cervical cancer group and healthy controls. There were no significant differences for the GSTO2 N142D genotype and allele frequencies between the patient (i.e., cervical cancer and HPV-positive) groups and controls.

Conclusion: The 140AD genotype, 140D allele, and 140AD/142NN combination genotype seem to confer a protective property in women's susceptibility to HPV 6, 16, 18, 16/18 infections and cervical cancer. However, the GSTO2 N142D polymorphism is not associated with HPV infections and cervical cancer. It would appear that GSTO1 A140D SNPs likely play a role in the level of susceptibility to HPV-related cervical cancer. (J Turk Ger Gynecol Assoc 2018; 19: 193-200)

Keywords: Human papilloma virus, cervical cancer, omega gene, polymorphism

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Introduction

Human papillomavirus (HPV) infections constitute a large portion of sexually-transmitted disease cases worldwide, and up to 70% of sexually active women are infected by HPV during their lifetime. HPVs are divided into high-risk and low-risk genotypes based on their level of association with malignancies (1-6).

Generally, 85% of the global burden of HPV infection is occurring in developing countries with high-risk areas such as those in Africa and South America, and North American and Western Asia bear a lower portion of the infection burden (7,8). The variation seen in the occurrence of HPV infection in different regions of the world demonstrates that although HPV is the main cause of cervical cancer, environmental and

Address for Correspondence: *Seyed Masoud Hosseini*
e.mail: Ma_Hosseini@sbu.ac.ir ORCID ID: orcid.org/0000-0001-7211-1920

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genetic factors such as genetic polymorphisms also affect the occurrence of this disease (8-10).

The human cytosolic glutathione-S-transferase (GST) super family contains at least 16 genes subdivided into eight distinct classes designated as Alpha, kappa, Mu, Omega, Pi, Sigma, Theta, and Zeta. The GST superfamily of antitoxic enzymes can catalyze the conjugation of glutathione to a wide variety of endogenous and exogenous compounds (8,11-13) and contribute in many important cellular reactions including the response to environmental stresses, cell proliferation, phase II metabolism, apoptosis, oncogenesis, tumor progression, and drug resistance (8,13). The over expression of these enzymes can induce apoptosis, which can affect cancer development (14). The presence of genetic diversity in this enzymatic super family can affect the antitoxic activities of these enzymes (8). Single nucleotide polymorphisms (SNPs) of this super family can affect the likelihood of cancer development and the chances of success for various treatments (14).

The GST omega (GSTO) class belongs to the GST enzyme super family, which has a cysteine amino acid in its active site. Two actively transcribed GST genes (GSTO1 and GSTO2) are located on the long arm of chromosome 10, and both genes contain 6 exons (13-15).

GSTO members are widely distributed in a range of mammalian tissue types including the liver, colon, heart, ovary, pancreas, prostate, and spleen (13,14). GSTOs have physiologic roles in multidrug resistance, oxidative stress response, and interleukin-1β activation. GSTO genes are polymorphic, and SNPs have been reported in the coding and noncoding regions of these genes. The gene frequency of different substitutions and their effects on enzyme function vary in different populations (14).

The most frequent missense polymorphism in the GSTO1 gene is the Ala140/Asp substitution. This substitution can be found in all populations. The Asp 140 variant has lower thiol transferase activity. The GSTO2 gene is really polymorphic, and 66 SNPs have been reported for this region to date. The most common substitution found across all populations is Asn142/Asp (14,16). SNPs of GSTOs play an important role in cancers such as breast cancer, hepatocellular carcinoma, bile duct carcinoma, urethral cancer, acute lymphoblastic leukemia, and non-small cell lung cancer (13,17).

Recently, there has been great interest in identifying new biomarkers that might provide better results in the earlier recognition of HPV infections and cervical cancer. Currently, no GSTO gene polymorphisms have been explored between HPV infection and genital cancers, but some studies have investigated the interaction between these SNPs and many diseases such as sporadic Alzheimer's disease, cerebrovascular atherosclerosis, and obstructive pulmonary disease (18).

In this study, we established the frequencies of the GSTO1 and GSTO2 genotypes and allele in an Iranian population. In addition, we investigated whether GSTO gene polymorphisms could influence the risk susceptibility of cervical cancer development in women with HPV genotypes.

Material and Methods

We collected and evaluated 50 liquid-based cytology (LBC) samples from patients admitted to Mohebe-Yas Hospital in Tehran, Iran, who were diagnosed with cervical intraepithelial neoplasia and cervical cancer. In addition, we also collected and evaluated 43 archived LBC samples from patients with neither cervical cancer nor HPV infection to serve as a negative control comparator, and 43 LBC samples that were positive for HPV genotypes from 2 private laboratories of Tehran, Iran. LBC samples were transferred to the molecular biology department of the health reference laboratory of the Ministry of Health and Medical Education and stored at -20 °C until they were analyzed. The study was approved by the University Ethics Committee. Informed consent was obtained from all subjects. Table 1 presents the demographic clinical data for all patient samples.

DNA extraction

Genomic DNA was extracted using a High Pure PCR Template Preparation Kit (Roche, Germany). Briefly, according to the manufacturer's instruction, initially LBCs were lysed, and then DNA binding buffer was added. We mixed them immediately

Table 1. Demographic clinical data of subjects

	Patients with CC	HPV (+) patients	Controls
Age/years			
Mean	44	32	33
Standard deviation	10.56	8.99	8.14
Range	23-70	20-58	20-55
HPV types			
HPV 16 (+)	35	6	-
HPV 18 (+)	31	1	-
HPV 16 and 18 co-infection	21	0	-
HPV 6 (+)	7	16	-
Other HPV types (+)*	49	25	-
Pathologic staging of CC			
CIN I	9	-	-
CIN II	6	-	-
CIN and ICC	35	-	-
Total	50	43	43

*Other HPV types including: HPV 11, 26, 31, 33, 35, 39, 45, 51, 52, 53, 54, 56, 58, 59, 61, 66, 68, 70, 82 and 89; CC: Cervical cancer; CIN: Cervical intraepithelial neoplasia; HPV: Human papilloma virus; ICC: Invasive cervical cancer

and incubated for 10 min at +70 °C until the cells were digested completely. Isopropanol was added and mixed well. We inserted a High Pure filter tube into one collection tube and transferred the remaining liquid sample with a pipet into upper buffer reservoir of the filter tube. After that, we put the entire High Pure Filter Tube assembly into a standard table-top centrifuge and centrifuged for 1 min at 8000×g. Then, we discarded the flow-through and the collection tube. We combined the filter tube with a new collection tube and added Inhibitor Removal Buffer to the upper reservoir of the filter tube. Centrifugation for 1 min at 8000×g was then performed. Hereafter, the protocol for washing and elution step was accomplished. At the end, micro centrifuge tubes contained the eluted DNA.

Polymerase chain reaction procedure

In this step, the GSTO1 and GSTO2 genes were amplified using a polymerase chain reaction (PCR) method. These genes were amplified using GSTO1 forward and reverse primers F: 5 '-GAA CTT GAT GCA CCC TTG GT-3' and R: 5 '-TGA TAG CTA GGA GAA ATA ATT AC-3. The primers for GSTO2 were F: 5 '-AGG CAG AAC AGG AAC TGG AA-3' and R: 5 '-GAG GGA CCC CTT TTT GTA CC-3' (15). The PCR reaction contained 15 μL of Master Mix® 2X (Ampliqon, Denmark) and 1 μL of forward and reverse primers, in which, 15 μL of Master Mix was taken for each sample and mixed with 10 μL of genomic DNA, so that the final mixture volume was 25 μL. PCR cycling was performed with initial denaturation at 94 °C for 5 min, followed by 35 cycles of amplification at 94 °C for 60 seconds, 62 °C for 60 seconds, 72 °C for 60 seconds, and finally at 72 °C for 10 min.

GSTO1*A140D polymorphism analysis

For the PCR-restriction fragment length polymorphism (PCR-RFLP) step to indicate C>A transversion polymorphism in exon 4 of the GSTO1 gene, we used the restricting enzyme of CaC8 I (New England BioLabs, USA). The PCR-RFLP mixture included 16 μL of distilled water, 0.7 μL of CaC8 I, three μL of 10X NEBuffer® (New England BioLabs, USA), and 10 μL of PCR products so that the final volume was around 30 μL. This mixture was stored at 37 °C for 1 hour for digestion. The digested products appeared in 3 different patterns: (I) wild-type (140AA) showing 254 fragments; (II) heterozygote (140AD), 68, 186, and 254 bp fragments; and (III) homozygote (140DD) demonstrating 68 and 186 bp fragments (Figure 1).

GSTO2*N142D polymorphism analysis

The A>G transition polymorphism at codon 142 in exon 4 of GSTO2 was shown via the use of the restricting enzyme MboI (New England BioLabs, USA). The PCR-RFLP mixture for each reaction was similar to that used in the GSTO1*A140D polymorphism analysis. The digested products were shown in 3 patterns: (I) wild-type homozygote (142NN) presenting 185 bp fragments; (II) heterozygote (142ND), 63, 122, and 185 bp fragments; and (III) homozygote (142DD) 122 and 63 fragments. Afterwards, electrophoresis was performed at 100 V for 40 minutes in 1X Tris/Borate/Ethylenediamine tetra acetic acid buffer and 3% agarose gel to detect PCR-RFLP patterns. Products were visualized under ultraviolet light (Figure 1).

Statistical analysis

We used the IBM SPSS Statistics for Windows, Version 23.0 (Released 2013. Armonk, NY: IBM Corp.) computer software for data analysis. One-way analysis of variance (post hoc, least significant difference method) was used to compare the mean value for age in the different groups employed. The crude and adjusted odds ratio (OR) and 95% confidence intervals (CI) were calculated using binary logistic regression. In addition, Pearson's chi-square test was used for comparing the relationship between GSTO1 and GSTO2 genotypes and for pathologic staging of CC. A p-value of <0.05 was considered significant. Allele frequencies of GSTO1 and GSTO2 genotype polymorphisms were calculated using the Hardy-Weinberg equilibrium. A chi-square test was employed to study the deviation from the Hardy-Weinberg equilibrium between the observed and expected genotype frequencies in the controls.

Results

The distribution between the HPV-positive group and controls were not significantly different for age ($p=0.679$). However, there was a significant difference ($p<0.001$) in ages between samples in the cervical cancer group and the HPV-negative controls.

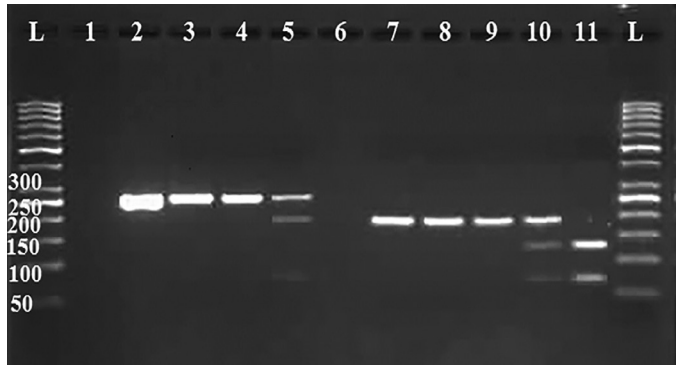

Figure 1. Lanes: (L) 50-bp DNA ladder; (1), Negative Control for GSTO1; (2), PCR product for GSTO1: 254bp fragment; (3 and 4), homozygote AA: 254 bp fragment; (5), heterozygote AD: 254, 186, and 68-bp fragments; (6), Negative Control for GSTO2; (7), PCR product for GSTO2: 185 bp fragment; (8 and 9), homozygote NN: 185-bp fragment; (10), heterozygote ND: 185, 122 and 63 fragments; (11), homozygote DD: 122 and 63 bp fragments; (L) 50-bp DNA ladder

PCR: Polymerase chain reaction

The GSTO1 A140D and GSTO2 N142D genotypic frequencies of the HPV-negative control group were in Hardy-Weinberg equilibrium ($\chi 2$= 1.91 and 0.452, respectively). Allele frequencies in the HPV-negative control population for the GSTO1 gene were 0.825 for the A allele and 0.175 for the D allele. The frequencies for the GSTO2 gene were 0.697 for the N allele and 0.303 for the D allele.

There was a significant difference for the 140 AD genotype and D allele frequency in the cervical cancer group compared with the HPV-negative control group. This indicates a protective property of the AD genotype and D allele. Calculating with binary logistic adjusted for age also revealed a significant difference for the 140AD/142NN combination genotype (p=0.016) with a protective function for this genotype in these groups. The OR analysis and 95% CI between the HPV-positive group and HPV-negative controls were not significantly different for genotypes and allele frequencies (Table 2).

HPV 16, HPV 18, and HPV 6 were the most prevalent subtypes in the cervical cancer group and the HPV-positive group. The sum of patients with HPV 16, HPV 18, HPV 16/18, and HPV 6 infections in the cervical cancer group and the HPV-positive groups were 41, 32, 21, and 23, respectively. The details of HPV genotyping results are not shown in this study. The relationship between these patients and those in the HPV-negative control group for these genotypes were calculated using binary logistic adjusted for age, like in the previous analysis.

In individuals positive for HPV 16, the frequencies of GSTO1 genotypes were 38 for AA, 3 for AD, and 0 for DD. This analysis revealed a significant protective attribute for the AD genotype [OR= 0.075; 95% CI: (0.015 to 0.386); p=0.002]. The frequencies of GSTO2 genotypes were 18 for NN, 19 for ND, 4 for DD, and 23 for ND/DD. We found no significant difference for GSTO2 N142D between the HPV 16-positive group and the HPV-negative controls (p>0.05). The GSTO1 A140D and GSTO2 N142D combination genotype frequencies were 16 for 140AA/142NN, 18 for 140AA/142ND, 4 for 140AA/142DD, 2 for 140AD/142NN, and 1 for 140AD/142ND. There was a significant difference in the 140AD/142NN combination genotype between patients positive for HPV 16 and those in the HPV-negative group [OR= 0.058; 95% CI: (0.007 to 0.503); p=0.01] with the protective role. There were no significant differences for other combination genotypes between patients positive for HPV 16 and those in the HPV-negative group (p>0.05).

For patients positive for HPV 18, the frequencies of GSTO1 genotypes were 28 for AA, 4 for AD, and 0 for DD. This analysis showed a significant protective role for the AD genotype [OR= 0.113; 95% CI: (0.024 to 0.525); p=0.005]. The frequencies of the GSTO2 genotypes were13 for NN, 18 for ND, 1 for DD, and 19 for ND/DD. We found no significant difference for GSTO2 N142D between HPV 18-positive samples and the HPV-negative control samples (p>0.05). The frequencies of GSTO1 A140D and GSTO2 N142D combination genotype were 11 for 140AA/142NN, 16 for 140AA/142ND, 1 for 140AA/142DD, 2 for 140AD/142NN, 2 for 140AD/142ND, and 0 for 140AD/142DD. There was a significant difference for the 140AD/142NN combination genotype between patients positive for HPV 18 and those in the HPV-negative group [OR= 0.058; 95% CI: (0.006 to 0.532); p=0.012] with the protective role. There were no significant differences for other combination genotypes between patients positive for HPV 18 and those in the HPV-negative group (p>0.05).

In the HPV 16/18 co-infection group, the frequencies of GSTO1 genotypes were 19 for AA, 2 for AD, and 0 for DD. The analysis indicated a significant protective role for the AD genotype [OR= 0.055; 95% CI: (0.006 to 0.462); p=0.008]. The frequencies of GSTO2 genotypes were 8 for NN, 12 for ND, 1 for DD, and 13 for ND/DD. We found no significant difference for GSTO2 N142D between the HPV 16/18 co-infection group samples and the HPV-negative controls (p>0.05). The GSTO1 A140D and GSTO2 N142D combination genotype frequencies were 7 for 140AA/142NN, 11 for 140AA/142ND, 1 for 140AA/142DD, 1 for 140AD/142NN, and 1 for 140AD/142ND. There was a significant difference in the protective role for the 140AD/142NN genotype between HPV 16/18-positive patients and HPV-negative controls [OR= 0.018; 95% CI: (0.01 to 0.403); p=0.011]. There was no significant difference for other combination genotypes between HPV 16/18-positive patients and HPV-negative controls (p>0.05).

In the samples positive for HPV 6, the frequencies of GSTO1 genotypes were 22 for AA, 1 for AD, and 0 for DD. This analysis showed a significant protective role for the AD genotype [OR= 0.056; 95% CI: (0.006 to 0.519); p=0.011]. The frequencies of GSTO2 genotypes were 10 for NN, 11 for ND, 2 for DD, and 13 for ND/DD. We found no significant difference for GSTO2 N142D between the HPV 6-positive patients and the HPV-negative controls (p>0.05). The frequencies of GSTO1 A140D and GSTO2 N142D combination genotypes were 9 for 140AA/142NN, 11 for 140AA/142ND, 2 for 140AA/142DD, and 1 for 140AD/142NN. We found no significant difference for these combination genotypes between HPV 6-positive patients and HPV-negative controls (p>0.05), although the result of the analysis for 140AD/142NN genotype was an OR of 0.094, with 95% CI of 0.008 to 1.06 (p=0.056). Therefore, although there was an association between this genotype and HPV6 infection, the association was not significant.

There was no significant association between GSTO1 and GSTO2 genotypes and pathologic staging of cervical cancer (Table 3). All patients with cervical intraepithelial neoplasia (CIN) grade I, II, and more than 82% of patients with CIN grade III and invasive cervical cancer were recognized with the

Table 2. Calculated OR and 95% CI with binary logistic regression and adjusted for age population study

	Patients with CC	Control group	OR	95% CI	p value
GSTO1 A140D					
AA	44 (88%)	28 (65%)	1 (Ref)	-	-
AD	6 (12%)	15 (35%)	0.151	0.041-0.558	0.005*
DD	0	0	-	-	-
A allele	0.94	0.825	1 (Ref)	-	-
D allele	0.06	0.175	0.302	0.112-0.818	0.018*
GSTO2 N142D					
NN	24 (48%)	20 (46.5%)	1 (Ref)	-	-
ND	25 (50%)	20 (46.5%)	1.56	0.586-4.18	0.372
DD	1 (2%)	3 (7%)	0.489	0.041-5.77	0.57
ND/DD	26 (52%)	23 (53%)	1.41	0.543-3.68	0.478
N allele	0.73	0.697	1 (Ref)	-	-
D allele	0.27	0.303	0.854	0.451-1.61	0.626
Combination GSTO1 A140D & GSTO2 N142D					
140AA/142NN	20 (40%)	12 (28%)	1 (Ref)	-	-
140AA/142ND	23 (46%)	14 (32.5%)	1.19	0.377-3.78	0.762
140AA/142DD	1 (2%)	2 (4.6%)	0.410	0.026-6.44	0.526
140AD/142NN	4 (8%)	8 (18.6%)	0.117	0.021-0.666	0.016*
140AD/142ND	2 (4%)	6 (14%)	0.291	0.040-2.11	0.223
140AD/142DD	0	1 (2.3%)	0	0	1
	HPV (+) group	**Control group**	**OR**	**95% CI**	**p value**
GSTO1 A140D					
AA	43 (100%)	28 (65%)	1 (Ref)	-	-
AD	0	15 (35%)	0	0	0.998
DD	0	0	-	-	-
A allele	1	0.825	1 (Ref)	-	-
D allele	0	0.175	0	0	0.998
GSTO2 N142D					
NN	14 (32.5%)	20 (46.5%)	1 (Ref)	-	-
ND	20 (46.5%)	20 (46.5%)	1.42	0.539-3.76	0.476
DD	9 (21%)	3 (7%)	4.27	0.947-19.26	0.059
ND/DD	29 (67.5%)	23 (53%)	1.79	0.711-4.53	0.216
N allele	0.557	0.697	1 (Ref)	-	-
D allele	0.443	0.303	1.827	0.976-3.41	0.06
Combination GSTO1 A140D & GSTO2 N142D					
140AA/142NN	14 (32.5%)	12 (28%)	1 (Ref)	-	-
140AA/142ND	20 (46.5%)	14 (32.5%)	1.36	0.47-3.95	0.56
140AA/142DD	9 (21%)	2 (4.6%)	4.36	0.762-25.01	0.098
140AD/142NN	0	8 (18.6%)	0	0	0.999
140AD/142ND	0	6 (14%)	0	0	0.999
140AD/142DD	0	1 (2.3%)	0	0	1
Total	**43**	**43**	**-**	**-**	**-**

*p value <0.05; CI: Confidence interval; OR: Odds ratio; CC: Cervical cancer

140AA genotype, and none were recognized with the 140DD and 142DD genotypes.

GSTO1 and GSTO2 genotype frequencies in individuals with HPV 11, 26, 31, 33, 35, 39, 45, 51, 52, 53, 54, 56, 58, 59, 61, 66, 68, 70, 82, and 89 infections are summarized in Table 4.

Discussion

This study investigated the association between GSTO1 A140D and GSTO2 N142D polymorphisms and susceptibility to HPV infection and cervical cancer progression in Iranian women. To the best of our knowledge, no other previous study has investigated this issue in Iranian populations. Cervical cancer is one of the more prevalent causes of death among women, leading to approximately 270,000 deaths annually (2,19,20). Persistent genital infections with different high-risk HPV genotypes, specifically HPV 16 and 18, lead to CIN and cervical cancer (1,21,22,23). It would seem that investigations should be performed on the recognition and development of genetic and epigenetic patterns as molecular prognostic biomarkers in the diagnosis of early stage of cervical cancer (15,24).

The GSTO enzyme is a new class in the GST super family which conjugated glutathione to electrophilic intermediates and detoxifies endogenous and exogenous compounds (15,25,26). GSTO1 is expressed in a wide range of human tissues and plays a role in apoptosis. This enzyme is a potential source of intracellular glutathione, which protects against cellular oxidative stresses (16). This protective aspect against cell toxicity may be weakened if enzymatic activity is reduced.

Table 3. Relationship between GSTO1 and GSTO2 genotypes and pathologic staging of patients with cervical cancer

	GSTO1				GSTO2			
	140AA	**140AD**	**140DD**	**p value**	**142NN**	**142ND**	**142DD**	**p value**
CIN I	9	0	0		3	5	1	
CIN II	6	0	0	0.232	3	3	0	0.275
CIN III/ ICC	29	6	0		18	17	0	
CIN: Cervical intraepithelial neoplasia; ICC: Invasive cervical cancer								

Table 4. GSTO1 and GSTO2 genotype frequency in women with HPV genotype infections

Other infections		**AA**	**AD**	**DD**	**NN**	**ND**	**DD**	**Number of patients**
HPV 52	HR	7	1	0	3	5	0	8
HPV 31	HR	8	1	0	5	3	1	9
HPV 56	HR	7	1	0	4	4	0	8
HPV 58	HR	6	1	0	5	2	0	7
HPV 59	HR	7	1	0	5	2	1	8
HPV 11	LR	7	1	0	4	4	0	8
HPV 33	HR	3	0	0	2	1	0	3
HPV 66	HR	7	0	0	4	3	0	7
HPV 54	LR	2	0	0	0	0	2	2
HPV 61	LR	1	0	0	1	0	0	1
HPV 45	HR	2	1	0	1	1	1	3
HPV 68	HR	6	1	0	1	6	0	7
HPV 35	HR	5	0	0	1	4	0	5
HPV 51	HR	7	1	0	4	3	1	8
HPV 39	HR	4	0	0	3	1	0	4
HPV 53	pHR	2	0	0	0	1	1	2
HPV 82	HR	1	0	0	0	0	1	1
HPV 70	LR	1	0	0	0	1	0	1
HPV 89	LR	1	0	0	0	0	1	1
HPV 26	HR	1	0	0	0	1	0	1
Total		**85**	**9**	**0**	**43**	**42**	**9**	**94**
HR: High-risk, LR: Low-risk, pHR: Probably high-risk, HPV: Human papilloma virus								

Some studies showed a significant reduction in thiol transferal activity resulting from aspartic acid substitution, whereas other studies found no significant reduction in the enzyme activity (16). The allele frequency of the GSTO1 A140D polymorphism in our study was similar to that reported by Ada et al. (16) in a Chinese population study that compared European, American, and other Asian populations.

The GSTO2 enzyme shows a new type of activity that is not seen in other GSTs including glutathione-dependent thiol transferase, monomethyl arsenate reductase, and dehydroascorbate reductase activity. The gene encoding GSTO2 is a polymorphic gene with a single nucleotide polymorphism causing an asn142Asp (N142D) substitution. This substitution may alter the function of the GSTO2 enzyme (15,17,27). The allele frequencies of the GSTO2 N142D polymorphism in our study are similar to those reported by Rezazadeh et al. (15) in comparison with Italy, Thailand, Japan, and Turkey.

Based on our findings, the 140AD genotype, 140D allele, and 140AD/142NN combination genotype seem to confer a protective property in women's susceptibility to HPV 6, 16, 18 and 16/18 infections and cervical cancer. To the best of our knowledge, there have been no investigations to determine association between the GSTO1 A140D and GSTO2 N142D and HPV infections or cervical cancer. However, Sanguansin et al. (18) suggested that the GSTO1*D140 variant genotype might play a protective role against head and neck cancer in the Thai population. In contrast, Ada et al. reported no significant association between the GSTO1 A140D polymorphism and susceptibility to non-small cell lung cancer in the Turkish population (16). Also, Rezazadeh et al. found that the frequency of GSTO1 A140D polymorphism was not associated with childhood pre-B acute lymphoblastic leukemia in the Iranian population (15).

In our study, the GSTO2 N142D polymorphism was not associated with HPV infections and cervical cancer. Sanguansin et al. (18) revealed that the frequency of GSTO2 genotype was not significantly different between patients with head and neck cancer and controls in the Thai population, which is in agreement with our results. Also, Rezazadeh et al. (15) showed that there was no significant association between pre-B acute lymphoblastic leukemia and GSTO2 N142D polymorphism in the Iranian population. However, Khosravi et al. (17) demonstrated that individuals with DD genotype were more susceptible to developing hepatic failure leading to liver transplantation.

Therefore, we suggest that GSTO1 A140D gene polymorphisms likely play an inconspicuous role in the level of susceptibility to HPV-related cervical cancer. Future studies with a larger number of patients should explore the additional effect of these polymorphisms with other HPV infections or cervical cancer risk factors.

Our study was limited by the relatively small number of patients evaluated. The clinical dataset from the subjects was another limitation of the study. Some important risk factors like smoking status, which have a critical role in GSTO polymorphism interactions and HPV infections or cervical cancer were missed because our patients were not new (they were collected in 2012), and we did not have more information from the subjects. In addition, approximately 65% of participants in the HPV-positive group were infected with low-risk HPV genotypes (HPV 6, n=16; other low-risk HPV types, n=2).

In conclusion, future investigations should be performed on larger groups of participants, especially on women with high-risk HPV genotypes and other sexually-transmitted infections in order to find any association between SNPs and cervical malignancies in developing countries. Cancer screening, particularly early diagnosis in the first stages can be helpful in national health programs using an approved molecular biomarker.

Ethics Committee Approval: *All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.*

Informed Consent: *This article does not contain any studies with animals performed by any of the authors. Informed consent was obtained from all patients.*

Peer-review: *Externally peer-reviewed.*

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References

1. Bharti AC, Shukla S, Mahata S, Hedau S, Das BC. Anti-human papillomavirus therapeutics: facts & future. Indian J Med Res 2009; 130: 296-310.
2. Jia H, Wang X, Long Z, Li L. Human papillomavirus infection and cervical dysplasia in female sex workers in Northeast China: an observational study. BMC Public Health 2015; 15: 695-700.
3. Pour BT, Hosseini SM, Kardi MT. Frequency of human papilloma virus genotypes in patient samples from Isfahan (Iran) using polymerase chain reaction reverse dot blot method. Jundishapur J Microbiol 2017; 10: 37672-4.
4. Shayanfar N, Babaheidarian P, Rahmani H, Azadmanesh K, Sohrabi A, Mohammadpour M, et al. Epidermodysplasia verruciformis

associated with plasmablastic lymphoma and hepatitis B virus infection. Acta Dermatovenerol Croat 2012; 20: 267-71.
5. Shayanfar N, Hosseini N, Panahi M, Azadmanesh K, Mohammad pour M, Kadivar M, et al. Detection of mucosal type human papillomavirus in cutaneous squamous cell carcinoma in Iran. Pathol Res Pract 2013; 209: 90-4.
6. Sohrabi A, Hajia M, Jamali F, Kharazi F. Is incidence of multiple HPV genotypes rising in genital infections? J Infect Public Health 2017; 10: 730-3.
7. Wang D, Wang B, Zhai JX, Liu DW, Sun GG. Glutathione S-transferase Ml and T1 polymorphisms and cervical cancer risk: A meta-analysis. Neoplasma 2011; 58: 352-9.
8. Zhen S, Hu CM, Bian LH. Glutathione S-transferase polymorphism interactions with smoking status and HPV infection in cervical cancer risk: an evidence-based meta-analysis. PLoS One 2013; 8: 83497.
9. Gao LB, Pan XM, Li LJ, Liang WB, Bai P, Rao L, et al. Null genotypes of GSTM1 and GSTT1 contribute to risk of cervical neoplasia: an evidence-based meta-analysis. PLoS One 2011; 6: 20157.
10. Settheetham-Ishida W, Yuenyao P, Kularbkaew C, Settheetham D, Ishida T. Glutathione S-transferase (GSTM1 and GSTT1) polymorphisms in cervical cancer in Northeastern Thailand. Asian Pac J Cancer Prev 2009; 10:365-8.
11. Coughlin SS, Hall IJ. Glutathione S-transferase polymorphisms and risk of ovarian cancer: A HuGE review. Genet Med 2002; 4: 250-7.
12. Hasan S, Hameed A, Saleem S, Shahid SM, Haider G, Azhar A. The association of GSTM1 and GSTT1 polymorphisms with squamous cell carcinoma of cervix in Pakistan. Tumour Biol 2015; 36: 5195-9.
13. Xu YT, Wang J, Yin R, Qiu MT, Xu L, Wang J, et al. Genetic polymorphisms in Glutathione S-transferase Omega (GSTO) and cancer risk: a meta-analysis of 20 studies. Sci Rep 2014; 4: 6578-85.
14. Board PG. The omega-class glutathione transferases: structure, function, and genetics. Drug Metab Rev 2011; 43: 226-35.
15. Rezazadeh D, Moradi MT, Kazemi A, Mansouri K. Childhood Pre-B acute lymphoblastic leukemia and glutathione S-transferase omega 1 and 2 polymorphisms. Int J Lab Hematol 2015; 37: 530-5.
16. Ada TG, Ada AO, Kunak SC, Alpar S, Gulhan M, Iscan M. Association between glutathione S-transferase omega 1 A140D polymorphism in the Turkish population and susceptibility to non-small cell lung cancer. Arh Hig Rada Toksikol 2013; 64: 61-7.
17. Khosravi M, Saadat I, Karimi MH, Geramizadeh B, Saadat M. Glutathione S-transferase omega 2 genetic polymorphism and risk of hepatic failure that lead to liver transplantation in Iranian population. Int J Organ Transpl Med 2013; 4: 16-20.
18. Sanguansin S, Petmitr S, O-Charoenrat P, Pongstaporn W. Association of glutathione S-transferase omega gene polymorphisms with progression of head and neck cancer. Mol Biol Rep 2012; 39: 10915-20.
19. Sohrabi A, Hajia M. Cervical cancer and genital infections: assessment of performance and validation in human papillomavirus genotyping assays in Iran, its neighboring countries and Persian Gulf area. Iran J Pathol 2017; 12: 35-44.
20. Sohrabi A, Mirab-Samiee S, Modarresi MH, Izadimood N, Azadmanesh K, Rahnamaye-Farzami M. Development of in-house multiplex real time PCR for human papillomavirus genotyping in Iranian women with cervical cancer and cervical intraepithelial neoplasia. Asian Pac J Cancer Prev 2014; 15: 6257-61.
21. Sohrabi A, Farzami R, Samiee SM, Modarresi MH. An overview on papillomaviruses as the main cause of cervical cancer, Iran. J Obstet Gynecol Infertil 2015; 18: 14-25.
22. Sohrabi A, Rahnamaye-Farzami M, Mirab-Samiee S, Mahdavi S, Babaei M. Comparison of in-house multiplex real time PCR, Diagcor Geno Flow HPV Array test and INNO-LiPA HPV genotyping extra assays with LCD-Array Kit for human papillomavirus genotyping in cervical liquid based cytology specimens and genital lesions in Tehran, Iran. Clin Lab 2016; 62: 615-9.
23. Hajia M, Sohrabi A. Possible Synergistic Interactions among Multiple HPV Genotypes in Women Suffering from Genital Neoplasia. Asian Pac J Cancer Prev 2018; 19: 785-9.
24. Sohrabi A, Mirab-Samiee S, Rahnamaye-Farzami M, Rafizadeh M, Akhavan S, Hashemi-Bahremani M, et al. C13orf18 and C1orf166 (MULAN) DNA genes methylation are not associated with cervical cancer and precancerous lesions of human papillomavirus genotypes in Iranian women. Asian Pac J Cancer Prev 2014; 15: 6745-8.
25. Ciba M, AK M, Karahalil B. Alpha-glutathione-s-transferase can be a biomarker for both drug-related toxicity as well as individual susceptibility. Minerva Psichiatrica 2016; 57: 62-71.
26. Helzlsouer KJ, Selmin O, Huang HY, Strickland PT, Hoffman S, Alberg AJ, et al. Association between glutathione S-transferase M1, P1, and T1 genetic polymorphisms and development of breast cancer. J Natl Cancer Inst 1998; 90: 512-8.
27. Saadat M. Genetic polymorphism of N142D GSTO2 and susceptibility to breast cancer: a meta-analysis. Mol Biol Res Commun 2012; 1:39-44.

Prognostic effect of isolated paraaortic nodal spread in endometrial cancer

Osman Türkmen[1], Derman Başaran[2], Alper Karalök[2], Günsu Cömert Kimyon[2], Tolga Taşçı[3], Işın Üreyen[4], Gökhan Tulunay[5], Taner Turan[2]

[1]Clinic of Gynecologic Oncology, Gaziantep Cengiz Gökçek Obstetrics and Children's Hospital, Gaziantep, Turkey
[2]Clinic of Gynecologic Oncology, University of Health Sciences, Etlik Zübeyde Hanım Women's Health Training and Research Hospital, Ankara, Turkey
[3]Clinic of Gynecologic Oncology, İstanbul Okmeydanı Training and Research Hospital, İstanbul, Turkey
[4]Clinic of Gynecologic Oncology, University of Health Sciences, Antalya Training and Research Hospital, Antalya, Turkey
[5]Clinic of Gynecologic Oncology, TOBB University Hospital, Ankara, Turkey

Abstract

Objective: To evaluate the prognostic effect of isolated paraaortic lymph node metastasis in endometrial cancer (EC).

Material and Methods: This retrospective study included patients with FIGO 2009 stage IIIC2 disease due to isolated paraaortic lymph node metastasis (LNM). Patients with sarcomatous histology, synchronous gynecologic cancers and patients with concurrent pelvic lymph node metastases or patients that have intraabdominal tumor spread were excluded. Kaplan-Meier method was used for calculation of progression free survival (PFS) and overall survival.

Results: One thousand six hundred and fourteen patients were operated for EC during study period. Nine hundred and sixty-one patients underwent lymph node dissection and 25 (2.6%) were found to have isolated LNM in paraaortic region and these constituted the study cohort. Twenty (80%) patients had endometrioid EC. Median number of retrieved lymph nodes from pelvic region and paraaortic region was 21.5 (range: 5-41) and 34.5 (range: 1-65), respectively. Median number of metastatic paraaortic nodes was 1 (range: 1-32). The median follow-up time was 15 months (range 5-94). Seven (28%) patients recurred after a median of 20 months (range, 3-99) from initial surgery. Three patients recurred only in pelvis, one patient had upper abdominal spread and 3 had isolated extraabdominal recurrence. Involvement of uterine serosa, positive peritoneal cytology and presence of adnexal metastasis were significantly associated with diminished PFS ($p<0.05$).

Conclusion: The presence of serosal involvement or adnexal involvement is as important as gross peritoneal spread and is related with poor survival in patients with isolated paraaortic nodal spread in EC. Chemotherapy should be the mainstay of treatment in this patient cohort which may eradicate systemic tumor spread. (J Turk Ger Gynecol Assoc 2018; 19: 201-5)

Keywords: Endometrial cancer, paraaortic lymph node metastasis, serosal involvement

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Introduction

Endometrial cancer (EC) is the most common gynecologic malignancy and the 4th most common cancer of women (1). Surgical staging of EC was recommended by the International Federation of Gynecology and Obstetrics (FIGO) in 1988 and has been re-validated in the most recent FIGO 2009 staging system (2,3). One of the most striking differences in the FIGO 2009 staging system was the division of the former stage IIIC of the FIGO 1988 system into two subgroups; stage IIIC1 and IIIC2. Patients with only positive pelvic lymphatic spread were defined as stage IIIC1 and those with paraaortic lymphatic spread irrespective of pelvic lymph nodes were named as stage IIIC2 (4). However, patients with stage IIIC2 disease

Address for Correspondence: *Osman Türkmen*
e.mail: osmanturkmen312@gmail.com ORCID ID: orcid.org/0000-0002-1470-7731

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constitute a very heterogeneous patient cohort in whom either pelvic extrauterine disease or positive pelvic lymph nodes may exist concurrently. Another important patient subgroup of FIGO stage IIIC2 disease, with an incidence varying between 1% and 6% among all patients with EC, comprises patients with isolated paraaortic lymph node metastases (5-9). Tumors of the uterine corpus may spread to the paraaortic region via lymphatic routes of obturator and external iliac chains or the lymphatic pathways through gonadal vessels (10). These findings indicate that even patients with stage IIIC2 disease due to isolated paraaortic lymph nodes may represent a heterogeneous patient group because of lymphatic spread patterns.

In this retrospective study we sought to define the clinical and surgical factors related to the prognosis of patients with isolated paraaortic lymph spread in EC.

Material and Methods

This retrospective study included patients with epithelial EC who underwent comprehensive surgical staging at the Gynecologic Oncology Department of our hospital from January 1992 to September 2014. The inclusion criterion was having FIGO 2009 stage IIIC2 disease due to isolated paraaortic lymph node metastasis (LNM). Patients with sarcomatous histology, synchronous gynecologic cancers, and patients with concurrent pelvic LNMs or intraabdominal tumor spread were excluded. Demographics, intraoperative findings, and surgicopathological results were accumulated from patient files, pathology reports, and electronic files.

All patients underwent hysterectomy and bilateral salpingo-oophorectomy and complete systematic lymph node dissection. Bilateral pelvic lymphadenectomy was performed to complete skeletonization, with all lymphatic tissue of the pelvic vascular structure and the obturator fossa. Between the aortic bifurcation and deep circumflex iliac vein, lymphatic tissues around the pelvic vascular structure and lymphatic tissue in obturator fossa were defined as pelvic lymph nodes. All lymphatic tissues around the vascular structure between the aortic bifurcation and renal vein were defined as para aortic lymph nodes. All surgical procedures were performed by gynecologic oncologists and pathologic evaluations were reported by a dedicated team of and gynecopathologists.

All patients received adjuvant treatment in forms of chemotherapy, radiotherapy or combined chemoradiation after initial surgery. The decision regarding the type of adjuvant radiotherapy was made by a gynecologic oncology council according to the patient's risk factors. Chemotherapy regimens included platinum and taxane- based protocols. External beam radiotherapy (EBRT) was given to the patients. EBRT was directed to the pelvis in the para-aortic region. Pelvic radiotherapy targeted the lower common iliacs, external iliacs, internal iliacs, parametrium, upper vagina, and paravaginal tissue. Extended-field radiotherapy included pelvic tissues and the para-aortic lymph node region up to the renal vessels. The external beam doses were 45 to 50 Gy. Adnexal and uterine serosal involvement and positivity of peritoneal cytology were accepted as non-nodal extrauterine disease. Progression-free survival (PFS) was defined as the time between surgery and relapse or the last follow-up. The duration until exitus because of the disease or until the follow-up was defined as overall disease-specific survival (OS). We defined recurrence distal to the linea terminalis as pelvic recurrence, recurrence between the pelvic inlet and diaphragm peritoneum as upper abdominal recurrence, and other recurrences such as recurrence in the liver parenchyma, skin, and bone were accepted as extra-abdominal recurrence. Ascites and peritonitis carcinomatosa were accepted as upper abdominal recurrence.

After adjuvant treatment was completed, patients were followed up every three months for the first 2 years, then every 6 months for 3 years, and annually thereafter. The follow-up routine included pelvic inspection and full abdominal ultrason ography. Chest X-rays were performed yearly unless there was clinical suspicion. Thoracic and/or abdominal computerized tomography was performed when necessary. Ca-125 levels were used in the follow-up.

SPSS (SPSS Inc, Chicago IL, USA) version 15.0 was used for statistical analyses. Kaplan-Meier analysis was used for the calculation of PFS and OS. Survival curves were compared using the log-rank test. Prognostic factors were evaluated using the Cox regression model. The cut-off for statistical significance was set at $p<0.05$. A multivariate model was not created because of the intercorrelations between parameters that were significant in the univariate analysis. This study was approved by the local ethics committee. Signed informed consent was given by all patients, which allowed us to use their clinical data.

Results

One thousand six hundred fourteen patients underwent surgery for EC during the study period. Of these, 42 patients were excluded because of having synchronous cancers. Nine hundred sixty-one out of 1572 patients underwent lymph node dissection and 25 (2.6%) were found to have isolated LNM in the paraaortic region, and these constituted the study cohort. The median age of the patients was 60 years (range, 44-77 years). The surgical and pathologic features of the patients are presented in Table 1. Twenty (80%) patients had endometrioid type of EC and 19 (76%) had lymphovascular space invasion in paraffin histologic sections. The median number of removed lymph nodes from the pelvic area and paraaortic area was 21.5 (range, 5-41) and 34.5 (range, 1-65), respectively. The median number of metastatic paraaortic nodes was 1 (range, 1-32).

Table 1. Characteristics of the patients with isolated paraaortic lymph node metastasis (n=25)

Parameters	n, median	%, range
Age (years)	60	44-77
Tumor histology		
Endometrioid	20	80
Serous	1	4
Clear cell	3	12
Mixed	1	4
Tumor differentiation		
Grade 1	7	28
Grade 2	11	44
Grade 3	7	28
Lymphovascular space invasion		
Positive	19	76
Negative	6	24
Tumor size (mm)	45	20-85
Total number of harvested lymph nodes	56	5-93
Total number of paraaortic lymph nodes	34.5	1-65
Total number of pelvic lymph nodes	21.5	5-41
Patients with positive peritoneal cytology	3	12
Cervical stromal/glandular invasion	7	28
Adnexal metastasis	5	20
Serosal invasion	3	12

All patients with isolated paraaortic LNM received adjuvant treatment; 14 had radiotherapy, 6 received chemotherapy, and 5 underwent combined chemoradiation (sandwich protocol). Complete response was documented in all patients after adjuvant treatment. The median follow-up time was 15 months (range, 5-94). Seven (28%) patients had recurrence after a median of 20 months (range, 3-99) from the initial surgery. Three patients had recurrence only in the pelvis, one patient had upper abdominal spread, and 3 had isolated extraabdominal recurrence. Two (8%) patients died of disease. Univariate analysis showed that uterine serosal involvement, positivity of peritoneal cytology, presence of adnexal metastasis and presence of non-nodal extrauterine disease were significantly associated with diminished PFS (Table 2, 3). The median OS was 15 months. Only lymphovascular space invasion but not age, uterine serosal invasion, adnexal metastasis, non-nodal extrauterine disease, tumor histology, myometrial invasion, grade, type of adjuvant treatment, number of metastatic paraaortic lymph nodes, cervical stromal/glandular invasion and preoperative CA125 level were found to be associated with OS.

Table 2. Characteristics of the patients with recurrent disease

Parameters	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Age (years)	77	59	60	40	67	72	60
Tumor type	Clear cell	Endometrioid	Clear cell	Endometrioid	Endometrioid	Endometrioid	Endometrioid
Depth of myometrial invasion	<1/2	<1/2	≥1/2	≥1/2	≥1/2	≥1/2	≥1/2
Grade	3	1	3	2	2	2	2
Peritoneal cytology	Negative	Negative	Positive	Negative	Negative	Negative	Positive
LVSI	Positive	Positive	Positive	Negative	Positive	Positive	Positive
Cervical invasion	Positive	Negative	Positive	Negative	Negative	Negative	Negative
Adnexal metastasis	Negative	Negative	Positive	Positive	Negative	Positive	Negative
Serosal invasion	Negative	Negative	Positive	Negative	Negative	Positive	Negative
Adjuvant treatment	ChemoTx	RadioTx	ChemoTx + RadioTx	ChemoTx + RadioTx	RadioTx	RadioTx	RadioTx
Recurrence site	Vaginal cuff	Pelvic	Upper abdomen	Liver	Pelvic	Lung	Extraabdominal
Time to recurrence (months)	7	33	3	24	23	20	10

ChemoTx: Chemotherapy; RadioTx: Radiotherapy; LVSI: Lymphovascular space invasion

Table 3. Univariate analysis of surgical factors associated with disease-free survival

Parameters	DFS (months)	p
Uterine serosal invasion		0.007
Yes	7	
No	15	
Peritoneal cytology		0.002
Positive	7	
Negative	23	
Adnexal metastasis		0.021
Yes	8	
No	15	
Non-nodal extrauterine disease		0.002
Present	8	
Absent	15	
Age		0.153
Endometrioid vs non-endometrioid histology		0.75
Myometrial invasion <1/2 vs myometrial invasion ≥1/2		0.901
Grade 1 vs. grade 2 vs. grade 3		0.506
Type of adjuvant treatment (radiotherapy vs chemotherapy vs sandwich)		0.967
Number of total harvested lymph nodes		0.253
Number of metastatic paraaortic lymph nodes		0.056
Lymphovascular space invasion		0.752
Cervical stromal/glandular invasion		0.697
Preoperative CA125 level		0.643
DFS: Disease-free survival		

Discussion

The incidence of paraaortic LNM in EC and the prognostic factors of patients with stage IIIC2 EC have been the subject of various studies in the literature; however, there are limited data on the prognosis of isolated paraaortic LNM in EC (5-9,11,12). Todo et al. (13) conducted a study to evaluate the prognosis of stage IIIC EC. A total of 93 patients with stage IIIC EC were classified into three groups: group 1 consisted of patients who underwent pelvic and paraaortic lymphadenectomy and were positive for pelvic LNM (stage IIIC1), group 2 underwent only pelvic lymphadenectomy and had positive pelvic spread (at least stage IIIC1), and patients in group 3 had positive paraaortic lymph nodes (stage IIIC2). The 5-year survival rates were 89.3% in group 1, 46.5% in group 2, and 59.9% in group 3. Group 2 (p=0.0001) and group 3 (p=0.0016) had significantly diminished overall survival rates compared with group 1. They showed that metastatic lymph node count, lymphadenectomy level, and type of adjuvant therapy were significantly and independently associated with overall survival. Marchetti et al. (14) showed that a combined approach with chemotherapy and radiotherapy might improve recurrence-free survival compared with radiation or chemotherapy alone for stage IIIC EC. In the present study, metastatic node count in the paraaortic region and adjuvant treatment modality options were not related with PFS in patients with isolated paraaortic LNM (p=0.056 and p=0.967, respectively).

The prognostic role of uterine factors in stage IIIC EC was evaluated by Hoekstra et al. (15). Their study included 54 patients with pelvic spread and 31 patients with paraaortic (±pelvic) spread. In multivariate analysis, age, clear cell and serous h istology, and invasion rate were significantly related with OS; age and non-endometrioid histology were related with PFS. In the present study, presence of deep myometrial invasion (except serosal invasion) and non-endometrioid histology was not related with diminished survival. This finding is probably the result of the homogeneity of patients with isolated paraaortic LNM in our study because depth of myometrial invasion and non-endometrial histology are proved factors for lymphatic spread (16,17).

The significance of extranodal disease in stage IIIC EC was evaluated in a Surveillance, Epidemiology, and End Results (SEER) database analysis by Garg et al. (18). In patients with IIIC2 disease, high-risk factors such as grade III disease (p<0.001), non-endometrioid histologic types (p=0.01), and extrauterine disease (p<0.001) were common when compared with patients with stage IIIC1 disease. However, further analysis showed that in patients with extranodal disease, area of nodal metastasis had no effect on survival (HR=0.92; 95% CI: 0.74-1.14) and patients with positivity of peritoneal cytology and adnexal/serosal metastasis. These results support our findings that patients with isolated paraaortic LNM had unfavorable survival in the positivity of extranodal disease. Serosal involvement (p=0.007), positivity of peritoneal cytology (p=0.002), and adnexal metastasis (p=0.0021) were related with worse survival in our study. When the patients were classified into two groups according to the presence of extranodal disease, patients with no extranodal disease had significantly better outcomes (p=0.002). Therefore, one can conclude that patients with isolated paraaortic nodes have poor survival when the disease spreads intraperitoneally.

The major limitation of the current study is the inherent drawbacks from its retrospective design. However, histologic examination by experienced gynecologic pathologists, expert gynecologic oncologists, the high lymph node counts from all node bearing regions, and the relatively high number of cases

with isolated paraaortic LNM are some strong sides of this study.

In our study, only lymphovascular space invasion was associated with OS. Lymphovascular space invasion (LVSI) has two components. In our cohort, all patients had lymphatic spread so a lymphatic component of LVSI was present in all patients. Four of the seven recurrences were out of the lymphatic region. The vascular component of LVSI is associated with extraabdominal recurrence and upper abdominal recurrence (19). The effect of LVSI on OS may occur because of the vascular component of LVSI.

In conclusion, our stu dy showed that peritoneal spread including adnexal metastasis, uterine serosal involvement, and positive peritoneal cytology rather than tumor histology or uterine factors determine the prognosis in patients with isolated positive paraaortic lymph nodes in EC.

Ethics Committee Approval: *Ethical approval was approved by Etlik Zübeyde Hanım Women's Health Training and Research Hospital Ethical Committee by the number of 18.11.2016-16/3.*

Informed Consent: *Informed consent was signed by the all patients that allows our institution to use their clinical data.*

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References

1. Society AC. American Cancer Society: Cancer Facts and Figures 2012. American Cancer Society Atlanta, GA; 2012.
2. Morrow CP, Bundy BN, Kurman RJ, Creasman WT, Heller P, Homesley HD, et al. Relationship between surgical-pathological risk factors and outcome in clinical stage I and II carcinoma of the endometrium: a Gynecologic Oncology Group study. Gynecol Oncol 1991; 40: 55-65.
3. Pecorelli S. Revised FIGO staging for carcinoma of the vulva, cervix, and endometrium. Int J Gynaecol Obstet 2009; 105: 103-4.
4. Creasman W. Revised FIGO staging for carcinoma of the endometrium. Int J Gynaecol Obstet 2009; 105: 109.
5. Abu-Rustum NR, Gomez JD, Alektiar KM, Soslow RA, Hensley ML, Leitao MM Jr, et al. The incidence of isolated paraaortic nodal metastasis in surgically staged endometrial cancer patients with negative pelvic lymph nodes. Gynecol Oncol 2009; 115: 236-8.
6. Kumar S, Podratz KC, Bakkum-Gamez JN, Dowdy SC, Weaver AL, McGree ME, et al. Prospective assessment of the prevalence of pelvic, paraaortic and high paraaortic lymph node metastasis in endometrial cancer. Gynecol Oncol 2014; 132: 38-43.
7. Chiang AJ, Yu KJ, Chao KC, Teng NN. The incidence of isolated para-aortic nodal metastasis in completely staged endometrial cancer patients. Gynecol Oncol 2011; 121: 122-5.
8. Kang S, Lee J, Lee J, Kim J, Cho C, Kim S, et al. The incidence of isolated paraaortic nodal metastasis in surgically staged endometrial cancer patients: A multi-center retrospective study of 397 patients. Gynecol Oncol 2012; 125: S167.
9. Abu-Rustum NR, Chi DS, Leitao M, Oke EA, Hensley ML, Alektiar KM, et al. What is the incidence of isolated paraaortic nodal recurrence in grade 1 endometrial carcinoma? Gynecol Oncol 2008; 111: 46-8.
10. Mariani A, Dowdy SC, Cliby WA, Gostout BS, Jones MB, Wilson TO, et al. Prospective assessment of lymphatic dissemination in endometrial cancer: a paradigm shift in surgical staging. Gynecol Oncol 2008; 109: 11-8.
11. Gadducci A, Cosio S, Fabrini MG, Guerrieri ME, Greco C, Genazzani AR. Analysis of failures in patients with FIGO stage IIIc1-IIIc2 endometrial cancer. Anticancer Res 2012; 32: 201-5.
12. Turan T, Hizli D, Sarici S, Boran N, Gundogdu B, Karadag B, et al. Is it possible to predict para-aortic lymph node metastasis in endometrial cancer? Eur J Obstet Gynecol Reprod Biol 2011; 158: 274-9.
13. Todo Y, Kato H, Minobe S, Okamoto K, Suzuki Y, Konno Y, et al. A validation study of the new revised FIGO staging system to estimate prognosis for patients with stage IIIC endometrial cancer. Gynecol Oncol 2011; 121: 126-30.
14. Marchetti C, Pisano C, Mangili G, Lorusso D, Panici PB, Silvestro G, et al. Use of adjuvant therapy in patients with FIGO stage III endometrial carcinoma: a multicenter retrospective study. Oncology 2011; 81: 104-12.
15. Hoekstra AV, Kim RJ, Small W Jr, Rademaker AW, Helenowski IB, Singh DK, et al. FIGO stage IIIC endometrial carcinoma: prognostic factors and outcomes. Gynecol Oncol 2009; 114: 273-8.
16. Mariani A, Dowdy SC, Cliby WA, Gostout BS, Jones MB, Wilson TO, et al. Prospective assessment of lymphatic dissemination in endometrial cancer: a paradigm shift in surgical staging. Gynecol Oncol 2008; 109: 11-8.
17. Havrilesky LJ, Cragun JM, Calingaert B, Synan I, Secord AA, Soper JT, et al. Resection of lymph node metastases influences survival in stage IIIC endometrial cancer. Gynecol Oncol 2005; 99: 689-95.
18. Garg G, Morris RT, Solomon L, Toy EP, Kruger M, Clary K, et al. Evaluating the significance of location of lymph node metastasis and extranodal disease in women with stage IIIC endometrial cancer. Gynecol Oncol 2011; 123: 208-13.
19. Visser NCM, Werner HMJ, Krakstad C, Mauland KK, Trovik J, Massuger LFAG, et al. Type of vascular invasion in association with progress of endometrial cancer. APMIS 2017; 125: 1084-91.

Outcome after prenatal diagnosis of fetal urinary tract abnormalities: A tertiary center experience

Ayşegül Özel, Ebru Alıcı Davutoğlu, Hakan Erenel, Mehmet Fatih Karslı, Sevim Özge Korkmaz, Rıza Madazlı

Department of Obstetrics and Gynecology, Perinatology Unit, İstanbul University Cerrahpaşa Faculty of Medicine, İstanbul, Turkey

Abstract

Objective: With the widespread use of ultrasonography for fetal screening, the detection and management of congenital urinary tract abnormalities has become crucial. In this study, we aimed to describe the clinical approaches in patients with prenatally detected urinary tract abnormalities.

Material and Methods: This study is a retrospective, single-center study performed at a perinatology unit of a university hospital, between 2010 and 2016. The outcomes of 124 patients who were prenatally diagnosed as having urinary tract abnormalities are reported. Variables included in the analysis were fetal sex, birth week and weight, persistency, and necessity surgery after birth for renal pelvic dilatation. Low-risk renal pelvic dilatation was determined as an anterior-posterior (AP) diameter of 4-7 mm at 16-28 weeks, 7-10 mm after 28 weeks, whereas high-risk dilatation was defined as AP measurements of ≥7 mm at 16-28 weeks, ≥10 mm after 28 weeks, respectively.

Results: The majority of patients consisted of male fetuses with bilateral pelviectasis (62.9%, 20.2%, respectively). The mean age was 28.8±6.4 years. The mean gestational age at birth was 34.2±7.8 weeks. The mean birth weight was 2593±1253.3 g. The need for surgery was greater in high-risk patients than in low-risk patients (58.3% vs. 8.7%) ($p<0.002$).

Conclusion: Patients with high-risk antenatal renal pelvic dilatation require surgical treatment after delivery. Close prenatal and postnatal follow-up is mandatory in specialized centers. Perinatologists, neonatologists, pediatricians and pediatric nephrologists, and radiologists should treat these children with a multidisciplinary approach. (J Turk Ger Gynecol Assoc 2018; 19: 206-9)

Keywords: Prenatal, fetal, urinary tract, pelviectasis

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Introduction

With a prevalence of 0.1-2.3%, urinary tract abnormalities are the most frequent findings on prenatal ultrasound (US) (1). The detection of these conditions in utero has permitted their early management. Nevertheless, patients are worried about abnormal findings on fetal US. Parents become strongly concerned and interested in prognosis, need of surgery and associated risks for their unborn baby (2,3). Oligohydramnios, bladder outlet obstruction, renal cysts, extra renal pathologies, prematurity, and low birth weight are adverse prognostic factors for postnatal outcome. It is still a matter of debate as to which specific prognostic factors predict termination of pregnancy (4). Prenatal pelvic dilatation has prognostic value and some studies suggested that it was correlated with the postnatal need for surgical treatment (5,6).

Congenital urinary tract abnormalities may develop at the level of the kidney (e.g., dysplasia and hypoplasia), collecting system (e.g., hydronephrosis and megaureter), bladder (e.g., ureterocele and vesicoureteral reflux), or urethra [e.g., posterior urethral valves (PUV)]. There is a continuous advance in the understanding of the genetic basis, pathophysiology, and natural history of these abnormalities (7). Renal pelvic dilatation of the

Address for Correspondence: *Rıza Madazlı*
e.mail: madazli@superonline.com ORCID ID: orcid.org/0000-0002-6400-1964

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fetus may be a cue for urinary tract abnormalities, ranging from obstruction to vesicoureteric reflux. However, pelviectasis may also be a marker for aneuploidy with increased incidence in fetuses with trisomy 21 (8,9); isolated urinary tract abnormalities have a low association with karyotypic abnormalities. Severe renal pelvic dilatation is associated with postnatal pathology and often requires surgical treatment in childhood (10).

In this retrospective study, we investigated the clinical course of prenatally diagnosed fetuses with urinary tract abnormalities and the relationship between fetal pyelectasis and need for postnatal treatment.

Material and Methods

The records of 124 patients with prenatally detected congenital urinary tract abnormalities who were examined at the perinatology unit of a university hospital in İstanbul between 2010 and 2016 were reviewed, retrospectively. Ethics Committee approval was received. Each fetus underwent a detailed sonographic examination for detecting fetal organ abnormalities. The presence of renal, ureteral, and bladder abnormalities and volume of amniotic fluid, date (weeks of pregnancy) of first diagnosis, fetal sex, prenatal invasive genetic tests, and birth week and weight were recorded. Registers of birth of 16 patients could not be accessed. Fetuses with renal pelvic dilatation were also evaluated. The fetuses were divided into two groups as low-risk and high-risk. Low-risk renal pelvic dilatation was defined as an anterior-posterior (AP) diameter (in the transverse plane) of 4-7 mm at 16-28 weeks, and 7-10 mm after 28 weeks, whereas high-risk dilatation was defined as AP measurements of ≥7 mm at 16-28 weeks, and ≥10 mm after 28 weeks (11). If renal pelviectasis were found bilaterally, the largest diameter dilatation was used to classify the patient. The presence of unilateral or bilateral dilatation was also recorded. We learned about postnatal persistency and need for surgery through telephone interviews with their parents.

Statistical analysis

We used SPSS® software, version 20.0 (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp) to analyze the collected data. Data are summarized as mean ± standard deviation or numeric (%) as appropriate.

Results

Retrospective data of 124 patients were analyzed. The clinical characteristics of the patients are summarized in Table 1. There were higher proportions of males and patients with bilateral pelviectasis (62.9%, 20.2%, respectively). The mean age of the mothers was 28.8±6.4 years. The mean gestational age at birth was 34.2±7.8 weeks. The mean birth weight was 2593±1253.3 g.

There were 4 (3.2%) neonatal exitus; 2 of which had bilateral renal agenesia, one neonate had infantile polycystic kidney, and one neonate had PUV. There were 6 (4.8%) spontaneous abortus and intrauterine demise; 2 of which had infantile polycystic kidney, two had megacystisis, one had unilateral dysplastic kidney, and one had PUV. There were 16 (12.9%) terminations of pregnancy; 6 fetuses had megacystisis, 4 had infantile polycystic kidney, two had PUV (one of which had

Table 1. The main characteristics of the patients

		n, %
Fetal sex		
	Male	78 (62.9)
	Female	33 (26.6)
	Unspecified	13 (10.5)
Amniotic fluid		
	Oligohydramnios	27 (21.8)
	Normal	97 (78.2)
Prenatal diagnosis		
	Bilateral pelviectasis	25 (20.2)
	Unilateral pelviectasis	15 (12.1)
	Multicystic dysplastic	15 (12.1)
	Ureteropelvic junction obstruction	12 (9.7)
	Megacystisis	11 (8.9)
	Infantile polycystic kidneys	9 (7.3)
	Duplicated collecting system	8 (6.5)
	Posterior ureteral valves	7 (5.6)
	Bladder extrophy	5 (4.0)
	Hyperechoic kidneys	2 (1.6)
	Renal cortical cyst	2 (1.6)
	Pelvic kidney	2 (1.6)
	Unilateral renal agenesia	2 (1.6)
	Bilateral renal agenesia	2 (1.6)
	Unilateral dysplastic kidney	2 (1.6)
	Horseshoe kidney	1 (0.8)
	Others	3 (2.4)
		Mean ± standard deviation
Age	28.8±6.4	
Birth week		34.2±7.8
Birth weight (g)		2593±1253.3
Age at diagnosis (week)		24.0±6.6

trisomy 18), one had renal agenesia, one had bladder extrophy, one was multicystic dysplastic, and one had hyperechoic kidney. The birth records of the 16 patients could not be accessed.

Of the 41 patients with renal pelvic dilatation, 25 (60.9%) were low-risk, and 16 (39.1%) were high-risk. Of the 35 cases for which follow-up data were available, postnatal persistency and requirement for surgery within the first year of life were evaluated (Table 2). Renal pelvic dilatation was persistent in 15 (65.2%) patients who were defined as low-risk. Renal pelvic dilatation was persistent in all patients who were defined as high-risk. The need for surgery was significantly greater in high-risk patients than in low-risk patients (58.3% vs. 8.7%) ($p<0.002$).

Discussion

Congenital abnormalities of the kidney and urinary tract account for 30-50% of all fetal anomalies. They occur with a prevalence of 1 in 70-1000 live births as the most common prenatal diagnoses. In addition, these abnormalities are the most common reason for chronic kidney disease in childhood (12). There is a wide spectrum of fetal anomalies, ranging from mild unilateral pelvic dilatation to severe bilateral renal and urinary tract malformations (13). Postnatal management of infants with a history of antenatal pelviectasis remains controversial, especially with regards to fetal intervention, diagnostic criteria, postnatal recommendations, and therapeutic management (14). The parents of fetuses with prenatal pelviectasis may be more concerned that their children will need advanced investigation and treatment after delivery, and how their renal function will be in the future. They are less interested in the most accurate diagnosis, but they are interested in the prognosis.

Unilateral pelviectasis typically requires no specific interventions during the prenatal period beyond close serial imaging. Bilateral pelviectasis, on the other hand, can be present in the context of clinically significant urinary tract obstruction such as PUV or urethral atresia, as well as in non-obstructing entities such as prune-belly syndrome or high-grade vesicoureteral reflux (2).

There are variable identification and classification schemes for the definition of hydronephrosis. An AP pelvic diameter of ≥4 mm at the first trimester is the most commonly used cut-off to indicate pelviectasis (15-17). Ouzounian et al. (18) showed that fetal pelvic dilatation of 8 mm provided the best combination of sensitivity and specificity, at 91% and 72%, respectively. A study showed that a third trimester AP pelvis diameter of ≥7 mm was the strongest ultrasound (US) criterion to predict postnatal kidney pathologies (19). In order to predict prognosis in this study, we used a grading system that was defined at a consensus meeting (11). Low-risk renal pelvic dilatation was defined as an AP diameter of 4-7 mm at 16-28 weeks, and 7-10 mm after 28 weeks, whereas high-risk dilatation was defined as AP measurements of ≥7 mm at 16-28 weeks, and ≥10 mm after 28 weeks. The postnatal surgery rate was 58.3% in fetuses with high-risk renal pelvic dilatation. All fetuses with high-risk were persistent in the postnatal period, but this was 65.2% among fetuses with low risk.

There are two previous reports examining the incidence of postnatal surgery associated with antenatal pelviectasis, and both showed similar results (5,15). Sairam et al. (15) and Wollenberg et al. (5), demonstrated that 34% and 36%, respectively, of the fetuses with AP diameters ≥10 mm on US examination needed surgery. Our results were similar to the first study by Grignon et al. (20), but they reported a higher rate of surgical treatment (60%) in fetuses with AP diameter ≥10 mm. Differences in criteria used to indicate surgery probably account for the differences in surgical treatment rates. John et al. (21) showed that fetuses with AP diameters ≥19 mm after 33 weeks' gestation had a significant risk of postnatal surgery. They also reported a spontaneous recovery rate of 25% at three months after birth, including children with fetal hydronephrosis defined as AP diameter ≥4 mm until 33 weeks' gestation and AP diameter ≥7 mm thereafter.

Table 2. Postnatal persistency and surgery rates in the low and highrisk groups

	Low-risk n, %	High-risk n, %	p
Postnatal persistency			
Yes	15 (65.2)	12 (100)	0.02
No	8 (34.8)	0 (0)	
Postnatal need for surgery			
Yes	2 (8.7)	2 (8.7)	7 (58.3)
No	21 (91.3)	21 (91.3)	5 (41.7)

Study limitations

There are potential limitations associated with the retrospective design in our study. In addition, the small number of cases is another limitation. Therefore, we could not use multivariate analysis to describe possible prognostic factors.

Our investigation showed that two-thirds (58%) of patients with high-risk antenatal renal pelvic dilatation required surgical treatment after delivery. Close prenatal and postnatal follow-up is mandatory in specialized centers. Perinatologists, neonatologists, pediatricians and pediatric nephrologists, and radiologists should have a multidisciplinary approach for these children. The ability to effectively determine fetuses with high-risk pelviectasis in the antenatal period would provide

correct postnatal management. It is important for minimizing unnecessary parental anxiety and postnatal renal damage.

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Ethics Committee Approval: *This study was retrospective.*

Informed Consent: *It was taken.*

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References

1. Gunn TR, Mora JD, Pease P. Antenatal diagnosis of urinary tract abnormalities by ultrasonography after 28 weeks' gestation: incidence and outcome. Am J Obstet Gynecol 1995; 172: 479-86.
2. Carr MC, Kim SS. Prenatal management of urogenital disorders. Urol Clin North Am 2010; 37: 149-58.
3. Kleinveld JH, Timmermans DR, de Smit DJ, Ader HJ, Van der WG, Ten Kate LP. Does prenatal screening influence anxiety levels of pregnant women? A longitudinal randomised controlled trial. Prenat Diagn 2006; 26: 354-61.
4. Hogan J, Dourthe ME, Blondiaux E, Jouannic JM, Garel C, Ulinski T. Renal outcome in children with antenatal diagnosis of severe CAKUT. Pediatr Nephrol 2012; 27: 497-502.
5. Wollenberg A, Neuhaus TJ, Willi UV, Wisser J. Outcome of fetal renal pelvic dilatation diagnosed during the third trimester. Ultrasound Obstet Gynecol 2005; 25: 483-8.
6. Sanna-Cherchi S, Ravani P, Corbani V, Parodi S, Haupt R, Piaggio G, et al. Renal outcome in patients with congenital anomalies of the kidney and urinary tract. Kidney Int 2009; 76: 528-33.
7. Quirino IG, Diniz JS, Bouzada MC, Pereira AK, Lopes TJ, Paixão GM, et al. Clinical Course of 822 Children with Prenatally Detected Nephrouropathies Clin J Am Soc Nephrol 2012; 7: 444-51.
8. Dagklis T, Plasencia W, Maiz N, Duarte L, Nicolaides KH. Choroid plexus cyst, intracardiac echogenic focus, hyperechogenic bowel and hydronephrosis in screening for trisomy 21 at 11 + 0 to 13 + 6 weeks. Ultrasound Obstet Gynecol 2008; 3: 132-5.
9. Staebler M, Donner C, Van Regemorter N, Duprez L, De Maertelaer V, Devreker F, et al. Should determination of the karyotype be systematic for all malformations detected by obstetrical ultrasound? Prenat Diagn 2005; 25: 567-73.
10. Kumar S, Walia S, Ikpeme O, Zhang E, Paramasivam G, Agarwal S, et al. Postnatal outcome of prenatally diagnosed severe fetal renal pelvic dilatation. Prenat Diagn 2012; 32: 519-22.
11. Nguyen HT, Benson CB, Bromley B, Campbell JB, Chow J, Coleman B, et al. Multidisciplinary consensus on the classification of prenatal and postnatal urinary tract dilation (UTD classification system). J Pediatr Urol 2014; 10: 982-98.
12. Nef S, Neuhaus TJ, Spartà G, Weitz M, Buder K, Wisser J, et al. Outcome after prenatal diagnosis of congenital anomalies of the kidney and urinary tract. Eur J Pediatr 2016; 175: 667-76.
13. Soares CM, Diniz JS, Lima EM, Silva JM, Oliveira GR, Canhestro MR, et al. Clinical outcome of children with chronic kidney disease in a pre-dialysis interdisciplinary program. Pediatr Nephrol 2008; 23: 2039-46.
14. Yamaçake KG, Nguyen HT. Current management of antenatal hydronephrosis. Pediatr Nephrol 2013; 28: 237-43.
15. Sairam S, Al-Habib A, Sasson S, Thilaganathan B. Natural history of fetal hydronephrosis diagnosed on mid-trimester ultrasound. Ultrasound Obstet Gynecol 2001; 17: 191-6.
16. Adra AM, Mejides AA, Dennaoui MS, Beydoun SN. Fetal pyelectasis: is it always physiologic? Am J Obstet Gynecol 1995; 173: 1263-6.
17. Kitagawa H, Pringle KC, Stone P, Flower J, Murakami N, Robinson R. Postnatal follow-up of hydronephrosis detected by prenatal ultrasound: the natural history. Fetal Diagn Ther 1998; 13: 19-25.
18. Ouzounian JG, Castro MA, Fresquez M, al-Sulyman OM, Kovacs BW. Prognostic significance of antenattally detected fetal pyelectasis. Ultrasound Obstet Gynecol 1996; 7: 424-8.
19. Ismaili K, Hall M, Donner C, Thomas D, Vermeylen D, Avni FE, et al. Results of systematic screening for minor degrees of fetal renal pelvis dilatation in an unselected population. Am J Obstet Gynecol 2003; 188: 242-6.
20. Grignon A, Filion R, Filiatrault D, Robitaille P, Homsy Y, Boutin H, et al. Urinary tract dilatation in utero: classification and clinical applications. Radiology 1986; 160: 645-7.
21. John U, Kahler C, Schultz S, Mentzel HJ, Vogt S, Misselwitz J. The impact of fetal renal pelvic diameter on postnatal outcome. Prenat Diagn 2004; 24: 591-5.

The value of autopsy to determine the cause of maternal deaths in Turkey

Hüseyin Levent Keskin, Yaprak Engin Üstün, Sema Sanisoğlu, Selma Karaahmetoğlu, Ayşe Özcan, Şevki Çelen, Saniye Tontuş, Yusuf Üstün, Veli Ongun, İrfan Şencan

General Directorate of Mother and Child Health and Family Planning, Ministry of Health of Turkey, Ankara, Turkey

Abstract

Objective: To analyze the value of autopsy reports for determining the cause of maternal deaths in Turkey.

Material and Methods: In this descriptive retrospective study, the case files of 992 maternal deaths, except for accidental causes, that occurred in Turkey between 2012 and 2016 were reviewed. An autopsy examination was performed in 177 (17.8%) of the cohort. When the files were reviewed, maternal descriptive data and the cause of maternal mortality according to the autopsy reports were recorded.

Results: The mean age at death was 31.5±6.6 years. No exact cause of maternal death was identified after autopsy in 44 (24.9%) of the 177 cases. An exact cause of death could be determined in 133 (75.1%); 34.5% (n=61) were due to direct causes, and 40.7% (n=72) were due to indirect causes. The leading direct causes of maternal deaths were obstetric hemorrhage (13.0%) and obstetric (pulmonary and amniotic fluid) embolism (12.4%). The main cause among the indirect causes was ruptured aortic aneurysm and/or dissection of aorta (8.5%). Among the subjects with no clinical diagnosis based on the clinical course before death (n=96), the exact cause of death could not be determined at autopsy in 19 (19.8%) cases. The exact or possible cause of death was identified at autopsy in 80.3% (n=77) cases with no clinical diagnosis. Among the cases who had antemortem diagnoses based on the clinical course (n=81), the final diagnosis at autopsy was compatible with the clinical diagnosis in 48 (59.3%) subjects.

Conclusion: Maternal autopsy examination provides an exact cause of death in most cases and is still a valuable tool for understanding the cause of maternal mortality. (J Turk Ger Gynecol Assoc 2018; 19: 210-4)

Keywords: Maternal mortality, autopsy, maternal death

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Introduction

Maternal mortality is an important public health problem with socioeconomic and clinical components.

The annual number of maternal deaths decreased by 43% from approximately 532,000 in 1990 to an estimated 303,000 in 2015 (1). By 2030, every country should reduce its maternal mortality ratio (MMR) by at least two thirds from the 2010 baseline, and no country should have an MMR higher than 140 deaths per 100,000 live births (2). The MMR of Turkey between 2007 and 2009 was 19.7 per 100,000 live births (3).

The major complications that account for nearly 75% of all maternal deaths are severe hemorrhage, infections, hypertensive disorders of pregnancy, complications from delivery, and unsafe abortion. However, many maternal deaths are still not identified (2). Accurate determination of causes of maternal deaths is critical for effective prevention. Autopsy remains the gold standard evaluation for maternal deaths.

Our aim was to evaluate maternal death autopsies in a five-year period in Turkey.

Material and Methods

In this descriptive retrospective study, the case files of all pregnancy-associated deaths recorded in Turkey between 2012 and 2016 were reviewed. Maternal deaths with autopsy results

Address for Correspondence: *Hüseyin Levent Keskin*
e.mail: hlkeskin@yahoo.com ORCID: orcid.org/0000-0002-2268-3821

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were included. Exclusion criteria were late maternal deaths and death by suicide.

The Turkish Statistical Institute (TURKSTAT) has been collecting data on the number of deaths and causes of death using the vital registration (VR) system since 2009 in details of ICD-10 codes, and underlying cause is the main concern as the World Health Organization suggests. The VR system of TURKSTAT collects data through forms that include a check box to mark whether the death was a maternal death. Maternal deaths are also discussed by the Ministry of Health. The Maternal Mortality Review Committee was formed by the Ministry of Health in 2007. All maternal deaths in Turkey must be reported to the Committee at the Ministry of Health. Identifying the cause and preventability of maternal mortality includes medical hospital records, death certificates, autopsy reports, local and national registries, and verbal autopsy. Verbal autopsy was performed routinely for every death.

The definition of maternal death was based on that used in the ICD-MM. The causes of maternal mortality are grouped into direct obstetric and indirect causes. According to the classification, maternal deaths are: Direct obstetric deaths resulting from natural obstetric complications of pregnancy, labor and puerperium or from obstetric interventions. Indirect obstetric deaths resulting from previously existing diseases or diseases that developed during pregnancy but not due to obstetric causes and worsened by pregnancy. The MMR was calculated as the number of maternal deaths to the number of births in the past one year. Clinical and pathologic autopsy results were evaluated. External examination, in situ examination, gross and microscopic examinations were performed in each case.

Maternal age, gravida, place of death (home, hospital) were recorded. The distribution of sociodemographic and clinical parameters was summarized using descriptive statistics, such as frequencies and rates, across the pregnancy continuum.

Results

From 2012 to 2016, a total of 992 maternal deaths were recorded in Turkey. The MMR during the 5-year study period was 15.1/100,000 live births. Of these women, 177 (17.8%) underwent an autopsy.

Mean age was 31.5±6.6 (range, 16-48) years. The median gravida was 3 (range, 1-12), and the median parity was 2 (range, 1-11). In 41 cases (23.2%), the index pregnancy was the first pregnancy and 26.6% (n=47) of deaths occurred in nulliparous women.

Death occurred while the pregnancy was ongoing in 64 (36.2%) cases, and after the pregnancy had ended in 86 (48.6%) cases. Twelve pregnant women were in the 1st trimester, 24 in the 2nd, and 28 women were in the 3rd trimester when death occurred. In 6 cases, death occurred after the pregnancy had ended in the 1st or 2nd trimester (spontaneous miscarriage or medical/legal termination). The remaining 86 (48.6%) women died after giving birth (29 through vaginal route, 57 via cesarean section). In 21 (11.9%) cases, the pregnancy was ended after performing perimortem cesarean section.

Twenty-six (14.7%) of the deaths happened at home, and 20 (11.3%) were admitted to the hospital as already exitus. Most of the deaths (n=131, 74%) were pronounced at a hospital. Sixty women were admitted under cardiopulmonary resuscitation after arrest or with general condition disturbance. In 71 cases, death occurred when they were in the hospital for giving birth or under the treatment for any disorders during the pregnancy or in the postpartum period.

The cause of maternal death was undetermined in 44 cases (24.9%) at the end of autopsy (Table 1).

Although in 44 (24.9%) out of 177 cases the exact cause of maternal death was undetermined at the end of autopsy, the exact cause of death could be detected in 133 (75.1%) (Table 1); 34.5% (n=61) were due to direct causes, and 40.7% (n=72) were due to indirect causes. The leading causes of the direct maternal death were obstetric hemorrhage (13.0%) and obstetric (pulmonary and amniotic fluid) embolism (12.4%). The main cause among the indirect causes was ruptured aortic aneurysm and/or dissection of aorta (8.5%) (Table 1).

Among the subjects who had no clinical diagnosis based on the antemortem clinical course before death (n=96), the exact cause of death could not be determined after autopsy in 19 (19.8%) cases. However, the exact or possible cause of death was identified in 80.3% (n=77) of cases (Table 2). The most common cause of death in those cases were ruptured aortic aneurysm and/or dissection of aorta (n=15) and pulmonary embolism (n=14) (Table 2).

Among the women who had an antemortem diagnosis based on the clinical course (n=81), the final diagnosis was compatible with the clinical course in 48 (59.3%) cases, and the autopsy diagnosis was incompatible with the clinical diagnosis in 8 (9.9%) cases. However, in 25 (30.9%) out of 81 cases with an antemortem diagnosis based on clinical findings, the exact cause of death could not be determined at autopsy.

In 23 (13%) cases, although the exact cause of the death was clearly defined, judicial autopsy was performed because of medicolegal issues.

Of the 7 cases diagnosed as amniotic fluid embolism (AFE) after autopsy, five were diagnosed as embolism with clinical findings before death occurred; however, no specific clinical diagnosis was considered as a differential diagnosis during the antemortem period in two cases. AFE was considered according to the antemortem clinical course in 8 cases -out of 44 with no definite cause of death were determined at autopsy-

although the exact cause of death could not be identified at autopsy.

In addition, in 7 out of 44 cases with no exact cause of death determined at autopsy, epilepsy featured in their medical history and the cause of maternal mortality was accepted as sudden, unexpected death in epilepsy.

Discussion

Every year approximately 300,000 women die because of the complications of giving birth (4). Determination of the etiologies of maternal mortality should be a priority to achieve a significant reduction in maternal mortality. A reliable ascertainment of the

Table 1. Causes of maternal deaths based on autopsy results (n=177)

Cause of death				n (%)
Direct maternal death (n=61)	Obstetric embolism	Pulmonary thromboembolism		15 (8.5%)
		Amniotic fluid embolism		7 (4.0%)
	Complications of hypertensive disorders	Intracranial hemorrhage		2 (1.1%)
		Unspecified		3 (1.7%)
	Obstetric hemorrhage	Uterine rupture		5 (2.8%)
		Others (atonic bleeding, pl. previa, abruptio placenta, pl. adhesion anomaly, genital laceration)		18 (10.2%)
	Ectopic pregnancy, ruptured			1 (0.6%)
	Complication of cesarean section	Injury of arteria epigastrica inferior		1 (0.6%)
		Injury of infundibulopelvic ligament		1 (0.6%)
		Injury of uterine artery		1 (0.6%)
		Intrabadominal abcess/peritonitis		1 (0.6%)
	Chorioamnonitis			3 (1.7%)
	Pelvic thrombophlebitis			1 (0.6%)
	Cerebrovenous sinus thrombosis			1 (0.6%)
	Intrauterine death followed by hemorrhage			1 (0.6%)
Indirect maternal death (n=72) (non-obstetric disorders complicating pregnancy, childbirth and the puerperium)	Diseases of the circulatory system	Ruptured aortic aneurysm and/or dissection of aorta		15 (8.5%)
		Acute myocard infarction/acute coronary syndrome		5 (2.8%)
		Coronary artery rupture		1 (0.6%)
		Hypertrophic cardiomyopathy		3 (1.7%)
		Myocarditis/endocarditis		3 (1.7%)
		Congenital anomaly	Bicuspit aorta, fibrosis	1 (0.6%)
			Intramyocardial coronary artery	1 (0.6%)
		Intraabdominal hemorhage	Splenic vessel rupture	5 (2.8%)
			Renal artery rupture	1 (0.6%)
			Unspecified origin	3 (1.7%)
		Intracranial hemorrhage		5 (2.8%)
	Epilepsy			3 (1.7%)
	Infections	Pyelonephritis		1 (0.6%)
		Pneumonia		8 (4.5%)
		Sepsis, unspecified origin		2 (1.1%)
	Diseases of the digestive system	Appendicitis, perforated		1 (0.6%)
		Toxic megacolon, sepsis		1 (0.6%)
		Gastrointestinal tract hemorrhage caused to aspiration of blood		1 (0.6%)
	Liver malignant metastasis (primary unknown)			1 (0.6%)
	Anaphylaxis due to diclofenac sodium			1 (0.6%)
	Cardiac and/or lung disease, unspecified			8 (4.5%)
Undetermined at autopsy				**44 (24.9%)**
Total				**177 (100%)**

causes of maternal mortality requires an autopsy (5). Its value was revealed in the study of Sonderegger-Iseli et al. (6) with clinical discrepancies in up to 30% of cases.

In our study, maternal autopsy improved the understanding of the cause of deaths in nearly half of the cases.

Castillo et al. (7) found that the minimally invasive autopsy method could be an important implementation to decide the etiologies of maternal death, especially for indirect maternal mortality causes, most of which are infectious diseases. Minimally invasive autopsy, which is made up of the evaluation of samples of basic organs and fluids in terms of histology and microbiology, could improve the value of the currently used procedures including verbal autopsies and clinical records, which have been revealed to have a high level of imprecision. Hasegawa et al. (8) reported that in most cases autopsy provided an exact cause of death, the necessity of autopsies should be more widely accepted, and autopsies should be performed more frequently in Japan.

Kavatkar et al. (9) showed that certain final pathogenetic mechanisms such as disseminated intravascular coagulation, acute renal failure, shock, congestive cardiac failure and hepatic encephalopathy led to maternal death. In the present study, the most frequent cause of mortality found at autopsy were aortic aneurysm rupture and pulmonary embolism.

Autopsies of maternal death have greater importance than other deaths because these reports are used to make recommendations for ameliorating clinical obstetric practice and defining the cause of death.

In conclusion, maternal autopsy examination provides an exact cause of death in most cases and is still a valuable tool for understanding the cause of maternal mortality.

Table 2. The causes of maternal deaths identified by maternal autopsy without any clinical course information or suspicion before death (n=96)

Cause of death				**n (%)**
Direct maternal death (n=24)	Obstetric embolism	Pulmonary thromboembolism		14 (14.6%)
		Amniotic fluid embolism		2 (2.1%)
	Uterine rupture			4 (4.2%)
	Ectopic pregnancy, ruptured			1 (1.0%)
	Complication of cesarean section	Injury of arteria epigastrica inferior		1 (1.0%)
		Intrabadominal abcess/peritonitis		1 (1.0%)
	Pelvic thrombophlebitis			1 (1.0%)
Indirect maternal death (n=53) (non-obstetric disorders complicating pregnancy, childbirth and the puerperium)	Diseases of the circulatory system	Ruptured aortic aneurysm and/or dissection of aorta		15 (15.6%)
		Acute myocard infarction/acute coronary syndrome		5 (5.2%)
		Coronary artery rupture		1 (1.0%)
		Hypertrophic cardiomyopathy		3 (3.1%)
		Myocarditis		2 (2.1%)
		Congenital anomaly	Bicuspit aorta, fibrosis	1 (1.0%)
			Intramyocardial coronary artery	1 (1.0%)
		Intraabdominal hemorhagea	Splenic vessel rupture	4 (4.2%)
			Renal artery rupture	1 (1.0%)
			Unknown origin	2 (2.1%)
		Intracranial hemorrhage		4 (4.2%)
	Epilepsy			2 (2.1%)
	Pneumonia			6 (6.3%)
	Diseases of the digestive system	Appendicitis, perforated		1 (1.0%)
		Gastrointestinal tract hemorrhage caused to aspiration of blood		1 (1.0%)
	Liver malignant metastasis (primary unknown)			1 (1.0%)
	Cardiac and/or lung disease, unspecified			3 (3.1%)
Undetermined at autopsy				19 (19.8%)
Total				**96 (100%)**

Ethics Committee Approval: *Retrospective study.*

Informed Consent: *Not applicable.*

Peer-review: *Externally peer-reviewed.*

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References

1. Trends in maternal mortality: 1990 to 2015: estimates by WHO, UNICEF, UNFPA, World Bank Group and the United Nations Population Division.
2. Strategies towards ending preventable maternal mortality (EPMM). Geneva: World Health Organization; 2015 (http://www.everywomaneverychild.org/images/EPMM_final_report_2015.pdf, accessed 5 November 2015).
3. Engin-Üstün Y, Çelen Ş, Özcan A, Sanisoğlu S, Karaahmetoğlu S, Gül R, et al. Maternal mortality from cardiac disease in Turkey: a population-based study. J Matern Fetal Neonatal Med 2012; 25: 2451-3.
4. You D, Hug L, Ejdemyr S, Idele P, Hogan D, Mathers C, et al. United Nations Inter-agency Group for Child Mortality Estimation (UN IGME) Global, regional, and national levels and trends in under-5 mortality between 1990 and 2015, with scenario-based projections to 2030: a systematic analysis by the UN Inter-agency Group for Child Mortality, 2015.
5. Fligner CL, Murray J, Roberts DJ. Synergism of verbal autopsy and diagnostic pathology autopsy for improved accuracy of mortality data. Popul Health Metr 2011; 9: 25.
6. Sonderegger-Iseli K, Burger S, Muntwyler J, Salomon F. Diagnostic errors in three medical eras: a necropsy study. Lancet 2000; 355: 2027-31.
7. Castillo P, Hurtado JC, Martínez MJ, Jordao D, Lovane L, Ismail MR, et al. Validity of a minimally invasive autopsy for cause of death determination in maternal deaths in Mozambique: An observational study. PLoS Med 2017; 14: e1002431.
8. Hasegawa J, Wakasa T, Matsumoto H, Takeuchi M, Kanayama N, Tanaka H, et al. Analysis of maternal death autopsies from the nationwide registration system of maternal deaths in Japan. J Matern Fetal Neonatal Med 2018; 31: 333-8.
9. Kavatkar AN, Sahasrabudhe NS, Jadhav MV, Deshmukh SD. Autopsy study of maternal deaths. Int J Gynaecol Obstet 2003; 81: 1-8.

The assessment of thyroid autoantibody levels in euthyroid patients with polycystic ovary syndrome

Sema Hepşen[1], Melia Karaköse[2], Erman Çakal[1], Sanem Öztekin[3], İlknur Ünsal[1], Pınar Akhanlı[1], Bekir Uçan[1], Mustafa Özbek[1]

[1]Clinic of Endocrinology and Metabolism, University of Health Sciences, Dışkapı Yıldırım Beyazıt Training and Research Hospital, Ankara, Turkey

[2]Clinic of Endocrinology and Metabolism, Sivas Numune Hospital, Sivas, Turkey

[3]Clinic of Internal Medicine, University of Health Sciences, Dışkapı Yıldırım Beyazıt Training and Research Hospital, Ankara, Turkey

Abstract

Objective: Thyroid hormone abnormalities are commonly seen in polycystic ovary syndrome (PCOS) and have considerable effects on comorbidities. The association with PCOS and thyroid autoimmunity which lead to thyroid pathologies are not revealed clearly. We targeted to commentate anti-thyroid peroxidase (anti-TPO), anti-thyroglobulin (anti-TG) antibody levels and thyroid autoimmunity in PCOS.

Material and Methods: One hundred eighty four patients who got the diagnosis of PCOS regard to the revised 2003 Rotterdam criteria were embodied in this study. One hundred six age-matched female volunteers were included in the control group. Characteristics, biochemical parameters, thyroid hormone and autoantibody levels of groups were investigated.

Results: Although; we did not find out a statistically significant difference in TSH and sT4 levels between two groups ($p>0.05$), anti-TPO and anti-TG antibody levels were determined higher in PCOS group significantly ($p<0.001$). Anti-TPO Ab and anti-TG Ab positivity prevalence of PCOS patients were significantly higher as against to controls ($p<0.001$; $p=0.01$).

Conclusion: Not only thyroid hormone levels but also thyroid autoantibody levels should be screened during the investigation of PCOS and the patients with positive results need to be followed up carefully in the long run. (J Turk Ger Gynecol Assoc 2018; 19: 215-9)

Keywords: Polycystic ovary syndrome, autoimmunity, anti-thyroid peroxidase antibody, anti-thyroglobulin antibody

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Introduction

Polycystic ovary syndrome (PCOS) is a prevalent endocrinologic disorder affect women at the fertility period (1). PCOS is identified with menstrual irregularity, hyperandrogenism, and infertility (2). Obesity, metabolic syndrome, dyslipidemia, insuline resistance, type 2 diabetes mellitus, and cardiovascular disorders are the most common comorbidities related to this syndrome (3-6).

Autoimmune thyroid disease prevalence in women is 4% and rises up to 15% in the event of existing thyroid autoantibody positivity (7). Thyroid hormone dysfunctions and thyroid autoimmunity cause abnormalities on sex hormone metabolism, menstrual irregularities and consequently infertility (8,9).

Anti-thyroid peroxidase antibodies (anti-TPO Ab) and anti-thyroglobulin antibodies (anti-TG Ab) are fundamental markers of thyroid autoimmunity. A study by Poppe et al. (8) demonstrated that thyroid auto antibodies are significantly

Address for Correspondence: *Sema Hepşen*
e.mail: semahepsen@gmail.com ORCID ID: orcid.org/0000-0002-8375-7409

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higher in infertile patients. Close follow up of thyroid hormones are considered important in patients with PCOS because of being the most common reason of medically treatable infertility (10). The latest studies revealed that autoimmune thyroid diseases have an increased prevalence in PCOS patients (11,12). From this point of view, not only thyroid hormones are substantial for PCOS follow-up but also thyroid antibodies can be guiding for probable thyroid diseases.

The target of this study is to assess thyroid autoantibodies and thyroid hormone levels in PCOS patients by taking into consideration with present or probable thyroid hormone dysfunctions can affect patient's clinical conditions and fertility substantially.

Material and Methods

Our study includes 184 patients who got PCOS diagnose in regard to the revised 2003 Rotterdam criteria at the Endocrinology and Metabolism and Internal Medicine departments of our institution between January 2014-April 2015 (13). The definition criteria include at least two of the three following subheadings after exclusion of related disorders; oligo or anovulation, clinical and/or biochemical signs of hyperandrogenism and ultrasonographic demonstration of polycystic ovary appearance (13).

One hundred six age-matched healthy female volunteers who menstruate regularly were included in the study as the control group. Individuals who have the diagnosis as hyperprolactinemia, congenital adrenal hyperplasia, androgen-secreting tumours, Cushing syndrome, hypertension, hepatic or renal insufficiency, diabetes mellitus and concurrent thyroid dysfunction were excluded from the study. Being in pregnancy or breastfeeding period and using drugs which affect glucose tolerance and lipid levels were other exclusion criterias. The age range was between 18-41 for all participants. The study protocol was granted by the Ethics Department and each individual signed a written informed consent form. Clinical and anthropometric data including body mass index (BMI) and waist/hip ratio were ascertained for each participant.

Biochemical parameters of all individuals were studied after 12 hours fasting at 2^{th}-5^{th} days of the follicular phase. Chemiluminescent immunoassay method was used to assess fasting blood glucose levels (Advia Centaur XP, Siemens Healthcare Diagnostic Inc., Tarrytown USA). The serum insulin levels were studied by electrochemiluminescent immunoassay method (Elecsys 2010, Cobas, Roche Diagnostic, Mannheim, Germany). Insulin resistance was qualified by the homeostasis model assessment formula (14).

Thyroid stimulating hormone (TSH) and free T4 (fT4) levels were quantified via chemiluminescent microparticle immunoassay (Abbott, Architect i2000, Abbott Laboratories Diagnosis Division, IL, USA). Chemiluminescent competitive immunoassay (Advia Centaur XP, Siemens, Tarrytown, USA) was used for the measurement of anti-thyroglobulin antibody (anti-TG Ab) and anti-TPO Ab levels. Reference range was as follows for each: TSH: 0.35-4.94 μIU/mL, fT4: 0.7-1.48 ng/dL, anti-TG: 0-60 U/mL, anti-TPO: 0-57 U/mL. Levels above the upper limits of anti-TPO Ab and anti-TG Ab were considered as positive.

Carotid intima-media thickness (CIMT) was estimated by the noninvasive high-resolution ultrasound of the common carotid arteries (Hitachi, Japan; EUB 7000) with 13 MHz linear probe. The carotid intima-media thickness was defined as the distance between the blood-intima and media-adventitia boundaries and the mean value of consecutive three measurements was taken baseline for CIMT. Measurements were carried out from the localization of 1-centimeter distance after the internal carotid arterial bifurcation, where the hemodinamia had been affected minimum, on B-mode imaging. The same researcher performed all measurements.

Statistical analysis

The statistical analysis was carried out with the SPSS statistical software (version 18; SPSS, Chicago, IL, USA). Kolmogorov-Smirnov analysis was done to access normality of the variables. Sample t-tests and Mann-Whitney U test was used for the comparison of two group's distributed variables. Continuous variables were tested by Pearson correlation coefficient and Spearman's rho correlation coefficient test was done to assess the non-normally distributed variables. P values of <0.05 were determined statistically significant.

Results

One hundred eighty four patients with PCOS and 106 controls were recruited in the study. Mean age was 23.9±5.6 for PCOS group and 24.3±4.3 for controls, ($p>0.05$). BMI, waist-hip ratio, fasting blood glucose, fasting insulin, HOMA-IR, triglyceride (TG), low-density lipoprotein cholesterol (LDL-C) and CIMT were higher in PCOS patients ($p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.01$ respectively). We didn't find a significant difference in total cholesterol (TC) levels between two groups ($p>0.05$), high-density lipoprotein cholesterol (HDL-C) was found lower in PCOS group significantly ($p<0.001$). Principal data of two groups were represented in Table 1.

We did not determine a statically significant difference in TSH and fT4 between the groups ($p>0.05$). We defined that anti-TPO Ab and anti-TG Ab levels were higher in PCOS group in contrast with controls significantly ($p<0.001$). Thyroid function tests belong to two groups were demonstrated in Table 2.

Anti-TPO Ab was positive in 55 (37.9%) subjects of PCOS group and 11 (11.1%) subjects of controls (Table 3). As also, subjects

with positive anti-TG Ab were 22 (15.3%) in PCOS group and 5 (5.1%) in controls (Table 3). Odd's ratio was calculated as 4.88 for anti-TPO Ab positivity (CI 95%: 2.40-9.95) and 3.39 for anti-TG Ab positivity (CI 95%: 1.24-9.28) (Table 3). Anti-TPO Ab and anti-TG Ab positivity prevalence were determined significantly higher in PCOS patients (respectively; p<0.001, p=0.013) (Table 3). We did not ascertain any correlation between thyroid autoantibody levels and BMI, waist-hip ratio, CIMT and other biochemical parameters. CIMT had a positive correlation with BMI (p<0.001; r=0.350), waist-hip ratio (p=0.023; r=0.194), HOMA-IR (p<0.001; r=0.310) and a negative correlation with HDL-C levels (p<0.01; r=-0.215) (Table 4).

Discussion

PCOS is the most common reason for medically treatable anovulatory dysfunction (10). Therefore, accurate diagnosis, treatment, and follow-up are substantially important in this patient group. Thyroid function tests are one of the primary

Table 1. The clinical and biochemical data of patients with PCOS and controls

Variable	PCOS (n=184)	Controls (n=106)	p
Age, years (mean ± SD)	23.9±5.6	24.3±4.3	>0.05
BMI, kg/m² (minimum-maximum)	26.4 (16-44)	22.1 (16-40.4)	<0.001
Waist/hip ratio (minimum-maximum)	0.83 (0.41-1.1)	0.73 (0.63-0.9)	<0.001
Fasting glucose, mg/dL (mean ± SD)	84±8.6	80±8.2	<0.001
Fasting insulin, IU/mL (minimum-maximum)	2.8 (1.2-10)	1.8 (1-6.1)	<0.001
HOMA-IR (minimum-maximum)	2.85 (0.46-18)	1.85 (0.39-7.32)	<0.001
Total cholesterol, mg/dL (minimum-maximum)	170 (120-286)	166 (121-236)	>0.05
Triglyceride, mg/dL (minimum-maximum)	94 (28-353)	73 (29-216)	<0.001
HDL-C, mg/dL (minimum-maximum)	51 (30-97)	59 (33-110)	<0.001
LDL-C, mg/dL (minimum-maximum)	100 (50-168)	84 (51-136)	<0.001
CIMT (mm) (minimum-maximum)	0.49 (0.33-0.66)	0.48 (0.33-0.6)	<0.01

BMI: Body mass index; HOMA-IR: Homeostasis model assessment insulin resistance index; LDL-C: Low-density lipoprotein cholesterol; HDL-C: High-density lipoprotein cholesterol; CIMT: Carotid intima media thickness; SD: Standard deviation; PCOS: Polycystic ovary syndrome

Table 2. Thyroid function test results of groups

Variable (minimum-maximum)	PCOS (n=184)	Controls (n=106)	p
TSH (μIU/mL)	2 (0.34-19.8)	1.8 (0.8-5.6)	>0.05
fT4 (ng/mL)	1.1 (0.59-2)	1.1 (0.9-1.46)	>0.05
Anti-TG (IU/mL)	26 (0.9-524)	20 (10-308)	<0.001
Anti-TPO (IU/mL)	52 (0.2-1300)	10 (10-1000)	<0.001

TSH: Thyroid-stimulating hormone; fT4: free T4; Anti-TG: Anti-thyroglobulin; Anti-TPO: Anti-thyroid peroxidase; PCOS: Polycystic ovary syndrome

Table 3. Thyroid autoantibody positivity and odds ratio for PCOS and control groups

	PCOS (n=184)	Controls (n=106)	p	OR (95% CI)
Anti-TG (%)	15.3%	5.1%	0.013	3.39 (1.24-9.28)
Anti-TPO (%)	37.9%	11.1%	<0.001	4.8 (2.40-9.95)

Anti-TG: Anti-thyroglobulin; Anti-TPO: Anti-thyroid peroxidase; CI: Confidence interval; OR: Odds ratio; PCOS: Polycystic ovary syndrome

Table 4. Parameters and their correlations with CIMT

	p	r
BMI, kg/m²	<0.001	0.350
Waist/hip ratio	0.023	0.194
HOMA-IR	<0.001	0.310
HDL, mg/dL	<0.01	-0.215

CIMT: Carotid intima media thickness; BMI: Body mass index; HOMA-IR: Homeostasis model assessment insulin resistance index; HDL: High-density lipoprotein

studies in the evaluation of menstrual dysfunctions and concurrent thyroid abnormalities for the correct diagnosis of PCOS.

Calvar et al. (12) represented that autoimmune thyroiditis and subclinical hypothyroidism are five times higher in PCOS group than controls. Different studies demonstrated that autoimmune thyroiditis, subclinical and clinical hypothyroidism are associated with PCOS and they recommended to evaluate thyroid function tests periodically in this patient group (11,15). Our study revealed that thyroid autoantibodies are highly positive in patients with PCOS although normal thyroid hormone levels. Calvar et al. (12) established a positive correlation between thyroid dysfunctions and HOMA-IR. In our study, there was no correlation between thyroid autoantibody levels and other parameters.

Du and Li (16) demonstrated a meta-analysis of 6 studies including 726 PCOS patients and 879 controls to evaluate the relationship between PCOS and thyroid autoimmunity. The results showed that autoimmune thyroid disease and thyroid autoantibody levels are higher in PCOS and they conceived that PCOS can be a disorder based on an autoimmune background (16).

Obesity is a metabolic disorder associated with PCOS with the prevalence of 35-70% (17). Many studies concluded that PCOS patients have higher fasting plasma glucose, HOMA-IR, LDL-C, TG and lower HDL-C levels (5,6,18,19). We determined that BMI, waist-hip ratio, fasting blood glucose, fasting insulin, HOMA-IR, TG and LDL-C levels are higher; HDL-C level was lower significantly in PCOS group as against to controls. Carotid intima-media thickness is an important identifier for premature atherosclerosis and different studies confirmed that CIMT is significantly higher in PCOS patients who have an increased risk for cardiovascular morbidities (20,21). In our study, CIMT was statically significant higher in PCOS patients and had a positive correlation with BMI, waist/hip ratio, and HOMA-IR similarly.

Infertility is a difficult issue in PCOS patients. Thyroid autoimmunity is associated with infertility, miscarriage, probable thyroid disorders during pregnancy and in the postpartum period (7). These disorders may also cause complications as gestational hypertension, preeclampsia, preterm delivery, postpartum haemorrhage and lower birth weight (9). Poppe et al. (8) reported that TSH and anti-TPO antibodies are significantly higher in infertile patients with different reasons (endometriosis, tubal and ovarian pathologies). Another study demonstrated that autoimmune thyroid disease prevalence in infertile women is 16% and statically significantly higher than controls (22). Bellver et al. (23) represented that autoimmune thyroid disease is higher in PCOS patients and have a strong correlation with unexplained infertility and implantation failure (23). Ott et al. (24) also pointed out the relationship between PCOS patients with higher anti-TPO levels and insufficient therapy response in infertile patients who administered clomiphene citrate and metformin.

In conclusion; we showed that thyroid autoantibody positivity prevalence of euthyroid PCOS patients is 3.5 times higher with respect to control group. Although thyroid hormone level evaluation is fundamental for PCOS follow-up and treatment, thyroid autoantibody assessment is mostly neglected. From all these close relations with PCOS and thyroid dysfunctions, we suggest evaluating both thyroid autoantibodies and hormone levels in PCOS patients at the initial visit and euthyroid patients with positive results for autoantibodies should be followed up closely for the possible thyroid disorders and relevant complications.

Ethical Committee Approval: *Ethics committee approval of this study was assumed from the Ethics Committee of Dışkapı Yıldırım Beyazıt Training and Research Hospital.*

Informed Consent: *The written informed consent form was received from all individual participants included in the study.*

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References

1. Azziz R, Woods KS, Reyna R, Key TJ, Knochenhauer ES, Yildiz BO. The prevalence and features of the polycystic ovary syndrome in an unselected population. J Clin Endocrinol Metab 2004; 89: 2745-9.
2. Geisthövel F, Rabe T. The ESHRE/ASRM consensus on polycystic ovary syndrome (PCOS)-an extended critical analysis. Reprod Biomed Online 2007; 14: 522-35.
3. Ehrmann DA, Barnes RB, Rosenfield RL, Cavaghan MK, Imperial J. Prevalence of impaired glucose tolerance and diabetes in women with polycystic ovary syndrome. Diabetes Care 1999; 22: 141-6.
4. Apridonidze T, Essah PA, Iuorno MJ, Nestler JE. Prevalence and characteristics of the metabolic syndrome in women with polycystic ovary syndrome. J Clin Endocrinol Metab 2005; 90: 1929-35.

5. Lo JC, Feigenbaum SL, Yang J, Pressman AR, Selby JV, Go AS. Epidemiology and adverse cardiovascular risk profile of diagnosed polycystic ovary syndrome. J Clin Endocrinol Metab 2006; 91: 1357-63.
6. Legro RS, Kunselman AR, Dunaif A. Prevalence and predictors of dyslipidemia in women with polycystic ovary syndrome. Am J Med 2001; 111: 607-13.
7. Kennedy RL, Malabu UH, Jarrod G, Nigam P, Kannan K, Rane A. Thyroid function and pregnancy: before, during and beyond. J Obstet Gynaecol 2010; 30: 774-83.
8. Poppe K, Glinoer D, Van Steirteghem A, Tournaye H, Devroey P, Schiettecatte J, et al. Thyroid dysfunction and autoimmunity in infertile women. Thyroid 2002; 12: 997-1001.
9. Medenica S, Nedeljkovic O, Radojevic N, Stojkovic M, Trbojevic B, Pajovic B. Thyroid dysfunction and thyroid autoimmunity in euthyroid women in achieving fertility. Eur Rev Med Pharmacol Sci 2015; 19: 977-87.
10. Kousta E, White DM, Cela E, McCarthy MI, Franks S. The prevalence of polycystic ovaries in women with infertility. Hum Reprod 1999; 14: 2720-3.
11. Kachuei M, Jafari F, Kachuei A, Keshteli AH. Prevalence of autoimmune thyroiditis in patients with polycystic ovary syndrome. Arch Gynecol Obstet 2012; 285: 853-6.
12. Calvar CE, Bengolea SV, Deutsch SI, Hermes R, Ramos G, Loyato M. High frequency of thyroid abnormalities in polycystic ovary syndrome. Medicina (B Aires) 2015; 75: 213-7.
13. Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod 2004; 19: 41-7.
14. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985; 28: 412-9.
15. Novais J de S, Benetti-Pinto CL, Garmes HM, Jales RM, Juliato CR. Polycystic ovary syndrome and chronic autoimmune thyroiditis. Gynecol Endocrinol 2015; 31: 48-51.
16. Du D, Li X. The relationship between thyroiditis and polycystic ovary syndrome: a meta-analysis. Int J Clin Exp Med 2013; 6: 880-9.
17. Leibel NI, Baumann EE, Kocherginsky M, Rosenfield RL. Relationship of adolescent polycystic ovary syndrome to parental metabolic syndrome. J Clin Endocrinol Metab 2006; 91: 1275-83.
18. Palmert MR, Gordon CM, Kartashov AI, Legro RS, Emans SJ, Dunaif A. Screening for abnormal glucose tolerance in adolescents with polycystic ovary syndrome. J Clin Endocrinol Metab 2002; 87: 1017-23.
19. Karaköse M, Hepsen S, Çakal E, Saykı Arslan M, Tutal E, Akın Ş, et al. Frequency of nodular goiter and autoimmune thyroid disease and association of these disorders with insulin resistance in polycystic ovary syndrome. J Turk Ger Gynecol Assoc 2017; 18: 85-9.
20. Allameh Z, Rouholamin S, Adibi A, Mehdipour M, Adeli M. Does carotid intima-media thickness have relationship with polycystic ovary syndrome? Int J Prev Med 2013; 4: 1266-70.
21. Abali R, Celik C, Tasdemir N, Guzel S, Alpsoy S, Yuksel A, et al. The serum protein α2-Heremans-Schmid glycoprotein/fetuin-a concentration and carotid intima-media thickness in women with polycystic ovary syndrome. Eur J Obstet Gynecol Reprod Biol 2013; 169: 45-9.
22. Unuane D, Velkeniers B, Anckaert E, Schiettecatte J, Tournaye H, Haentjens P, et al. Thyroglobulin autoantibodies: is there any added value in the detection of thyroid autoimmunity in women consulting for fertility treatment? Thyroid 2013; 23: 1022-8.
23. Bellver J, Soares SR, Alvarez C, Muñoz E, Ramírez A, Rubio C, et al. The role of thrombophilia and thyroid autoimmunity in unexplained infertility, implantation failure and recurrent spontaneous abortion. Hum Reprod 2008; 23: 278-84.
24. Ott J, Aust S, Kurz C, Nouri K, Wirth S, Huber JC, et al. Elevated antithyroid peroxidase antibodies indicating Hashimoto's thyroiditis are associated with the treatment response in infertile women with polycystic ovary syndrome. Fertil Steril 2010; 94: 2895-7.

The effect of nutrient supplementation in the management of polycystic ovary syndrome-associated metabolic dysfunctions: A critical review

Elif Günalan[1], Aylin Yaba[2], Bayram Yılmaz[1]
[1]Department of Physiology, Yeditepe University School of Medicine, İstanbul, Turkey
[2]Department of Histology and Embryology, Yeditepe University School of Medicine, İstanbul, Turkey

Abstract

Polycystic ovary syndrome (PCOS) is complex heterogeneous disorder that has several aspects in terms of pathology such as metabolic, endocrine, reproductive, and psychological. However, the etiology of PCOS remains poorly understood. Several studies suggest that insulin resistance and hyperandrogenism play a central role in the progression of PCOS pathophysiology. Therefore, common treatment strategies of PCOS are based on lifestyle modification, which include exercise, diet, and nutrient supplementation therapy. Recent studies have recommended some nutrients such as vitamins, minerals, and vitamin-like nutrients for the therapy of PCOS because each has at least one functional property in PCOS-induced pathways. Therefore, it is claimed that the cause of PCOS could be vitamin or mineral deficiency. This review aims to provide a critical literature survey on nutritional supplementation for the treatment of PCOS-associated endocrine and metabolic dysfunctions and discuss the role of nutrients in the management of PCOS in view of the clinical trials and experimental studies. (J Turk Ger Gynecol Assoc 2018; 19: 220-32)
Keywords: Polycystic ovary syndrome, insulin resistance, hyperandrogenism, metabolic dysfunctions, dietary supplements

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Introduction

The polycystic ovary syndrome (PCOS) is one of the most common endocrine diseases that affects 5 to 10% of women of adolescent and reproductive age (1,2). PCOS was first defined by Stein and Leventhal in 1935. The basic characteristic trait of PCOS is hyperandrogenism as a result of excessive androgen secretion or activity (3). However, hyperandrogenism is not the only diagnostic criteria for PCOS. According to the Rotterdam criteria, PCOS is defined by the existence of at least two of three criteria, which are hyperandrogenism, chronic anovulation, and polycystic ovaries on ultrasound findings (4). Later, the Androgen Excess and PCOS Society reported that there should be the presence of hyperandrogenism and ovarian dysfunction (anovulation and polycystic ovaries) for PCOS (5). Patients with PCOS have various symptoms including menstrual dysfunction, hyperinsulinemia, infertility, glucose intolerance, type 2 diabetes, hirsutism, obesity, acne, metabolic syndrome, increased risk for the development of cardiovascular diseases, endometrium cancer, anxiety, obstructive sleep apnea, and abnormalities of lipid profile (6,7).

Although there are extensive studies in the literature, the cause of PCOS remains unclear due to poorly understood interactions between genetic and environmental factors (8). Reproductive neuroendocrine defects, impaired ovarian steroidogenesis, insulin resistance (IR), and increased cortisol metabolism-related adrenal hyperandrogenism can be among the causes of PCOS (9-11). Recent studies suggest that IR contributes to both metabolic and reproductive disturbances. Therefore, IR has a central role in the pathogenesis of PCOS (12). Briefly, insulin is considered as a key hormone for hyperandrogenism in the PCOS pathophysiology via two different pathways: 1)

Address for Correspondence: *Aylin Yaba*
e.mail: aylin.ucar@yeditepe.edu.tr ORCID ID: orcid.org/0000-0001-6781-9983

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insulin stimulates androgen production of theca cells with luteinizing hormone (LH) and elevated androgen production leads to hirsutism, acne, and anovulatory infertility. 2) Hyperandrogenism associated function of insulin is inhibition of sex hormone-binding globulin (SHBG) synthesis in the liver (13). SHBG is a plasma protein for androgen and estrogens and so decreased SHBG levels can lead to hyperandrogenism in PCOS. At the metabolic perspective, insulin plays a key role in regulating glucose metabolism, blocking of lipolysis, and activation of aminoacid transportation (14). Various nutrients have regulatory roles in the insulin signaling pathway and androgen synthesis.

Providing sufficient nutrients and energy for growth and reproduction depend on the definition of the optimal nutrient composition. It is clear that nutrition-associated signaling pathways play a central role in the regulation of ovarian follicle growth and ovulation rates (15). In particular, deficiencies of myo-inositol and vitamin D can lead to PCOS pathogenesis-related complications (16-18). Therefore, nutritional supplementation may contribute to overcome complications of PCOS such as immature oocyte, IR, hyperandrogenism, and oxidative stress. This review addresses current knowledge about the efficacy of nutrients in the treatment of PCOS in view of experimental studies and clinical studies.

Vitamin Supplements

Vitamin A is a fat soluble vitamin also known as retinol. Vitamin A-derived metabolites such as retinoids, retinoic acid, and retinol contribute to antioxidant activity, steroid metabolism, oocyte nuclear maturation, and inhibition of cumulus cell apoptosis (19,20). It is known that retinoic acid synthesis-related genes are expressed differentially in theca interna cells isolated from patients with PCOS (21). To examine the effects of retinol and retinoids, derivatives of retinol were applied into theca interna cell culture obtained from PCOS and healthy women. All trans retinol-treated theca interna cells gave rise to increased dehydroepiandestrone levels and mRNA accumulation of cytochrome P450 17α hydrxylase (CYP17) involved in androgen production and retinol biosynthesis (22). Obesity and abnormal glucose metabolism are associated with elevated retinol-binding protein 4 (RBP4) levels in overweight women with PCOS (23). Another RBP4-based study reported the measurement of RBP4 expression in isolated subcutenous and omental adipose tissue from women with PCOS. The authors suggested that elevated 17β estrodiol could contribute to the altered gonadal and adrenal steroid profile via upregulation of the RBP4 gene (24).

B group vitamins; most studies focus on B6, B12, and folic acid in this group due to the increasing role of homocysteine (Hcy) in PCOS. In this mechanism, Hcy is an essential amino acid derived from dietary methionine and elevated total plasma Hcy levels lead to an increased risk for cardiovascular and reproductive symptoms in PCOS (25). In addition, other metabolic pathways required for growth of cell and tissue are closely associated with Hcy (26). Folic acid, vitamin B6, and vitamin B12 have significant roles in Hcy regulation. In the physiopathology of PCOS, a positive correlation has been reported between IR and elevated Hcy levels (27,28). Kaya et al. (29) demonstrated that IR, obesity, and increased Hcy levels were related to low serum insulin B12 concentrations in women with PCOS. In order to reduce elevated levels of serum Hcy, folic acid supplementation for three months produced effective results, especially in women without IR. However, a dose-dependent effect of folic acid supplementation is not known (30). Regular exercise has also been suggested to decrease plasma Hcy concentrations in the pathophysiology of PCOS. According to the study of Randeva et al. (31) regular exercise for a period of six months provides significantly lower plasma Hcy levels in young obese and overweight women with PCOS.

Many women with PCOS have to use insulin-sensitizing agents such as metformin for improving insulin sensitivity. Metformin inhibits the binding intrinsic factor-B_{12} complex and its receptor, and also serum vitamin B12 and folic acid levels decrease during metformin therapy (32). In addition, metformin increases Hcy levels; therefore it gives rise to the long-term risk of cardiovascular diseases in women with PCOS (33). The interaction between metformin and B group vitamins has been explained by two studies: the first report showed that daily administration of folic acid or B group vitamins could be effective in reducing elevated Hcy levels in women with PCOS in short-term metformin therapy. However, the authors also suggested that supplements of vitamins had no effects on androgen and lipid levels in the pathophysiology of PCOS (34). The second report showed that the use of metformin with folate supplementation for six months had beneficial effects on vascular endothelium. This treatment provides reduced Hcy levels, thus it can be effective in the management of the long-term complications of PCOS such as cardiovascular disorders (35).

Inositol and its metabolites are known as sugar alcohols and also belong to B complex vitamins. In addition, inositol has 9 stereoisomers such as myo-, cis-, allo-, epi-, muco-, neo-, scyllo-, D-chiro and L-chiro- forms (36). Inositol-derived metabolites have essential roles in insulin sensitivity as second messengers, lipid synthesis, signal transduction, oocyte maturation, oogenesis, cell morphogenesis, and cytoskeleton organization (37). According to randomized controlled studies involving inositol supplementation in women with PCOS, inositol provides improvement in almost all pathologic conditions

in PCOS such as recovery of reproductive abnormalities, decreased androgen levels, and improved insulin levels (38).

Interestingly, combined treatment of inositol isomers such as myo-inositol (MI) and D-chiro inositol (DCI) should be applied at a certain ratio, which is known as the plasma physiologic ratio (MI/DCI: 40/1) (39). Otherwise, immature oocytes can appear, and the efficacy of inositol is decreased in the pathophysiology of PCOS (40). Some studies have claimed that these pathologic conditions may be accounted for by the 'DCI paradox' (41). Briefly, MI is found in the follicle-stimulating hormone (FSH) signaling mechanism and homeostasis of glucose uptake, and DCI is prompted to insulin-associated androgen synthesis. Epimerase plays a functional role in conversion of MI to CDI depending on insulin levels and also intake of inositol isomerase, except the physiologic ratio can lead to decreased MI and increased CDI levels. When hyperinsulinemia occurs in the pathogenesis of PCOS, elevated epimerase activity can lead to abnormalities in the FSH signaling pathway; therefore, immature oocytes and hyperandrogenism may develop (42).

Contributions of MI to treatment in women with PCOS are reviewed in Table 1 (43-50). According to the current literature, treatment of MI provides healing in hyperandrogenism and IR- associated parameters, and also improvement of the lipid profile.

Vitamin D is so essential vitamin for skeletal growth, regulation of serotonin synthesis, bone mineral density, dental health, lower extremity functions, and regulation of calcium (Ca) and phosphorus metabolism. In addition, previous studies reported

Table 1. Effects of myo-inositol compounds in women with PCOS

References	Patients	Treatment	Outcomes
Nestler et al. (43)	44 obese women with PCOS (placebo group n=22, inositol group n=22)	Oral administration of 1200 mg of D-chiro inositol per day for 7-8 weeks	• Plasma triglyceride ↓ • Diastolic and systolic pressure ↓ • DHEA-S ↓ • SHBG ↑
Baillargeon et al. (44)	19 obese women with PCOS	For 4-8 weeks, oral administration of metformin therapy (n=10) (500 mg orally thrice daily) and placebo group (n=9)	• Improvement of insulin mediated release of DCI-IPG
Gerli et al. (45)	92 women with oligomenorrhea and polycystic ovaries	For 12-16 weeks, 400 mcg folic acid intake in placebo group (n=47) and 400 mcg folic acid + 4 g inositol intake in treatment group (n=45)	• Higher ovulation rate • Weight loss • Folicular maturation • Circulating HDL ↑
Papaleo et al. (46)	25 women with PCOS who have oligo or amenorrhea since childbearing age	Myo-inositol + folic acid (inofolic) (2 g twice a day) for 6 months	• Improvement in menstrual cyclicity • Replacement of healthy ovarian activity • Serum free testesterone ↓
Genazzani et al. (47)	20 overweight women with PCOS	Group A (n=10); 2 g myo-inositol + 200 μg folic acid per day Group B (n=10); 200 μg folic acid per day for 12 weeks	• Circulating LH, T, PRL and insulin level ↓ • Ratio of LH/FSH ↓ • Restroration of menstrual cyclicity
Costantino et al. (48)	42 women with PCOS from reproductive age (18-40 years)	Placebo group (n=19): 400 mcg folic acid alone; experiment group (n=23): 4 g myo-inositol + 400 mcg folic acid for 12-16 weeks	• Insulin and androgen level ↓ • Improved glucose tolerance
Minozzi et al. (49)	155 women with PCOS	12 weeks' treatment: placebo group (n=75) oral contraceptive pills (OCP) intake, and the treatment group OCP + myo-inositol (4 g/day) intake	• Insulin sensitivity ↑ • Recovery of hirsutism • Androgen synthesis ↓ • HDL cholesterol level ↑ • LDL cholesterol level ↓
Morgante et al. (50)	Insulin resistant women with PCOS (n=15)	Low dose step-down gonadotropin regimen + Redestop (1500 mg inositol, 100 mg lactoferrin)	• Improved clinical outcomes • Pregnancy rate ↑ • Number of follicles >15 mm in diameter ↓ • Cancellation rate ↓

DHEA-S: Dehydroepiandestrone sulfate; PCOS: Polycystic ovary syndrome; SHBG: Sex hormone-binding globulin; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; T: Testosterone; LH: Luteinizing hormone; PRL: Prolactin; DCI-IPG: D-chiro-inositol-containing-inositolphosphoglycan; FSH: Follicle-stimulating hormone; ↓ decreasing; ↑ increasing

that vitamin D might be a significant and independent predictor of IR (51). Vitamin D levels decrease in obese patients when compared with non-obese people owing to IR. Regarding to PCOS, a recently published review by Krul-Poel et al. (52) about the role of vitamin D in metabolic disturbances of PCOS confirmed an association between vitamin D and metabolic disturbances. Thereby, it was found that women with PCOS (who are obese) had significantly decreased 25-dehydroxy vitamin D levels (53). Moreover, a cross-sectional study reported that lower D vitamin was linked with IR as a result of the pathophysiology of PCOS (54).

Researchers focus on vitamin D supplementation for the treatment of women with PCOS to show an interaction between vitamin D deficiency and PCOS. A recent study about vitamin D replacement therapy in which vitamin D3 was administered for three weeks in 11 subjects with PCOS suggested some beneficial effects on IR, but no changes in androgen levels were observed (55). In addition, Kotsa et al. (56) used a vitamin D3 analogue (alphacalcidol) in order to determine the effect of vitamin D in the treatment of PCOS. Their findings showed an increased first phase of insulin secretion, decreased serum triglyceride (TG) levels, and increased serum high-density lipoprotein (HDL) cholesterol profile.

The molecular mechanism between vitamin D supplementation and improvement of PCOS is currently unknown. However, a recent report claimed that vitamin D3 replacement treatment in women with PCOS improved some biochemical parameters by increasing in amount of soluble receptor for Advanced Glycosylated Ends (AGEs). Therefore, vitamin D3 inhibits inflammatory progress in the pathogenesis of PCOS. Moreover, vitamin D3 treatment plays a vital role in folliculogenesis due to decreasing elevated anti-mullerian hormone levels (57). Interestingly, Jafari-Sfidvajani et al. (58) demonstrated that vitamin D supplementation in women with PCOS caused no statistically significant differences in the androgen profile when combined with a low-calorie diet; however, an improvement in menstrual frequency was observed.

Vitamin E is a lipid-soluble vitamin and free radical scavenger that regulates the balance between antioxidant and oxidant systems (59). In addition, new evidence confirmed that vitamin E could improve endometrial thickness in women with unexplained infertility, and the effects were attributed to its anticoagulant and antioxidant effects (60). Moreover, cotreatment of coenzyme q10 and vitamin E for 8 weeks in patients with PCOS provided improvement in SHBG concentrations (61). Another study showed that vitamin E (400 IU) and omega-3 fatty acid (1000 mg) co-supplementation in women with PCOS for 12 weeks provided significant improvement in IR and androgen levels (62).

Supplementation of Vitamin-like Nutrients in PCOS

Alpha-Lipoic Acid (a-LA) is a free radical scavenger, an essential cofactor in the citric acid cycle, and a regulatory agent of body weight (63,64). Interestingly, Masharani et al. (65) found that controlled release of α-LA administered to six non-diabetic women with PCOS was not related with elevation in plasma antioxidant potency or reduction in plasma oxidation metabolites. To investigate the role of α-LA and DCI (DCA) in the short-term management of PCOS, both metabolites were given to 46 women (26 women with PCOS and 20 female controls) for 180 days. They suggested that some reproductive characteristics improved including menstrual cycles, decreased number of ovarian cysts, and increased progesterone levels. At the metabolic perspective, IR significantly improved in the subjects wiht PCOS, and impaired lipid metabolism was significantly changed (66).

Bioflavonoids consist of polyphenolic compounds, which are found in plants. Flavonoids have antioxidant, antidiabetic, antiestrogenic, anti-inflammatory, and antiproliferative properties (67). Bioflavonoids consist of various metabolites, some of which provide improvement of the pathogenesis of PCOS at different levels. For instance, Oh et al. (68) analyzed six flavonoid classes (anthacyanides, flovan-3-oils, flavanones, flavones, flavonols and isoflavones) in terms of their contribution to the treatment of metabolic syndrome in PCOS pathophysiology. The authors suggested that only flavonol consumption was the most effective treatment of metabolic syndrome in PCOS when compared with the other groups (68). Romualdi et al. (69) showed that 36 mg/d soy isoflavone genistein treatment in women with PCOS for three months provided a significantly improved lipid profile. However, other characteristic traits of PCOS such as hyperinsulinemia, anthropometric measurements, hyperandrogenism, and reproductive abnormalities did not change significantly (69). On the contrary, in an experimental study on rats, Shah and Patel (70) reported improved ovarian and uterine morphologic appearences, increased LH levels, and significantly decreased insulin and testesterone in PCOS following quercetin treatment, a bioflavonoid with antioxidant activity. They considered that quarcetin was functional in phosphatidylinositole-3-kinase (PI3K) inhibition and therefore PI3K could be beneficial target for a novel therapy approach of PCOS (70).

Carnitine is a quaternary ammonium compound found in fatty acid metabolism, oxidative stress mechanisms, and glucose metabolism (71). According to a clinical study, non-obese women with PCOS have significantly decreased serum total L-carnitine levels when compared with healthy women (72). Fenkci et al. (72) considered that lower L-carnitine level could be linked with hyperandrogenism and IR. Consistently,

some antidiabetic agents that are used for PCOS treatment are associated with carnitine metabolism. For instance, piaglitazone administration for 16 weeks in obese premenoupausal patients with PCOS led to increased fasting concentrations of free carnitine (73). Moreover, Dunning and Robker (74) claimed that L-carnitine influenced oocyte quality because L-carnitine provides transport of fatty acids and regulation of energy production, which have a central role in promoting oocyte maturation. Immature oocytes can be a source of metabolic and endocrine malfunctions in PCOS (75). A randomized clinical trial in clomiphene-resistant women with PCOS reported that using both clomiphene citrate and L-carnitine provided thicker endometrium, higher estradiol concentrations, higher pregnancy rates, and improved lipid profiles compared with clomiphene citrate treatment alone (76). Another study demonstrated that L-carnitin supplementation (250 mg per day) for 12 weeks had beneficial effects within mental health and oxidative stress parameters (77).

Mineral Supplements

Mineral supplements are among the dietary supplements that are expexted to provide improvement of metabolic profile, mental health, ovulation, and menstrual cyclicity. Recent studies about PCOS focused on mineral supplementation in order to remove pathologic situations from PCOS.

Calcium is an essential micronutrient and is involved in egg activity, oocyte maturation, progression of follicular development, and regulation of cell division in mammalian oocytes (78-80). Furthermore, Ca deficiency could be related to risk of obesity because the insulin signaling pathway is Ca dependent (81). Therefore, it is considered that abnormalities of Ca concentrations could be associated with IR and promoting PCOS pathologies. Biochemical studies have shown that decreased Ca levels are observed in obese women with PCOS when compared with healthy women. Ca homeostasis depends on vitamin D receptor (VDR), parathyroid hormone (PTH), and Ca-sensing receptor (CaSR). In addition, adiponectin concentration is strongly associated with Ca and vitamin D levels (82). To determine the role of the polymorphisms of Ca homeostasis-linked factors in initiating PCOS, VDR, PTH, CaSR, insulin receptor, and adiponectin genes were analyzed and compared with PCOS-associated biochemical parameters. Consequently, polymorphisms of VDR are related to increased LH and reduced SHBG levels and the gene variant of CaSR is linked to higher homeostatic model assessment-IR (HOMA-IR) and IR (83). Combined supplementation of vitamin D 100,000 IU/month, Ca 1000 mg/day, and metformin 1500 mg/day for 6 months in 100 infertile patients with PCOS resulted in significanly reduced body mass index (BMI). In addition, menstrual cyclicity, follicular maturation, and pregnancy rates were affected positively, but the alterations were not statistically significant (84).

Chromium is an essential mineral that has an essential role in carbohydrate and lipid metabolism. Chromium has been widely studied in the treatment of hyperglycemia, especially type 2 diabetes, because chromium deficiency leads to disorders in glucose homeostasis and IR (85). There is also evidence to confirm that women with PCOS showed decreased chromium levels, which was linked to IR (86). A pilot study suggested that with daily supplementation of 200 μg chromium for three months, women with PCOS showed improved glucose tolerance, but it did not affect reproductive function and hormonal disturbances (87). Another study involving 64 women with PCOS showed that daily 200 μg chromium supplementation for eight weeks caused significant decreases in serum insulin levels, HOMA-IR, HOMA-B, TGs, very-low-density lipoprotein (VLDL) cholesterol, and total cholesterol concentration. In addition, Jamilian and Asemi (88) showed that a significantly increased quantitative insulin sensitivity check index (QUICKI) score in women with PCOS compared with placebo. However, circulating LDL, HDL, cholesterol levels, and fasting plasma glucose levels were not altered in the treatment group (88).

The effect of chromium within androgen level depends on the treatment amount and duration of chromium treatment. According to a double-blind, randomized clinical study, chromium picolinate (200 μg/day) treatment in 46 patients with clomiphene citrate-resistant PCOS for 3 months gave rise to increased insulin sensitivity. However, there were no findings about a relationship between applied chromium and androgen levels (89).

In contrast, Amr and Abdel-Rahim (90) administered high doses of chromium picolinate (1000 μg/day) treatment to adolescent girls with PCOS for 6 months. At the end of the study, improvement of oligo/amenorrhea, decreased number of total follicles, lower free testesterone levels, and smaller ovarian volume were obtained in ultrasonographic views and biochemical analyses.

Magnesium is the one of the most predominant intracellular cations (91). Magnesium regulates ATP-generation, ATP-use, transphosphorylation reactions, DNA and RNA synthesis, insulin metabolism, ion homeostasis, membrane structure, cytoskeletal function, and cell growth (92). In addition, magnesium is associated with entry of Ca into the neuron because magnesium is a Ca antogonist and a voltage-dependent blocker of the N-methyl-D-aspartate channel (93,94). This property provides protection for neurons against cell death. Therefore, magnesium supplementation is used generally in neurologic disorders including depression-related diseases such as PCOS, as well hypertension, cardiovascular diseases, and diabetes (95,96). However, only a few studies

have suggested a relationship between serum magnesium level and the pathogenesis of PCOS. Lower serum magnesium level and higher Ca/Mg ratios in women with PCOS due to IR have been reported. No significant correlation between Mg levels and steroid hormones was found (97). The effects of magnesium levels in PCOS pathology remains unclear.

Selenium is an effective essential element against oxidative stress and is required for the embryonic gonodal development and function of reproductive tissues (98). Biochemical studies have shown that women with PCOS have lower selenium level compared with controls. Coskun et al. (99) suggested that accumulation of free radicals was detected in PCOS women due to insufficient selenium level, which leads to increased androgen levels including LH and total testesterone (99). In this regard, selenium supplementation in the form of immunomodulatory drug (IMOD) was administered for 21 days to hyperandrogenism-induced PCOS female rats. IMOD reduced tumor necrosis factor-α production and increased antioxidant capacity (100).

Another aspect of selenium intake is related to glucose and fat metabolism because selenium possesses insulin-like activities (101,102). There were two clinical studies about the effect of selenium supplementation in women with PCOS in terms of IR. In the first study, 70 women with PCOS were randomly divided into two groups, one received 200 μg per day selenium supplements (n=35) and the other placebo (n=35). After 8 weeks of intervention, they reported a reduction in serum insulin levels, HOMA-IR, HOMA-B, and increased QUICKI. Also, selenium intake showed decreased serum TGs and VLDL-C concentrations when compared with placebo (103). Another study included 200 μg selenium supplementation (n=20) and placebo (n=20) per day for 8 weeks in 40 infertile women with PCOS. At the end of the study, the authors measured the insulin and lipid-related gene expression levels such as PPAR-γ, GLUT-1, and LDLR from lymphocytes in the subjects. The results showed that selenium supplementation could be a candidate for *in vitro* fertilization due to significantly increased expression levels of PPAR-γ and GLUT-1 and decreased expression levels of LDLR (102).

Zinc is an another essential trace element found in the metabolism of lipid, carbohydrates, and protein, which is responsible for the function of over 300 enzymes. It is a component of more than 200 enzymes (104). In particular, zinc ions play crucial roles in insulin metabolism including the synthesis, storage, secretion, conformational integrity, function, and action of insulin, and also zinc ions produce an insulin-like effect (105). For this reason, insufficiency of zinc gives rise to diabetes, obesity, glucose intolerance, lipidemia, hyperglycemia, and hypertriglyceridemia (106,107). Studies have shown that women with PCOS have lower zinc levels (108). It has been demonstrated that one of the reasons for IR in PCOS was related to decreased insulin-dependent tyrosine phosphorylation due to a post-receptor defect (109,110). Therefore, inadequate zinc levels could not stimulate insulin receptor tyrosine kinase in patients with PCOS. Zinc levels can play an important role in the development of IR in PCOS. Several studies suggested that zinc supplementation had therapeutic effects for the prevention of type 2 diabetes (111).

The pathology of PCOS involves risk of cardiovascular diseases in the long term due to altered lipid profiles including elevated trygliceride levels, decreased HDL levels, and increased LDL levels (112). It has been suggested that zinc deficiency in PCOS might be associated with abnormal lipid profiles. The effect of zinc supplementation in women with PCOS has been shown in recent clinical research (113). In this study, 50 mg/d of zinc as zinc sulphate or placebo was given to 60 women with PCOS for 8 weeks, as an adjunct to their pre-study oral estrogen-progestrone compound therapy. The results showed a significant reduction in levels of serum total cholesterol, LDL-C, TG, and TG/HDL-C ratio in the zinc group (113). Therefore, zinc supplementation can provide an effective adjunctive nutritional therapy with potential for improving lipid metabolism and IR in women with PCOS.

Other Supplements

Melatonin (MT) is a neuroendocrine hormone secreted from the pineal gland. It plays a central role in the regulation of circadian rhythm. High concentrations of MT have been found in follicular fluid, which affects physiologic processes in the ovaries such as folliculogenesis, follicular atresia, ovulation, steroidogenesis in theca cells, and corpus luteum formation due to its powerful free radical scavenger activity (114-116). Moreover, Wei et al. (117) reported that supplementation of MT at a low concentration supports nuclear maturation of oocytes *in vitro*. Therefore, MT may provide improvement of oocyte quality and increase pregnancy rates (118). Concentration of MT in pre-ovulatory follicular fluid is lower in women with PCOS. Kim et al. (119) suggested that MT administration may be useful in *in vitro* fertilization strategy and improve clinical outcomes of PCOS.

N-acetyl-L-cysteine (NAC) is the acylated form of L-cysteine amino acid and also one of the precursors of glutathione, an antioxidant substance (120). Liu et al. (121) showed that NAC administration supported oocyte quality through an anti-aging effect on mouse oocytes. In addition, NAC regulates insulin receptor function in eryhtrocytes and supports insulin secretion from the pancreatic β cells (122). Fulghesu et al. (123) investigated the effects of NAC administration for 5-6 weeks on insulin-associated parameters in obese and lean women. They determined a significant decrease in testesterone and androgen levels. In addition, increased

peripheral insulin sensitivity appeared in women with PCOS (123). Thus, both metformin and NAC have important effects on hyperandrogenism, hyperinsulinemia, and menstrual cyclicity in women with PCOS. Elnashar et al. (124) compared the effects of metformin and NAC on insulin and testesterone levels and ovulation success in women with clomiphene citrate-resistant PCOS. In fact, clomiphene citrate is used in the first-line treatment of PCOS as a stimulator of ovulation. However, resistance against clomiphene citrate in women with PCOS obstructs the possibility of pregnancy. As a consequence, it was suggested that metformin had more efficacy in ovulation rates (51.6%) and insulin sensitivity than NAC (124). Another clinical trial compared metformin use (500 mg three times daily) and NAC supplementation (600 mg three times daily) over a 24-week period. Both groups had equal efficacy in terms of decreased BMI and free testesterone levels, improved insulin sensitivity, menstrual cyclicity, and lower hirsutism scores. Moreover, metformin administration caused a decrease in total cholesterol levels and NAC supplementation led to reduction in both total cholesterol and LDL levels (125).

Omega 3 Fatty Acids are polyunsaturated fatty acids (PUFAs). α-linolenic acid, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) are the most commnly known members in this group. Each fatty acid has distinct metabolic and endocrine properties and PUFAs intake can be linked to reduced TG, whereas monounsaturated fatty acids (MUFAs) consumption leads to decreased testesterone level (126). Omega-3 fatty acids reduce oxidative stress, decrease hypertension, and improve lipid profiles and anti-inflamatory activity, and so they have potential role against cardiovascular disease risk (127,128). In recent years, omega-3 fatty acids have been considered as therapeutical agents for the treatment of PCOS. It has considered that the healing mechanism of omega-3 is associated with regulation of abnormal gene expression in the pathophysiology of PCOS. For instance, different doses (25-100 μg) of omega-3 EPA in granulosa cell culture resulted with higher insulin growth factor (IGF)-1 expression and lower cycloxogenase 2 (COX2) expression. It is clear that IGF-1 is an essential compound of follicular differentiation and COX-2 contributes to oocyte maturation (129).

The relationship between IR and omega-3 supplementation has been discussed by various researchers due to inconsistent findings. However, a meta-analysis about the effects of omega-3 in the IR-associated pathology of PCOS reported no association between intake of omega-3 and insulin sensitivity (130). Some clinical studies are briefly summarized in Table 2 (131-136).

Table 2. According to clinical studies, the role of omega-3 in the treatment of PCOS

References	Subjects	Treatment	Outcomes
Kasim-Karakas et al. (131)	17 women with PCOS	Habitual diet process were followed for 3 months and then dietary fats were exchanged with PUFAs for another 3 months	Improvement of metabolic and endocrine parameters: • Fasting glucose and free fatty acid ↑ • Ketone bodies ↑
Phelan et al. (132)	126 women with PCOS	104 women with PCOS for asssessment of fatty acid metabolism in PCOS: (n=22) n-3 PUFA supplemented group which were used for the evaluation of metabolic and endocrine parameters	• Atherogenic lipid profile ↓ • Bioavaliability of plasma testosterone concentration ↓
Kalgaonkar et al. (133)	32 women with PCOS	Subjects received 31 g fats such as MUFA-rich almond (n=16) or PUFA-rich walnut (n=16) for 6 weeks	• Walnut group: increased adiponectin and leptin; decreased LDL cholesterol and ApoB • Almond group: increased adiponectin; decreased FAI
Mohammadi and Rafraf (134)	64 PCOS women with 20-35 years old	Treatment group (n=32) received 4 g/day omega-3 fatty acids and another 32 women were found in the placebo group	Decreased cardiovascular risk: • Serum paraoxonase I activity ↑ • Improvement of lipid profile
Rafraf et al. (135)	61 overweight and obese PCOS patients with 20-35 years old	(n=30) treated group 1 g/day n-3 capsules four times and another (n=31) women with PCOS were given placebo for 8 weeks	Improvement of insulin resistence associated parameters
Oner and Muderris (136)	45 non-obese patients with PCOS	Subjects were revealed 1500 mg/d of omega-3 supplements for 6 months	• HOMA-IR and insulin level ↓ • LH and testosterone ↓ • SHBG and TNF-α ↑

HOMA-IR: Homeostatic model assessment-insulin resistance; PCOS: Polycystic ovary syndrome; MUFA: Monounsaturated fatty acid; PUFA: Polyunsaturated fatty acid; SHBG: Sex hormone binding globulin; TNF: Tumor necrosis factor; FAI: Fatty acid index; HDL: High density lipoprotein; LDL: Low density lipoprotein; T: Testosterone; LH: Luteinizing hormone; FSH: Folicule stimulating hormone; ↓ decreasing; ↑ increasing

Probiotics are living microbial dietary supplements found in dairy products and have synergism with the gut microbiota (137). Probiotics have beneficial effects in metabolism, especially under inflammatory conditions (138,139). According to recent studies, probiotic consumption improves fasting blood glucose and antioxidant status in patients with type 2 diabetes (140). In addition, Yadav et al. (141) showed that a probiotic-supplemented diet delayed the onset of glucose intolerence, hyperglycemia, hyperinsulinemia, and dyslipidemia in diabetic rats. Shoaei et al. (142) studied the effects of probiotic supplementation on pancreatic β cells and C-reactive protein (CRP) in patients with PCOS using multispecies probiotics for 8 weeks. The results of their study showed reduced fasting blood sugar and serum insulin levels in a crude model. Interestingly, CRP levels did not significantly change.

The etiology of PCOS has two pathologic conditions including a chronic state of inflammation and IR (143). Both conditions are associated with the dysbiosis of gut microbiota (DOGMA) theory. The background of DOGMA involves an imbalance in gut microbiota, i.e., increasing the transition of Gram-negative colonic bacteria into the systemic circulation. Therefore, a chronic inflammatory response occurs in the host. The inflammatory process affects insulin receptor function and PCOS-associated pathways such as androgen biosynthesis. Therefore, to overcome the pathophysiologic conditions of PCOS, probiotic supplements are recommended by some researchers (144,145). On this point, Guo et al. (146) performed fecal microbiota transplantation (FMT) and *lactobacillus* transplantation in rats with PCOS. At the end of the study, they reported that all rats in the FMT group had an improved estrous cycle and most of the lactobacillus-treated rats had decreased androgen biosynthesis (146).

The pathophysiology of PCOS is associated with various defects, including neuroendocrine defects, impaired ovarian steroidogenesis, IR, and increased cortisol metabolism-related adrenal hyperandrogenism. Although the triggering cause of PCOS is currently unknown, androgens and insulin are thought to be two key factors in its pathogenesis. Therefore, treatment of PCOS is required to overcome both hyperandrogenism and hyperinsulinemia. Nutrients act as cofactors in maintaining functions of insulin and androgen receptors. In this study, we focused on the efficacy of nutrient supplementation in management of PCOS because almost all vitamin and mineral deficiencies are seen in PCOS. In this process, published clinical and experimental studies that met specified criteria were extracted from PubMed, Web of Science, EmBASE, Google Scholar database from the last 25 years as accurately and precisely as possible. Articles were divided into treated nutrient

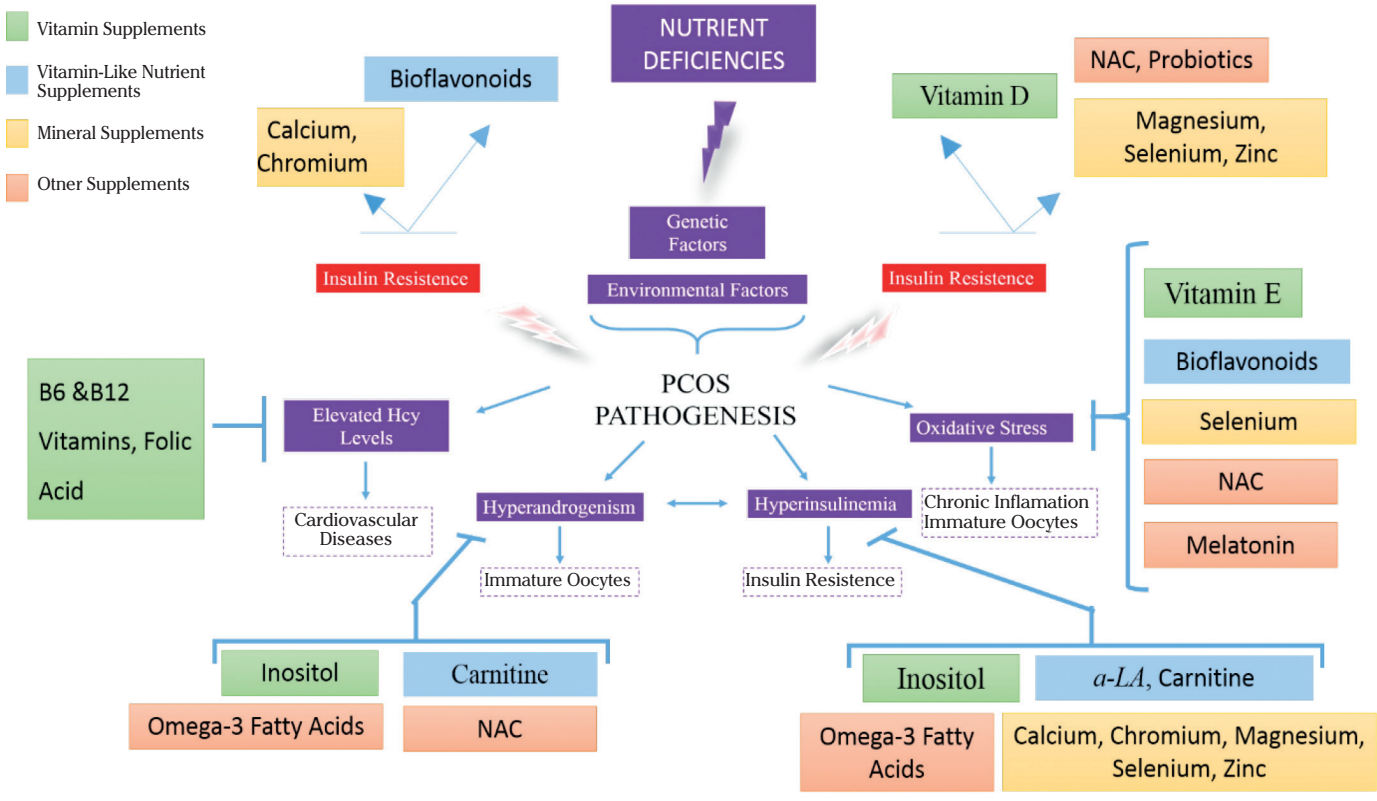

Figure 1. The effects of vitamins, minerals, vitamin-like substances and other supplements on the pathophysiology of PCOS
NAC: N-acetyl-L-cysteine; α-LA: α-linolenic acid; PCOS: Polycystic ovary syndrome

groups; vitamins, minerals, vitamin-like substances, and other nutrients and each substance was evaluated in terms of treated dose, duration, and effectiveness in terms of their ability to prevent PCOS complications. In addition, we summarized how supplementation of different vitamins, minerals, and other supplements contribute to prevent complications of PCOS (Figure 1).

Figure 1 indicates that interactions between genetic factors and some nutrient deficiencies cause PCOS pathophysiology-related symptoms such as elevated Hcy levels, oxidative stress, hyperandrogenism, and hyperinsulinemia. In particular, deficiencies of vitamin D, bioflavonoids, Ca, chromium, NAC, probiotics, magnesium, zinc, and selenium are associated with IR. Therefore, the treatment of women with PCOS with these supplements provides improvement for hyperinsulinemia and increased insulin sensitivity. Inositol, vitamin A, carnitine, omega-3 fatty acids, and NAC supplements affect hyperandrogenism. Inositol and omega 3 supplementation in particular help the recovery of PCOS with regard to metabolic and reproductive parameters. Apart from that, vitamin B6, B12, and folic acid have beneficial effects in abnormal Hcy levels and also vitamin E, α-linolenic acid, bioflavonoids, selenium, NAC, and MT supplements help to remove the oxidative stress of PCOS. Nevertheless, the safe use and effectiveness of herbal medicine and nutrient supplements, except for inositol and omega-3 fatty acid, have not been clearly demonstrated and more studies are needed in these areas (147).

Study Limitations

A limitation of our study was the huge number of related articles published: the doses, types, and combinations of supplemented nutrients are extremely different from each other, which depends on the investigated group, thus it makes the evaluation of the results difficult. Another limitation is the dose of nutrients used in the studies, as well as the insufficient diagnostic criteria used for PCOS. In addition, each woman with PCOS requires different supplementation depending on the signs and physiologic abnormalities. For instance, some patients have infertility due to PCOS, whereas others have endocrine and metabolic dysfunctions. However, most nutrient supplementation research focuses on the metabolic aspects of PCOS. Therefore, this review mostly focused on the therapeutic effects on the metabolic and endocrine dysfunctions instead of infertility, which is also a limiting factor in this study. Large, molecular scale up studies can be planned to illuminate the disrupted signaling pathways in PCOS. In this way, nutrients can be used effectively in the management of all aspects of PCOS via a molecular targeting strategy.

In conclusion, vitamin or mineral supplements can exert beneficial effects on PCOS-related symptoms such as immature oocytes, hyperinsulinemia, hyperandrogenism, increased BMI, cardiovascular disorders, and mental and psychological problems.

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References

1. Knochenhauer ES, Key TJ, Kahsar-Miller M, Waggoner W, Boots LR, Azziz R. Prevalence of the polycystic ovary syndrome in unselected black and white women of the southeastern United States: a prospective study. J Clin Endocrinol Metab 1998; 83: 3078-82.
2. Carreau AM, Baillargeon JP. PCOS in adolescence and type 2 diabetes. Curr Diab Rep 2015; 15: 564.
3. Stein JD, Andrews C, Musch DC, Green C, Lee PP. Sight-Threatening Ocular Diseases Remain Underdiagnosed Among Children Of Less Affluent Families. Health Aff (Millwood) 2016; 35: 1359-66.
4. Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod 2004; 19: 41-7.
5. Azziz R, Carmina E, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Futterweit W, et al. The Androgen Excess and PCOS Society criteria for the polycystic ovary syndrome: the complete task force report. Fertil Steril 2009; 91: 456-88.
6. Sheehan MT. Polycystic ovarian syndrome: diagnosis and management. Clin Med Res 2004; 2: 13-27.
7. Sirmans SM, Pate KA. Epidemiology, diagnosis, and management of polycystic ovary syndrome. Clin Epidemiol 2013; 6: 1-13.
8. Carmina E. Genetic and environmental aspect of polycystic ovary syndrome. J Endocrinol Invest 2003; 26: 1151-9.
9. Doi SA. Neuroendocrine dysfunction in PCOS: a critique of recent reviews. Clin Med Res 2008; 6: 47-53.
10. Takayama K, Fukaya T, Sasano H, Funayama Y, Suzuki T, Takaya R, et al. Immunohistochemical study of steroidogenesis and cell proliferation in polycystic ovarian syndrome. Hum Reprod 1996; 11: 1387-92.
11. Yaba A, Demir N. The mechanism of mTOR (mammalian target of rapamycin) in a mouse model of polycystic ovary syndrome (PCOS). J Ovarian Res 2012; 5: 38.
12. Diamanti-Kandarakis E, Dunaif A. Insulin resistance and the polycystic ovary syndrome revisited: an update on mechanisms and implications. Endocr Rev 2012; 33: 981-1030.
13. Ehrmann DA. Polycystic ovary syndrome. N Engl J Med 2005; 352: 1223-36.
14. Kahn CR. Banting Lecture. Insulin action, diabetogenes, and the cause of type II diabetes. Diabetes 1994; 43: 1066-84.

15. Yu J, Yaba A, Kasiman C, Thomson T, Johnson J. mTOR controls ovarian follicle growth by regulating granulosa cell proliferation. PLoS One 2011; 6: 21415.
16. Muscogiuri G, Policola C, Prioletta A, Sorice G, Mezza T, Lassandro A, et al. Low levels of 25(OH)D and insulin-resistance: 2 unrelated features or a cause-effect in PCOS? Clin Nutr 2012; 31: 476-80.
17. Thomson RL, Spedding S, Buckley JD. Vitamin D in the aetiology and management of polycystic ovary syndrome. Clin Endocrinol (Oxf) 2012; 77: 343-50.
18. Jakimiuk AJ, Szamatowicz J. (The role of inositol deficiency in the etiology of polycystic ovary syndrome disorders). Ginekol Pol 2014; 85: 54-7.
19. Pu Y, Wang Z, Bian Y, Zhang F, Yang P, Li Y, et al. All-trans retinoic acid improves goat oocyte nuclear maturation and reduces apoptotic cumulus cells during in vitro maturation. Anim Sci J 2014; 85: 833-9.
20. Deb GK, Dey SR, Bang JI, Lee JG, Kong IK. 9-cis Retinoic acid inhibits cumulus cell apoptosis during the maturation of bovine cumulus-oocyte-complexes. J Anim Sci 2012; 90: 1798-806.
21. Wood JR, Nelson VL, Ho C, Jansen E, Wang CY, Urbanek M, et al. The molecular phenotype of polycystic ovary syndrome (PCOS) theca cells and new candidate PCOS genes defined by microarray analysis. J Biol Chem 2003; 278: 26380-90.
22. Wickenheisser JK, Nelson-DeGrave VL, Hendricks KL, Legro RS, Strauss JF, 3rd, McAllister JM. Retinoids and retinol differentially regulate steroid biosynthesis in ovarian theca cells isolated from normal cycling women and women with polycystic ovary syndrome. J Clin Endocrinol Metab 2005; 90: 4858-65.
23. Hahn S, Backhaus M, Broecker-Preuss M, Tan S, Dietz T, Kimmig R, et al. Retinol-binding protein 4 levels are elevated in polycystic ovary syndrome women with obesity and impaired glucose metabolism. Eur J Endocrinol 2007; 157: 201-7.
24. Tan BK, Chen J, Lehnert H, Kennedy R, Randeva HS. Raised serum, adipocyte, and adipose tissue retinol-binding protein 4 in overweight women with polycystic ovary syndrome: effects of gonadal and adrenal steroids. J Clin Endocrinol Metab 2007; 92: 2764-72.
25. Yarali H, Yildirir A, Aybar F, Kabakci G, Bükülmez O, Akgül E, et al. Diastolic dysfunction and increased serum homocysteine concentrations may contribute to increased cardiovascular risk in patients with polycystic ovary syndrome. Fertil Steril 2001; 76: 511-6.
26. de la Calle M, Usandizaga R, Sancha M, Magdaleno F, Herranz A, Cabrillo E. Homocysteine, folic acid and B-group vitamins in obstetrics and gynaecology. Eur J Obstet Gynecol Reprod Biol 2003; 107: 125-34.
27. Loverro G, Lorusso F, Mei L, Depalo R, Cormio G, Selvaggi L. The plasma homocysteine levels are increased in polycystic ovary syndrome. Gynecol Obstet Invest 2002; 53: 157-62.
28. Badawy A, State O, El Gawad S, El Aziz OA. Plasma homocysteine and polycystic ovary syndrome: the missed link. Eur J Obstet Gynecol Reprod Biol 2007; 131: 68-72.
29. Kaya C, Cengiz SD, Satiroglu H. Obesity and insulin resistance associated with lower plasma vitamin B12 in PCOS. Reprod Biomed Online 2009; 19: 721-6.
30. Kazerooni T, Asadi N, Dehbashi S, Zolghadri J. Effect of folic acid in women with and without insulin resistance who have hyperhomocysteinemic polycystic ovary syndrome. Int J Gynaecol Obstet 2008; 101: 156-60.
31. Randeva HS, Lewandowski KC, Drzewoski J, Brooke-Wavell K, O'Callaghan C, Czupryniak L, et al. Exercise decreases plasma total homocysteine in overweight young women with polycystic ovary syndrome. J Clin Endocrinol Metab 2002; 87: 4496-501.
32. de Jager J, Kooy A, Lehert P, Wulffele MG, van der Kolk J, Bets D, et al. Long term treatment with metformin in patients with type 2 diabetes and risk of vitamin B-12 deficiency: randomised placebo controlled trial. BMJ 2010; 340: 2181.
33. Kilicdag EB, Bagis T, Zeyneloglu HB, Tarim E, Aslan E, Haydardedeoglu B, et al. Homocysteine levels in women with polycystic ovary syndrome treated with metformin versus rosiglitazone: a randomized study. Hum Reprod 2005; 20: 894-9.
34. Kilicdag EB, Bagis T, Tarim E, Aslan E, Erkanli S, Simsek E, et al. Administration of B-group vitamins reduces circulating homocysteine in polycystic ovarian syndrome patients treated with metformin: a randomized trial. Hum Reprod 2005; 20: 1521-8.
35. Palomba S, Falbo A, Giallauria F, Russo T, Tolino A, Zullo F, et al. Effects of metformin with or without supplementation with folate on homocysteine levels and vascular endothelium of women with polycystic ovary syndrome. Diabetes Care 2010; 33: 246-51.
36. Daughaday WH, Larner J, Hartnett C. The synthesis of inositol in the immature rat and chick embryo. J Biol Chem 1955; 212: 869-75.
37. Papaleo E, Unfer V, Baillargeon JP, Chiu TT. Contribution of myo-inositol to reproduction. Eur J Obstet Gynecol Reprod Biol 2009; 147: 120-3.
38. Unfer V, Carlomagno G, Dante G, Facchinetti F. Effects of myo-inositol in women with PCOS: a systematic review of randomized controlled trials. Gynecol Endocrinol 2012; 28: 509-15.
39. Carlomagno G, De Grazia S, Unfer V, Manna F. Myo-inositol in a new pharmaceutical form: a step forward to a broader clinical use. Expert Opin Drug Deliv 2012; 9: 267-71.
40. Dinicola S, Chiu TT, Unfer V, Carlomagno G, Bizzarri M. The rationale of the myo-inositol and D-chiro-inositol combined treatment for polycystic ovary syndrome. J Clin Pharmacol 2014; 54: 1079-92.
41. Carlomagno G, Unfer V, Roseff S. The D-chiro-inositol paradox in the ovary. Fertil Steril 2011; 95: 2515-6.
42. Nestler JE, Unfer V. Reflections on inositol(s) for PCOS therapy: steps toward success. Gynecol Endocrinol 2015; 31: 501-5.
43. Nestler JE, Jakubowicz DJ, Reamer P, Gunn RD, Allan G. Ovulatory and metabolic effects of D-chiro-inositol in the polycystic ovary syndrome. N Engl J Med 1999; 340: 1314-20.
44. Baillargeon JP, Iuorno MJ, Jakubowicz DJ, Apridonidze T, He N, Nestler JE. Metformin therapy increases insulin-stimulated release of D-chiro-inositol-containing inositolphosphoglycan mediator in women with polycystic ovary syndrome. J Clin Endocrinol Metab 2004; 89: 242-9.
45. Gerli S, Papaleo E, Ferrari A, Di Renzo GC. Randomized, double blind placebo-controlled trial: effects of myo-inositol on ovarian function and metabolic factors in women with PCOS. Eur Rev Med Pharmacol Sci 2007; 11: 347-54.
46. Papaleo E, Unfer V, Baillargeon JP, De Santis L, Fusi F, Brigante C, et al. Myo-inositol in patients with polycystic ovary syndrome: a novel method for ovulation induction. Gynecol Endocrinol 2007; 23: 700-3.
47. Genazzani AD, Lanzoni C, Ricchieri F, Jasonni VM. Myo-inositol administration positively affects hyperinsulinemia and hormonal parameters in overweight patients with polycystic ovary syndrome. Gynecol Endocrinol 2008; 24: 139-44.
48. Costantino D, Minozzi G, Minozzi E, Guaraldi C. Metabolic and hormonal effects of myo-inositol in women with polycystic ovary syndrome: a double-blind trial. Eur Rev Med Pharmacol Sci 2009; 13: 105-10.
49. Minozzi M, Costantino D, Guaraldi C, Unfer V. The effect of a combination therapy with myo-inositol and a combined oral contraceptive pill versus a combined oral contraceptive pill alone

on metabolic, endocrine, and clinical parameters in polycystic ovary syndrome. Gynecol Endocrinol 2011; 27: 920-4.
50. Morgante G, Orvieto R, Di Sabatino A, Musacchio MC, De Leo V. The role of inositol supplementation in patients with polycystic ovary syndrome, with insulin resistance, undergoing the low-dose gonadotropin ovulation induction regimen. Fertil Steril 2011; 95: 2642-4.
51. Alvarez JA, Ashraf A. Role of vitamin d in insulin secretion and insulin sensitivity for glucose homeostasis. Int J Endocrinol 2010; 2010: 351385.
52. Krul-Poel YH, Snackey C, Louwers Y, Lips P, Lambalk CB, Laven JS, et al. The role of vitamin D in metabolic disturbances in polycystic ovary syndrome: a systematic review. Eur J Endocrinol 2013; 169: 853-65.
53. Yildizhan R, Kurdoglu M, Adali E, Kolusari A, Yildizhan B, Sahin HG, et al. Serum 25-hydroxyvitamin D concentrations in obese and non-obese women with polycystic ovary syndrome. Arch Gynecol Obstet 2009; 280: 559-63.
54. Joham AE, Teede HJ, Cassar S, Stepto NK, Strauss BJ, Harrison CL, et al. Vitamin D in polycystic ovary syndrome: Relationship to obesity and insulin resistance. Mol Nutr Food Res 2016; 60: 110-8.
55. Selimoglu H, Duran C, Kiyici S, Ersoy C, Guclu M, Ozkaya G, et al. The effect of vitamin D replacement therapy on insulin resistance and androgen levels in women with polycystic ovary syndrome. J Endocrinol Invest 2010; 33: 234-8.
56. Kotsa K, Yavropoulou MP, Anastasiou O, Yovos JG. Role of vitamin D treatment in glucose metabolism in polycystic ovary syndrome. Fertil Steril 2009; 92: 1053-8.
57. Irani M, Minkoff H, Seifer DB, Merhi Z. Vitamin D increases serum levels of the soluble receptor for advanced glycation end products in women with PCOS. J Clin Endocrinol Metab 2014; 99: 886-90.
58. Jafari-Sfidvajani S, Ahangari R, Hozoori M, Mozaffari-Khosravi H, Fallahzadeh H, Nadjarzadeh A. The effect of vitamin D supplementation in combination with low-calorie diet on anthropometric indices and androgen hormones in women with polycystic ovary syndrome: a double-blind, randomized, placebo-controlled trial. J Endocrinol Invest 2018; 41: 597-607.
59. Palamanda JR, Kehrer JP. Involvement of vitamin E and protein thiols in the inhibition of microsomal lipid peroxidation by glutathione. Lipids 1993; 28: 427-31.
60. Cicek N, Eryilmaz OG, Sarikaya E, Gulerman C, Genc Y. Vitamin E effect on controlled ovarian stimulation of unexplained infertile women. J Assist Reprod Genet 2012; 29: 325-8.
61. Izadi A, Ebrahimi S, Shirzai S, Taghizadeh S, Parized M, Farzadi L, et al. Hormonal and metabolic effects of coenzyme q10 and/or vitamin E in patients with polycystic ovary syndrome. J Clin Endocrinol Metab 2018.
62. Ebrahimi FA, Samimi M, Foroozanfard F, Jamilian M, Akbari H, Rahmani E, et al. The Effects of Omega-3 Fatty Acids and Vitamin E Co-Supplementation on Indices of Insulin Resistance and Hormonal Parameters in Patients with Polycystic Ovary Syndrome: A Randomized, Double-Blind, Placebo-Controlled Trial. Exp Clin Endocrinol Diabetes 2017; 125: 353-9.
63. Biewenga GP, Haenen GR, Bast A. The pharmacology of the antioxidant lipoic acid. Gen Pharmacol 1997; 29: 315-31.
64. Lee WJ, Koh EH, Won JC, Kim MS, Park JY, Lee KU. Obesity: the role of hypothalamic AMP-activated protein kinase in body weight regulation. Int J Biochem Cell Biol 2005; 37: 2254-9.
65. Masharani U, Gjerde C, Evans JL, Youngren JF, Goldfine ID. Effects of controlled-release alpha lipoic acid in lean, nondiabetic patients with polycystic ovary syndrome. J Diabetes Sci Technol 2010; 4: 359-64.
66. Cianci A, Panella M, Fichera M, Falduzzi C, Bartolo M, Caruso S. d-chiro-Inositol and alpha lipoic acid treatment of metabolic and menses disorders in women with PCOS. Gynecol Endocrinol 2015; 31: 483-6.
67. Ross JA, Kasum CM. Dietary flavonoids: bioavailability, metabolic effects, and safety. Annu Rev Nutr 2002; 22: 19-34.
68. Oh JS, Kim H, Vijayakumar A, Kwon O, Choi YJ, Huh KB, et al. Association between dietary flavanones intake and lipid profiles according to the presence of metabolic syndrome in Korean women with type 2 diabetes mellitus. Nutr Res Pract 2016; 10: 67-73.
69. Romualdi D, Costantini B, Campagna G, Lanzone A, Guido M. Is there a role for soy isoflavones in the therapeutic approach to polycystic ovary syndrome? Results from a pilot study. Fertil Steril 2008; 90: 1826-33.
70. Shah KN, Patel SS. Phosphatidylinositide 3-kinase inhibition: A new potential target for the treatment of polycystic ovarian syndrome. Pharm Biol 2016; 54: 975-83.
71. Steiber A, Kerner J, Hoppel CL. Carnitine: a nutritional, biosynthetic, and functional perspective. Mol Aspects Med 2004; 25: 455-73.
72. Fenkci SM, Fenkci V, Oztekin O, Rota S, Karagenc N. Serum total L-carnitine levels in non-obese women with polycystic ovary syndrome. Hum Reprod 2008; 23: 1602-6.
73. Vigerust NF, Bohov P, Bjorndal B, Seifert R, Nygard O, Svardal A, et al. Free carnitine and acylcarnitines in obese patients with polycystic ovary syndrome and effects of pioglitazone treatment. Fertil Steril 2012; 98: 1620-6.
74. Dunning KR, Robker RL. Promoting lipid utilization with l-carnitine to improve oocyte quality. Anim Reprod Sci 2012; 134: 69-75.
75. Dumesic DA, Abbott DH. Implications of polycystic ovary syndrome on oocyte development. Semin Reprod Med 2008; 26: 53-61.
76. Ismail AM, Hamed AH, Saso S, Thabet HH. Adding L-carnitine to clomiphene resistant PCOS women improves the quality of ovulation and the pregnancy rate. A randomized clinical trial. Eur J Obstet Gynecol Reprod Biol 2014; 180: 148-52.
77. Jamilian H, Jamilian M, Samimi M, Afshar Ebrahimi F, Rahimi M, Bahmani F, et al. Oral carnitine supplementation influences mental health parameters and biomarkers of oxidative stress in women with polycystic ovary syndrome: a randomized, double-blind, placebo-controlled trial. Gynecol Endocrinol 2017; 33: 442-7.
78. Homa ST, Carroll J, Swann K. The role of calcium in mammalian oocyte maturation and egg activation. Hum Reprod 1993; 8: 1274-81.
79. Ullah G, Jung P, Machaca K. Modeling Ca2+ signaling differentiation during oocyte maturation. Cell Calcium 2007; 42: 556-64.
80. De Felici M, Dolci S, Siracusa G. An Increase of Intracellular Free Ca2+ Is Essential for Spontaneous Meiotic Resumption by Mouse Oocytes. J Exp Zool 1991; 260: 401-5.
81. Gedik O, Zileli MS. Effects of hypocalcemia and theophylline on glucose tolerance and insulin release in human beings. Diabetes 1977; 26: 813-9.
82. Mazloomi S, Sharifi F, Hajihosseini R, Kalantari S, Mazloomzadeh S. Association between Hypoadiponectinemia and Low Serum Concentrations of Calcium and Vitamin D in Women with Polycystic Ovary Syndrome. ISRN Endocrinol 2012; 2012: 949427.
83. Ranjzad F, Mahban A, Shemirani AI, Mahmoudi T, Vahedi M, Nikzamir A, et al. Influence of gene variants related to calcium homeostasis on biochemical parameters of women with polycystic ovary syndrome. J Assist Reprod Genet 2011; 28: 225-32.
84. Firouzabadi Rd, Aflatoonian A, Modarresi S, Sekhavat L, MohammadTaheri S. Therapeutic effects of calcium & vitamin D supplementation in women with PCOS. Complement Ther Clin Pract 2012; 18: 85-8.
85. Anderson RA. Chromium in the prevention and control of diabetes. Diabetes Metab 2000; 26: 22-7.

86. Chakraborty P, Ghosh S, Goswami SK, Kabir SN, Chakravarty B, Jana K. Altered Trace Mineral Milieu Might Play An Aetiological Role in the Pathogenesis of Polycystic Ovary Syndrome. Biol Trace Elem Res 2013; 152: 9-15.
87. Lucidi RS, Thyer AC, Easton CA, Holden AE, Schenken RS, Brzyski RG. Effect of chromium supplementation on insulin resistance and ovarian and menstrual cyclicity in women with polycystic ovary syndrome. Fertil Steril 2005; 84: 1755-7.
88. Jamilian M, Asemi Z. Chromium Supplementation and the Effects on Metabolic Status in Women with Polycystic Ovary Syndrome: A Randomized, Double-Blind, Placebo-Controlled Trial. Ann Nutr Metab 2015; 67: 42-8.
89. Amooee S, Parsanezhad ME, Ravanbod Shirazi M, Alborzi S, Samsami A. Metformin versus chromium picolinate in clomiphene citrate-resistant patients with PCOs: A double-blind randomized clinical trial. Iran J Reprod Med 2013; 11: 611-8.
90. Amr N, Abdel-Rahim HE. The effect of chromium supplementation on polycystic ovary syndrome in adolescents. J Pediatr Adolesc Gynecol 2015; 28: 114-8.
91. Touyz RM. Magnesium in clinical medicine. Front Biosci 2004; 9: 1278-93.
92. Saris NE, Mervaala E, Karppanen H, Khawaja JA, Lewenstam A. Magnesium. An update on physiological, clinical and analytical aspects. Clin Chim Acta 2000; 294: 1-26.
93. Bresink I, Danysz W, Parsons CG, Mutschler E. Different Binding Affinities of Nmda Receptor-Channel Blockers in Various Brain-Regions - Indication of Nmda Receptor Heterogeneity. Neuropharmacology 1995; 34: 533-40.
94. Iseri LT, French JH. Magnesium: nature's physiologic calcium blocker. Am Heart J 1984; 108: 188-93.
95. Eby GA, Eby KL. Rapid recovery from major depression using magnesium treatment. Med Hypotheses 2006; 67: 362-70.
96. Song Y, Manson JE, Cook NR, Albert CM, Buring JE, Liu S. Dietary magnesium intake and risk of cardiovascular disease among women. Am J Cardiol 2005; 96: 1135-41.
97. Young LT, Robb JC, Levitt AJ, Cooke RG, Joffe RT. Serum Mg2+ and Ca2+/Mg2+ ratio in major depressive disorder. Neuropsychobiology 1996; 34: 26-8.
98. Mirone M, Giannetta E, Isidori AM. Selenium and reproductive function. A systematic review. J Endocrinol Invest 2013; 36: 28-36.
99. Coskun A, Arikan T, Kilinc M, Arikan DC, Ekerbicer HC. Plasma selenium levels in Turkish women with polycystic ovary syndrome. Eur J Obstet Gynecold Reprod Biol 2013; 168: 183-6.
100. Rezvanfar MA, Rezvanfar MA, Ahmadi A, Shojaei-Saadi HA, Baeeri M, Abdollahi M. Molecular mechanisms of a novel selenium-based complementary medicine which confers protection against hyperandrogenism-induced polycystic ovary. Theriogenology 2012; 78: 620-31.
101. Stapleton SR. Selenium: an insulin-mimetic. Cell Mol Life Sci 2000; 57: 1874-9.
102. Zadeh Modarres S, Heidar Z, Foroozanfard F, Rahmati Z, Aghadavod E, Asemi Z. The Effects of Selenium Supplementation on Gene Expression Related to Insulin and Lipid in Infertile Polycystic Ovary Syndrome Women Candidate for In Vitro Fertilization: a Randomized, Double-Blind, Placebo-Controlled Trial. Biol Trace Elem Res 2018; 183: 218-25.
103. Jamilian M, Razavi M, Fakhrie Kashan Z, Ghandi Y, Bagherian T, Asemi Z. Metabolic response to selenium supplementation in women with polycystic ovary syndrome: a randomized, double-blind, placebo-controlled trial. Clin Endocrinol 2015; 82: 885-91.
104. Tubek S. Zinc supplementation or regulation of its homeostasis: advantages and threats. Biol Trace Elem Res 2007; 119: 1-9.
105. Chausmer AB. Zinc, insulin and diabetes. J Am Coll Nutr 1998; 17: 109-15.
106. Di Martino G, Matera MG, De Martino B, Vacca C, Di Martino S, Rossi F. Relationship between Zinc and Obesity. J Med 1993; 24: 177-83.
107. Bolkent S, Yanardag R, Bolkent S, Mutlu O, Yildirim S, Kangawa K, et al. The effect of zinc supplementation on ghrelin-immunoreactive cells and lipid parameters in gastrointestinal tissue of streptozotocin-induced female diabetic rats. Mol Cell Biochem 2006; 286: 77-85.
108. Guler I, Himmetoglu O, Turp A, Erdem A, Erdem M, Onan MA, et al. Zinc and Homocysteine Levels in Polycystic Ovarian Syndrome Patients with Insulin Resistance. Biol Trace Elem Res 2014; 158: 297-304.
109. Ciaraldi TP, el-Roeiy A, Madar Z, Reichart D, Olefsky JM, Yen SS. Cellular mechanisms of insulin resistance in polycystic ovarian syndrome. J Clin Endocrinol Metab 1992; 75: 577-83.
110. Dunaif A. Insulin resistance and the polycystic ovary syndrome: mechanism and implications for pathogenesis. Endocr Rev 1997; 18: 774-800.
111. Beletate V, El Dib RP, Atallah AN. Zinc supplementation for the prevention of type 2 diabetes mellitus. Cochrane Database Syst Rev 2007: CD005525.
112. Legro RS, Kunselman AR, Dunaif A. Prevalence and predictors of dyslipidemia in women with polycystic ovary syndrome. Am J Med 2001; 111: 607-13.
113. Ahmed Z, Tabrizi S, Li A, Houlden H, Sailer A, Lees AJ, et al. A case of Huntington's disease phenocopy characterised by pallido-nigro-luysian degeneration with brain-iron accumulation and p62-positive glial inclusions. Eur J Neurol 2010; 17: 379.
114. Adriaens I, Jacquet P, Cortvrindt R, Janssen K, Smitz J. Melatonin has dose-dependent effects on folliculogenesis, oocyte maturation capacity and steroidogenesis. Toxicology 2006; 228: 333-43.
115. Tamura H, Nakamura Y, Korkmaz A, Manchester LC, Tan DX, Sugino N, et al. Melatonin and the ovary: physiological and pathophysiological implications. Fertil Steril 2009; 92: 328-43.
116. Carlomagno G, Nordio M, Chiu TT, Unfer V. Contribution of myo-inositol and melatonin to human reproduction. Eur J Obstet Gynecol Reprod Biol 2011; 159: 267-72.
117. Wei D, Zhang C, Xie J, Song X, Yin B, Liu Q, et al. Supplementation with low concentrations of melatonin improves nuclear maturation of human oocytes in vitro. J Assist Reprod Genet 2013; 30: 933-8.
118. Tamura H, Nakamura Y, Terron MP, Flores LJ, Manchester LC, Tan DX, et al. Melatonin and pregnancy in the human. Reprod Toxicol 2008; 25: 291-303.
119. Kim MK, Park EA, Kim HJ, Choi WY, Cho JH, Lee WS, et al. Does supplementation of in-vitro culture medium with melatonin improve IVF outcome in PCOS? Reprod Biomed Online 2013; 26: 22-9.
120. Dickinson DA, Moellering DR, Iles KE, Patel RP, Levonen AL, Wigley A, et al. Cytoprotection against oxidative stress and the regulation of glutathione synthesis. Biol Chem 2003; 384: 527-37.
121. Liu J, Liu M, Ye X, Liu K, Huang J, Wang L, et al. Delay in oocyte aging in mice by the antioxidant N-acetyl-L-cysteine (NAC). Hum Reprod 2012; 27: 1411-20.
122. Santini MT, Cametti C, Indovina PL, Peterson SW. Membrane electrical properties associated with insulin receptor downregulation in human erythrocytes. Exp Hematol 1994; 22: 40-4.
123. Fulghesu AM, Ciampelli M, Muzj G, Belosi C, Selvaggi L, Ayala GF, et al. N-acetyl-cysteine treatment improves insulin sensitivity

in women with polycystic ovary syndrome. Fertil Steril 2002; 77: 1128-35.

124. Elnashar A, Fahmy M, Mansour A, Ibrahim K. N-acetyl cysteine vs. metformin in treatment of clomiphene citrate-resistant polycystic ovary syndrome: a prospective randomized controlled study. Fertil Steril 2007; 88: 406-9.
125. Oner G, Muderris, II. Clinical, endocrine and metabolic effects of metformin vs N-acetyl-cysteine in women with polycystic ovary syndrome. Eur J Obstet Gynecol Reprod Biol 2011; 159: 127-31.
126. Vargas ML, Almario RU, Buchan W, Kim K, Karakas SE. Metabolic and endocrine effects of long-chain versus essential omega-3 polyunsaturated fatty acids in polycystic ovary syndrome. Metabolism 2011; 60: 1711-8.
127. Calder PC. n-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases. Am J Clin Nutr 2006; 83(6 Suppl): 1505-19.
128. Poudyal H, Panchal SK, Diwan V, Brown L. Omega-3 fatty acids and metabolic syndrome: effects and emerging mechanisms of action. Prog Lipid Res 2011; 50: 372-87.
129. Shahnazi V, Zaree M, Nouri M, Mehrzad-Sadaghiani M, Fayezi S, Darabi M, et al. Influence of omega-3 fatty acid eicosapentaenoic acid on IGF-1 and COX-2 gene expression in granulosa cells of PCOS women. Iran J Reprod Med 2015; 13: 71-8.
130. Akinkuolie AO, Ngwa JS, Meigs JB, Djousse L. Omega-3 polyunsaturated fatty acid and insulin sensitivity: a meta-analysis of randomized controlled trials. Clin Nutr 2011; 30: 702-7.
131. Kasim-Karakas SE, Almario RU, Gregory L, Wong R, Todd H, Lasley BL. Metabolic and endocrine effects of a polyunsaturated fatty acid-rich diet in polycystic ovary syndrome. J Clin Endocrinol Metab 2004; 89: 615-20.
132. Phelan N, O'Connor A, Kyaw Tun T, Correia N, Boran G, Roche HM, et al. Hormonal and metabolic effects of polyunsaturated fatty acids in young women with polycystic ovary syndrome: results from a cross-sectional analysis and a randomized, placebo-controlled, crossover trial. Am J Clin Nutr 2011; 93: 652-62.
133. Kalgaonkar S, Almario RU, Gurusinghe D, Garamendi EM, Buchan W, Kim K, et al. Differential effects of walnuts vs almonds on improving metabolic and endocrine parameters in PCOS. Eur J Clin Nutr 2011; 65: 386-93.
134. Mohammadi E, Rafraf M. Benefits of omega-3 Fatty acids supplementation on serum paraoxonase 1 activity and lipids ratios in polycystic ovary syndrome. Health Promot Perspect 2012; 2: 197-204.
135. Rafraf M, Mohammadi E, Asghari-Jafarabadi M, Farzadi L. Omega-3 Fatty Acids Improve Glucose Metabolism without Effects on Obesity Values and Serum Visfatin Levels in Women with Polycystic Ovary Syndrome. J Am Coll Nutr 2012; 31: 361-8.
136. Oner G, Muderris II. Efficacy of omega-3 in the treatment of polycystic ovary syndrome. J Obstet Gynaecol 2013; 33: 289-91.
137. Roberfroid MB. Prebiotics and probiotics: are they functional foods? Am J Clin Nutr 2000; 71 (6 Suppl): 1682-7.
138. Fuller R. Probiotics in Human Medicine. Gut 1991; 32: 439-42.
139. Allen SJ, Jordan S, Storey M, Thornton CA, Gravenor M, Garaiova I, et al. Dietary supplementation with lactobacilli and bifidobacteria is well tolerated and not associated with adverse events during late pregnancy and early infancy. J Nutr 2010; 140: 483-8.
140. Ejtahed HS, Mohtadi-Nia J, Homayouni-Rad A, Niafar M, Asghari-Jafarabadi M, Mofid V. Probiotic yogurt improves antioxidant status in type 2 diabetic patients. Nutrition 2012; 28: 539-43.
141. Yadav H, Jain S, Sinha PR. Antidiabetic effect of probiotic dahi containing Lactobacillus acidophilus and Lactobacillus casei in high fructose fed rats. Nutrition 2007; 23: 62-8.
142. Shoaei T, Heidari-Beni M, Tehrani HG, Feizi A, Esmaillzadeh A, Askari G. Effects of Probiotic Supplementation on Pancreatic beta-cell Function and C-reactive Protein in Women with Polycystic Ovary Syndrome: A Randomized Double-blind Placebo-controlled Clinical Trial. Int J Prev Med 2015; 6: 27.
143. Goodarzi MO, Dumesic DA, Chazenbalk G, Azziz R. Polycystic ovary syndrome: etiology, pathogenesis and diagnosis. Nat Rev Endocrinol 2011; 7: 219-31.
144. Ahmadi S, Jamilian M, Karamali M, Tajabadi-Ebrahimi M, Jafari P, Taghizadeh M, et al. Probiotic supplementation and the effects on weight loss, glycaemia and lipid profiles in women with polycystic ovary syndrome: a randomized, double-blind, placebo-controlled trial. Hum Fertil (Camb) 2017; 20: 254-61.
145. Tremellen K, Pearce K. Dysbiosis of Gut Microbiota (DOGMA) - A novel theory for the development of Polycystic Ovarian Syndrome. Med Hypotheses 2012; 79: 104-12.
146. Guo Y, Qi Y, Yang X, Zhao L, Wen S, Liu Y, et al. Association between polycystic ovary syndrome and gut microbiota. Plos one 2016; 11: e0153196.
147. Arentz S, Smith CA, Abbott J, Bensoussan A. Nutritional supplements and herbal medicines for women with polycystic ovary syndrome; a systematic review and meta-analysis. BMC Complement Altern Med 2017; 17: 500.

What is your diagnosis?

A 25-year-old woman with unexplained infertility was admitted to our assisted reproductive technology clinic. Her fertility assessment was found to be normal. On her third menstrual day, the total antral follicle count was 15, anti-mullerian hormone level was 4.8 ng/mL, follicle-stimulating hormone level was 9 mIU/mL, and the estradiol level was 16 pg/mL. She had 4 previous in vitro fertilization attempts at various centers. In her first two cycles, no mature oocytes (M2) were revealed. Although 9 oocytes were collected in her second cycle, none was an M2 oocyte. In her third cycle, 13 oocytes were collected and 6 were M2. Only 1 was fertilized and embryo transfer was performed on the third day. In her fourth cycle, a dual trigger, consisting of a gonadotrophin-releasing hormone analogue and human chorionic gonadotrophin, was applied for the final oocyte maturation and ovulation trigger. A total of 19 oocytes were collected and none was M2; however, fertilization did not occur.

In her fifth cycle at our clinic, we initially checked all her previous fertility assessments, ovulation induction protocols, triggers administered, and reproductive outcomes. In light of her previous reports, we chose an antagonist protocol with 300 IU human menopausal gonadotrophin/day and applied a 'dual trigger'. The peak estradiol level on the oocyte trigger day was 1696 pg/mL and the total gonadotrophin dosage was 2400 IU. Eleven oocytes were collected, 1was M2, but fertilization did not ensue.

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Answer

Successful fertilization and embryonic development in humans needs union of sperm and a mature oocyte. For maturation, the oocyte has to undergo some changes including growth, and mRNA and proteins accumulation (1). During embryonic development, oocytes first undergo meiotic progression and become arrested in the diplotene stage of prophase I at the time of birth. Upon a surge in luteinizing hormone (LH) just before ovulation, oocytes resume meiosis and progress through the second meiotic cycle and arrest at metaphase II until fertilization, this is called oocyte maturation. Attaining this molecular competence requires multiple factors regulated by different signaling pathways.

Oocyte maturation arrest (OMA) is presumed to be due to inadequate LH activity, the defect in signaling mechanism surrounding cumulus cells or intrinsic oocyte factors (2). Although the exact mechanisms and causes of this disorder are not known, a genetic deficiency of regulatory proteins or genetic alterations of genes or protein expressions of regulatory proteins and enzymes, or oocyte-specific alterations of transcription factors may contribute (1,2). There are some treatment strategies as dual-trigger and double-trigger applications, but a definitive treatment modality is lacking; however, investigating the genetic basis of OMA will provide great insight into understanding the mechanisms of this disorder, as well as improving treatment strategies.

Although we reviewed all previous cycle reports and changed the ovulation induction protocol to an antagonist protocol co-treated with a dual trigger, we obtained only 1 mature oocyte out of 11.

We performed genetic testing in view of its possible genetic association. Thirty-six genes related with reproductive functions were analyzed and heterozygote mutations in PROKR2 p. T273M, FSH p. Ala307Thr and p. Ser680Asp genes were identified. Mutations in FSH p. Ala307Thr and p. Ser680Asp genes have been reported to be associated with poor ovarian reserve, and mutations in PROK2 genes were linked to hypogonadotropic hypogonadism (3), but the exact pathogenic mechanisms of these mutated genes and any association with OMA have not been elucidated thus far. In the literature, data regarding that subject is scarce and subject to investigation.

Address for Correspondence: *Nafiye Yılmaz*
e.mail: nafiyekarakas@gmail.com ORCID ID: orcid.org/0000-0002-4041-297X

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These findings may suggest some roles for these mutations in oocyte maturation arrest and expand our knowledge in terms of the genetic basis of female infertility. Unraveling molecular and genetic basis of OMA will help patients by improving diagnosis and our understanding of the disease. This will guide us in counseling patients about treatment outcomes, develop strategies to overcome this disorder, and allow for better informed decisions regarding treatment options and prevent unnecessary interventions.

Banu Seven Yüksel, Nafiye Yılmaz, Evin Nil Uğurlu, Cavidan Gülerman, Yaprak Engin-Üstün

Department of Obstetrics and Gynecology, University of Health Sciences, Ankara Dr. Zekai Tahir Burak Women Disease Training and Research Hospital, Ankara, Turkey

References

1. Ryu KY, Sinnar SA, Reinholdt LG, Vaccari S, Hall S, Garcia MA, et al. The Mouse Polyubiquitin Gene Ubb Is Essential for Meiotic Progression. Mol Cell Biol 2008; 28: 1136-46.
2. Sen A, Caiazza F. Oocyte maturation: a story of arrest and release. Front Biosci (Schol Ed) 2013; 5: 451-77.
3. Beall S, Brenner C, Segars J. Oocyte maturation failure: a syndrome of bad eggs. Fertil Steril 2010; 94: 2507-13.

How to facilitate laparoscopic extraperitoneal suture?

Erdoğan Nohuz[1,2], Nicolas Bourdel[2,3]

[1]Department of Obstetrics and Gynecological Surgery, Thiers Hospital, Thiers, France
[2]Université Clermont Auvergne, CHU Clermont-Ferrand, CNRS, SIGMA Clermont, Clermont-Ferrand, France
[3]Department of Obstetrics and Gynecology, University Hospital CHU Clermont-Ferrand, Place Lucie et Raymond Aubrac, Clermont-Ferrand, France

Abstract

To show a simplified technique of extraperitoneal laparoscopic suture. Step-by-step explanation of the technique using an educative video and pictures. This technique of extraperitoneal laparoscopic suture is highlighted through two laparoscopic procedures: a sacrocolpopexy (mesh fixation) for a pelvic organ prolapse and an ovariopexy after hysterectomy without adnexectomy (fibromatous uterus). This method avoids the need for repetitive use of the knot-pusher in performing extraperitoneal knots. Time saved in the operating room and limited gestures can theoretically contribute to decrease cost and improve safety. Although our intimate conviction goes in this direction, further studies are needed to better evaluate this procedure. Rehabilitating a process historically used during laparotomic procedures, this technique avoids iterative intra-abdominal gestures and expedites the knot-tying steps. (J Turk Ger Gynecol Assoc 2018; 19: 235-40)

Keywords: Laparoscopy, laparoscopic suture, suturing techniques, extracorporeal knots

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Introduction

Extraperitoneal laparoscopic sutures usually require iterative knots that are successively advanced into the abdominopelvic cavity with a knot pusher. Because this procedure can be tedious, we describe a simplified technique inspired by the Roeder's knot that may be applicable to any laparoscopic procedure requiring separate knots.

Interventions

This technique of extraperitoneal laparoscopic suture is highlighted through two surgical procedures: a mesh fixation during a sacrocolpopexy and an ovariopexy after interadnexal hysterectomy.

Sacrocolpopexy (mesh fixation) for a pelvic organ prolapse in a 61-year old patient (Figures 1-3): After the intervesical vaginal dissection is accomplished, a precut polypropylene mesh is secured to the anterior wall of the vagina and the uterine isthmus, using a braided non-absorbable polyester 2-0 suture of 90 cm of length with a half-circle needle of 26 mm (Ethibond®, Ethicon, Somerville, NJ, USA). Once this step is performed, the needle-holder introduced through the suprapubic operator port grasps the wire about 2 cm from its point of insertion on the needle and exits it. Thus, the needle is brought outside. A self-locking sliding knot is then made. To do this, a simple half-hitch knot is performed first. The end of the free strand (without the needle) makes three rounds around both suture limbs. A second half-knot is performed around one side of the suture limbs before the end of the free strand enters in the loop of the first half-hitch knot. By formalizing this knot, we obtain 1:3:1+1 (1 half-hitch, 3 winds and 2 locking half-hitches). This creates a sliding knot that will be lowered by simply pulling on the axial strand. The free strand is then cut to about one centimeter of the knot. Gentle but sustained traction allows the advancement of the knot, which is slid down the trocar into the abdominal cavity and comes to block itself once arrived at the

Address for Correspondence: *Erdoğan Nohuz*
e.mail: enohuz@yahoo.fr ORCID ID: orcid.org/0000-0002-8577-0682

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destination under permanent laparoscopic control. Once the knot is seated, the needle holder can maintain pressure on the knot to strengthen its tightening and lock it. The suture is then cut to a centimeter.

Ovariopexy in a 43-year-old woman after hysterectomy without adnexectomy (fibromatous uterus): The surgical procedure consists in the joigning of the round and utero-ovarian ligaments' stumps through a similar technique (Figures 4-8).

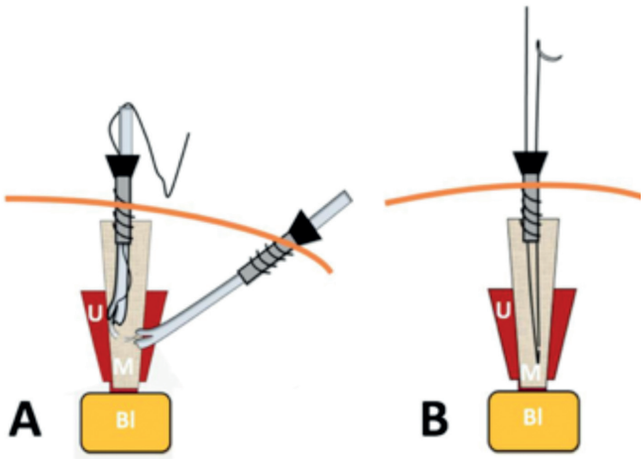


Figure 1. Operative steps of mesh fixation during a laparoscopic sacrocolpopexy Suture of the mesh to the uterine isthmus (a) and needle removal (b) (U: Uterus; M: Mesh; Bl: Bladder)

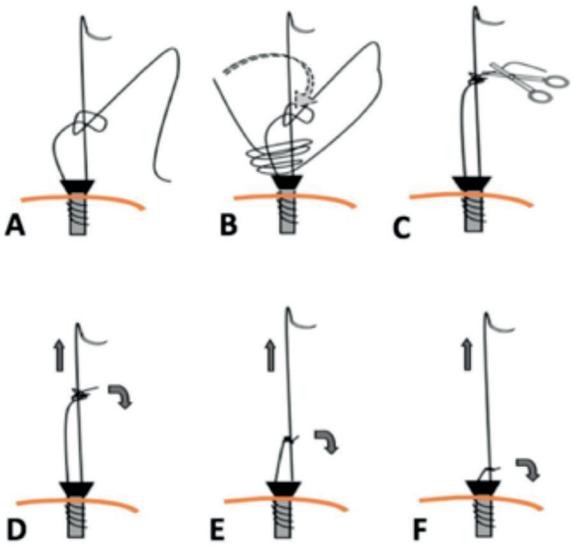


Figure 2. Operative steps of mesh fixation during a laparoscopic sacrocolpopexy Confection then advancement of the self-locking sliding knot (a-e)

Discussion

This technique avoids the need for repetitive use of the knot-pusher in performing extraperitoneal knots. Time saved in the operating room and limited gestures can theoretically contribute to decrease cost and improve safety. Although our intimate conviction goes in this direction, further studies are needed to better evaluate this procedure.

Knot safety depends mainly on the number of initial turns around the standing part and on the additional half-hitches to secure the knot afterwards (1,2). Three round turns seem sufficient if the ligature is braided, but 4 turns are needed to secure the knot if the suture material is slippery monofilament material. This method, which can thus contribute to improve safety as well as surgical ergonomics, is fully illustrated in the present work (graphical abstract and video). We also use this technique to perform an ovariopexy (accomplished after a laparoscopic inter-adnexal hysterectomy for benign pathology), which consists of the joining of the round and utero-ovarian ligaments' stumps. This ovariopexy is justified by the risk of ovarian torsion which can occur, probably due to the rare occurrence of adhesions and the fee long infundibulopelvic ligament that remains (3).

In order to secure this surgical procedure and reduce the risks of failure, prior practice on simulator seems necessary before its implementation.

Rehabilitating a process historically used during laparotomic procedures, this technique avoids iterative intra-abdominal gestures and expedite the knot-tying steps.

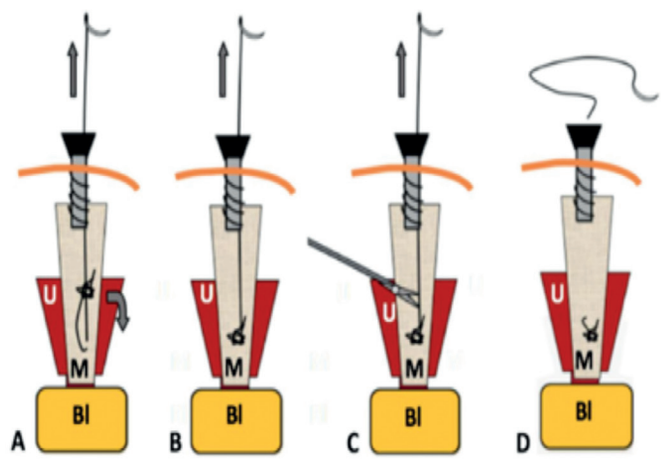


Figure 3. Operative steps of mesh fixation during a laparoscopic sacrocolpopexy Knot progression (a, b), section of the thread once the knot has arrived at destination and is tight (c) and wire removal (d)

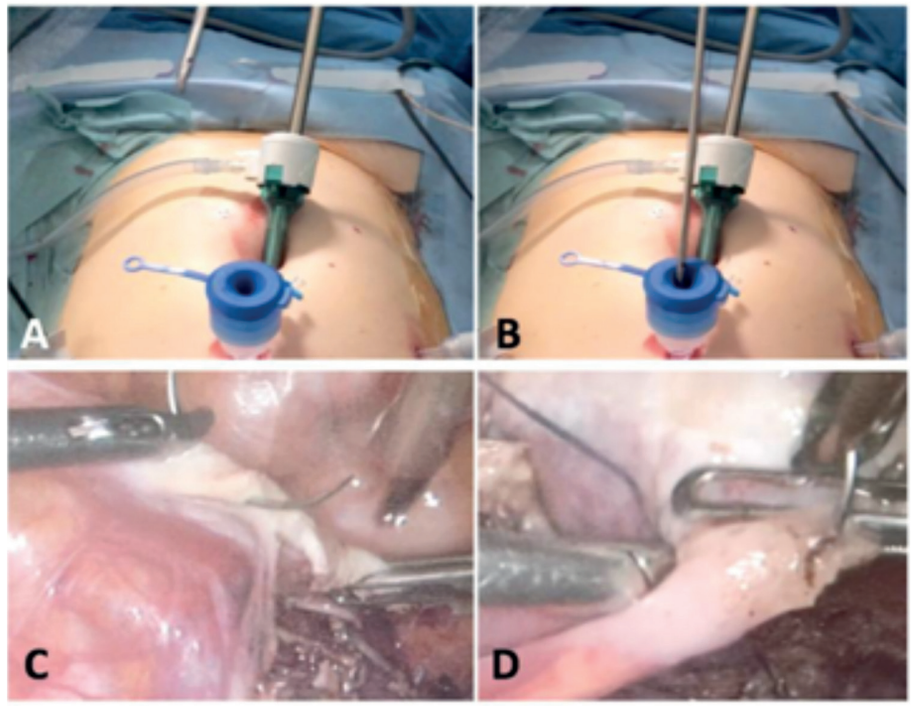


Figure 4. Operative steps during a laparoscopic ovariopexy
Introduction of the needle-holder loaded with the thread (a, b),
Ovariopexy consists of the joining of the round and utero-ovarian ligaments' stumps (c, d)

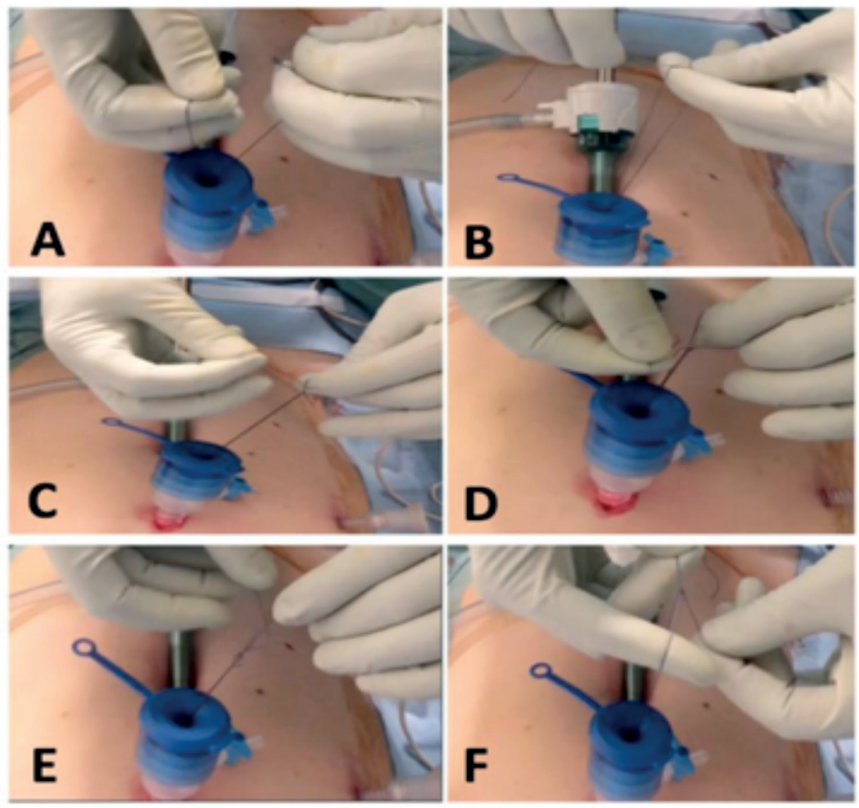


Figure 5. Operative steps during a laparoscopic ovariopexy
Confection of the self-locking sliding knot

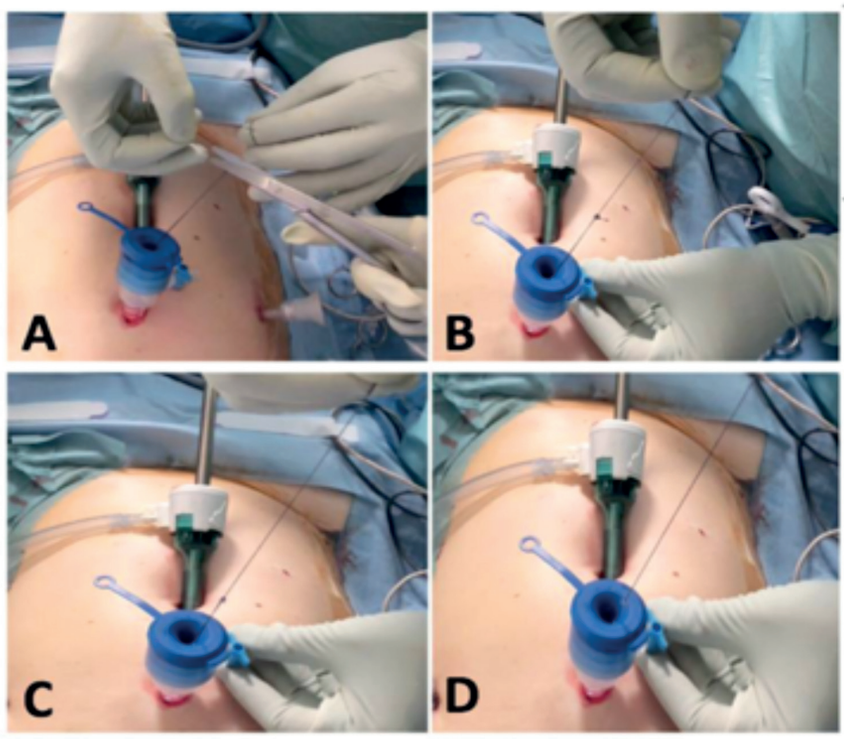


Figure 6. Operative steps during a laparoscopic ovariopexy
Section of the thread (a) and advancement of the knot (b-d)

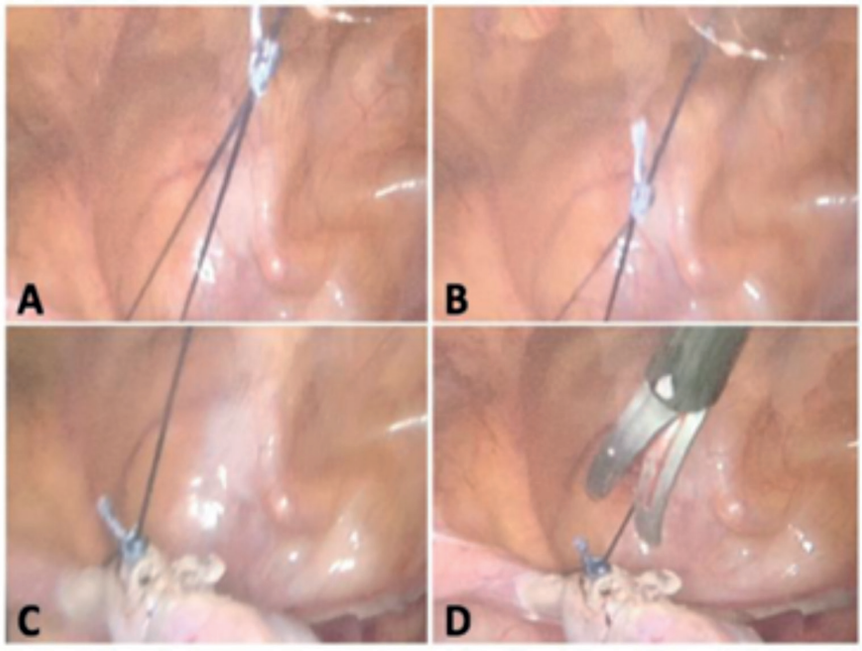
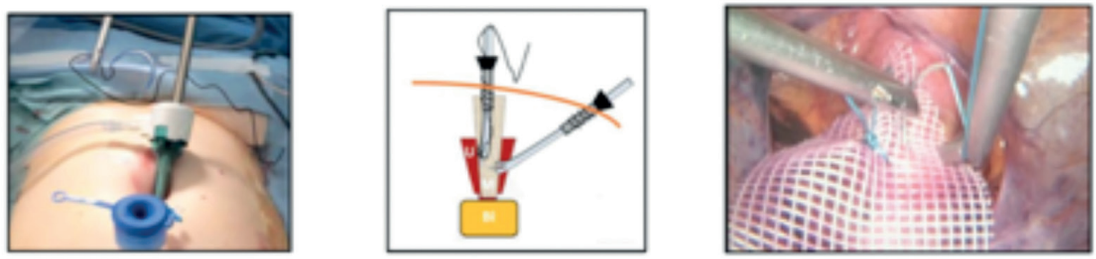


Figure 7. Operative steps during a laparoscopic ovariopexy
Once the knot has arrived at destination and is tight (a-c), the thread is cut (d)

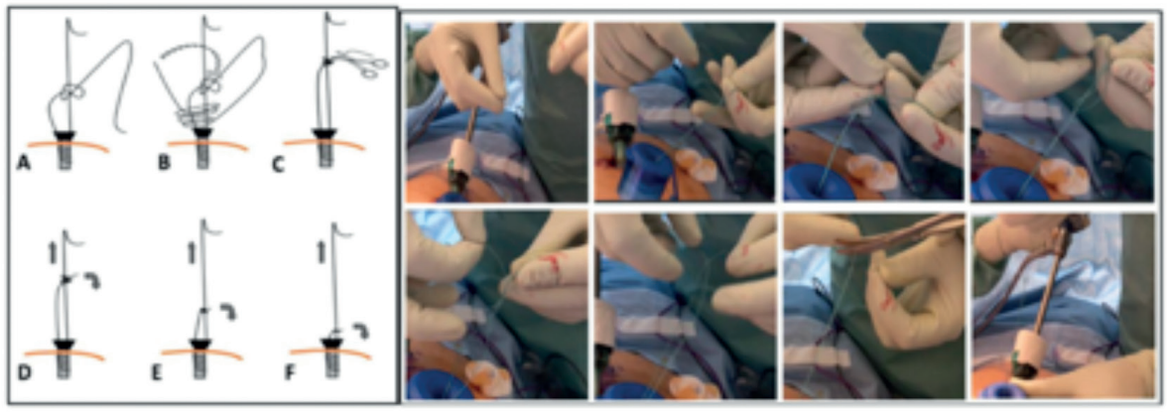
How to facilitate extraperitoneal suture during a laparoscopic sacrocolpopexy ?

1. Mesh fixation



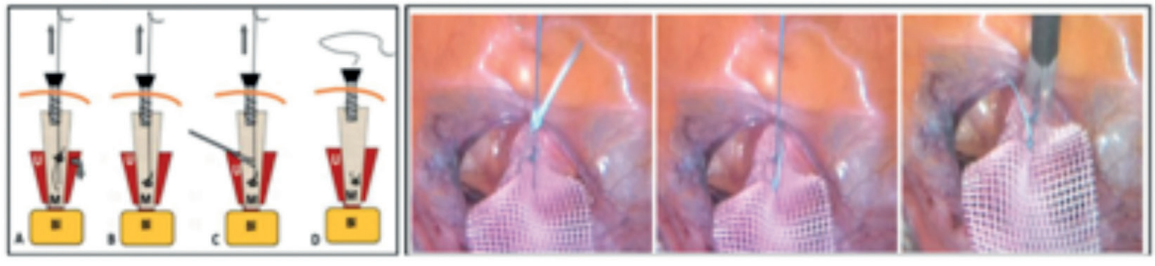
Suture of the mesh to the uterine isthmus. (U: Uterus; M: Mesh, BI: Bladder)

2. Confection then advancement of a self-locking knot and its extraperitoneal progression



A first half-hitch is performed. The end of the free strand (without the needle) makes three rounds around the both suture limbs. A second half knot is performed around one side of suture limbs before the end of the free strand enters in the loop of the first half-hitch knot.

3. Intraperitoneal knot progression



Section of the thread once the knot has arrived at destination and is tight.

Figure 8. Graphical abstract highlighting the surgical steps during a laparoscopic sacrocolpopexy

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Conflict of Interest: *No conflict of interest was declared by the authors.*

Financial Disclosure: *The authors declared that this study has received no financial support.*

References

1. Hage JJ. On the origin and evolution of the Roeder knot and loop-a geometrical review. Surg Laparosc Endosc Percutan Tech 2008; 18: 1-7.
2. Kadirkamanathan SS, Shelton JC, Hepworth CC, Laufer JG, Swain CP. A comparison of the strength of knots tied by hand and at laparoscopy. J Am Coll Surg 1996; 182: 46-54.
3. Mashiach R, Canis M, Jardon K, Mage G, Pouly JL, Wattiez A. Adnexal torsion after laparoscopic hysterectomy: description of seven cases. J Am Assoc Gynecol Laparosc 2004; 11: 336-9.

ERRATUM

DOI: 10.4274/jtgga.2017.0115

Kurdoğlu Z, Carr D, Harmouche J, Ünlü S, Kılıç GS. Short-term results of the efficacy of percutaneous tibial nerve stimulation on urinary symptoms and its financial cost. J Turk Ger Gynecol Assoc 2018; 19: 7-10.

The author order of the article has been corrected as following:

Kurdoğlu Z, Carr D, Harmouche J, Kurdoğlu M, Ünlü S, Kılıç GS. Short-term results of the efficacy of percutaneous tibial nerve stimulation on urinary symptoms and its financial cost. J Turk Ger Gynecol Assoc 2018; 19: 7-10.

2018 Referee Index

Acknowledgements for the Year 2018
(Reviewers contributed at the review process in 2018)

On behalf of the office staff and the Editorial Board of the *Journal of The Turkish German Gynecological Association*, we would like to thank to all of our reviewers of the past year for their outstanding contributions. Their thorough reviews and expertise enable our journal to improve its scientific quality. We certainly look forward to their ongoing support, suggestions and recommendations as to how to continue to advance the overall quality of the *Journal of The Turkish German Gynecological Association*.

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2018 Referee Index

2018 Subject Index

2018 Subject Index

2018 Author Index

2018 Author Index

2018 Author Index

CONGRESS CALENDER

INTERNATIONAL MEETINGS

(for detailed International Meeting please go website:
http://www.medical.theconferencewebsite.com/conferences/obstetrics-and-gynaecology)

November 11-15, 2018	**47th AAGL Global Congress on Minimally Invasive Gynecology 2018, Las Vegas, United States**
December 4-7, 2018	**French College of Obstetrıcs and Gynaecology 42nd National Congress 2018, Strasbourg, France**
December 5-8, 2018	**Pelvic Anatomy and Gynecologic Surgery Symposium 2018, Las Vegas, United States**
December 14-16, 2018	**Fertivision 14th Annual Congress of Indian Fertility Society 2018, Kerala, India**
January 2-6, 2019	**Obstetrics, Gynecology, Perinatal Medicine, Neonatology and the Law 35th Annual Conference 2019, Bahamas**
January 18-20, 2019	**Maternal-Fetal Imaging 2019: Advances in Ob-Gyn Ultrasound, San Antonio, United States**
January 28-31, 2019	**Arab Health Obs-Gyne Conference 2019, Dubai, United Arab Emirates**
February 3-6, 2019	**Mayo Clinic Ob/Gyn Clinical and Surgical Updates: Staying Current and Ahead of the Curve 2019, California, United States**
February 6-9, 2019	**23rd Annual Winter Conference on Clinical Issues in Ob/Gyn 2019, Oahu, United States**
February 7-9, 2019	**Gynaecologic Oncology Group Semi-Annual Meeting 2019, Phoenix, United States**
February 13-16, 2019	**Office Gynecology and Women's Health for the Primary Care Provider 26th Annual Conference 2019, Naples, FL, United States**
February 26-March 2, 2019	**Society of Urodynamics, Female Pelvic Medicine and Urogenital Reconstruction Winter Meeting 2019, Miami, FL, United States**
March 17-22, 2019	**Obstetrics and Gynecology 56th Annual Update 2019, Boston, MA, United States**
March 31-April 3, 2019	**Society of Gynecologic Surgeons 45th Annual Meeting 2019, Arizona, United States**
April 12-14, 2019	**Survival Skills for Today's Gynecologist 2019, Manhattan, United States**

CONGRESS CALENDER

NATIONAL MEETINGS

(for detailed International Meeting please go website:
http://www.kongre2017.com)

November 17-18, 2018	**2. Türk-Rus Ürojinekoloji Sempozyumu, İstanbul, Turkey**
November 21-25, 2018	**Ulusal Jinekolojik Onkoloji Kongresi, Antalya, Turkey**
December 14-15, 2018	**1. Uluslararası Hemşirelik ve İnnovasyon Kongresi, İstanbul, Turkey**
February 14-17, 2019	**3. Koru Gebelik, Lohusalık ve Doğum Kongresi, Bolu, Turkey**
February 20-24, 2019	**2. Minimal İnvazif Jinekolojik Cerrahi Kongresi, İstanbul, Turkey**
March 7-10, 2019	**14. Uludağ Jinekoloji ve Obstetrik Kış Kongresi, Bursa, Turkey**
March 13-17, 2019	**6. MESGE ve 8. Ulusal Jinekolojik Endoskopi Kongresi, Antalya, Turkey**
March 29-31, 2019	**7. Acıbadem Kadın Doğum Günleri, İstanbul, Turkey**
April 4-7, 2019	**Karadeniz Jinekoloji ve Obstetrik Kongresi, Samsun, Turkey**
April 18-19, 2019	**3. Uluslararası Kadın Çocuk Sağlığı ve Eğitimi Kongresi, Trabzon, Turkey**
April 18-21, 2019	**ÇİSED 4. Ulusal Cinsel Sağlık Kongresi, Antalya, Turkey**
April 24-28, 2019	**17. Ulusal Jinekoloji ve Obstetrik Kongresi, Antalya, Turkey**

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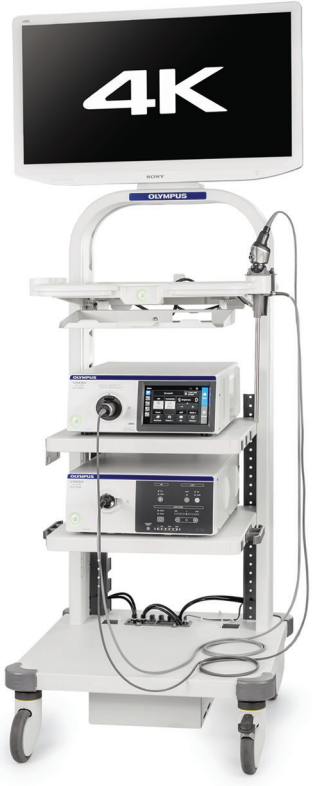

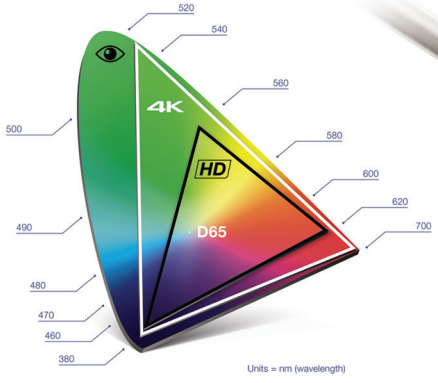

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Venöz veya arteriyel trombotik/tromboembolik olayların (örneğin derin ven trombozu, pulmoner emboli, miyokard enfarktüsü) veya serebrovasküler bir olayın varlığı ya da öyküsü, Tromboz prodromu varlığı veya öyküsü (örneğin geçici iskemik atak, anjina pektoris), Fokal nörolojik belirtili migren öyküsü, Vasküler tutulumlu diabetes mellitus, Venöz veya arteriyel tromboz için ciddi ya da bir çok risk faktörünün varlığı da kontrendikasyon olarak kabul edilir (Bkz. Uyarılar/Önlemler), Pankreatit veya şiddetli hipertrigliseridemi ile bağlantılı pankreatit öyküsü. Karaciğer fonksiyon değerleri normale dönmedikçe, ciddi karaciğer hastalığı öyküsü veya varlığı, Şiddetli veya akut böbrek yetmezliği, Karaciğer tümörü varlığı veya öyküsü (iyi veya kötü huylu), Eğer seks steroidlerinden etkileniyorsa genital organların veya memenin bilinen ya da şüpheli malign hastalıkları, Tanı konulmamış vaginal kanama, Bilinen gebelik veya şüphesi, Etkin ya da yardımcı maddelerden herhangi birine aşırı duyarlılık hali. **Uyarılar/Önlemler:** Dolaşım bozuklukları: Epidemiyolojik çalışmalar, kombine oral kontraseptif kullanımıyla miyokard enfarktüsü, inme, derin ven trombozu ve akciğer embolisi gibi arteriyel ve venöz trombotik/tromboembolik hastalıkların risk artışı arasında bir ilişki bulunduğunu belirtmektedirler. Bu olaylar ender olarak ortaya çıkmaktadır. Derin ven trombozu ve/veya pulmoner emboli şeklinde ortaya çıkan venöz tromboemboli (VTE) tüm kombine oral kontraseptiflerin kullanımı sırasında ortaya çıkabilir. Kombine oral kontraseptif kullananlarda, çok ender olarak, hepatik, mezenterik, renal, serebral veya retinal venler ve arterler gibi diğer kan damarlarında da tromboz bildirilmiştir. Kombine oral kontraseptif kullanımı ile bu olayların ortaya çıkması arasındaki nedensel ilişki halen tartışmalıdır. Venöz veya arteriyel trombotik/tromboembolik durumlar ya da serebrovasküler olay riski aşağıdaki faktörlerle artar: Yaş, Sigara kullanılması, Olası aile öyküsü, Obesite, Dislipoproteinemi, Hipertansiyon, Migren, Kalp kapak hastalığı, Atriyal fibrilasyon, Uzun süreli immobilizasyon. Lohusalık döneminde tromboemboli gelişimi riskinin arttığı göz önüne alınmalıdır. Kombine oral kontraseptiflerin kullanılması sırasında, migrenin sıklığında ve şiddetinde artış ortaya çıkması (bir serebrovasküler olayın habercisi olabilmesi açısından) ilacın derhal kesilmesi için bir neden olabilir. Tümörler: Bazı epidemiyolojik çalışmalarda uzun süre kombine oral kontraseptif kullananlarda servikal kanser riskinde artış görüldüğü bildirilmiştir. Ancak bu bulguların seksüel davranış ve human papilloma virus (HPV) gibi diğer faktörlerle bağlantısı da halen tartışılmaktadır. 54 epidemiyolojik çalışmayı kapsayan bir meta-analiz sonuçlarına göre halen oral kontraseptif kullanan kadınlarda meme kanserine rastlanma oranında hafif bir artış olduğu rapor edilmiştir. Bu risk artışı oral kontraseptif kullanımının kesilmesiyle birlikte 10 yıl içinde göreceli olarak ortadan kalkar. Meme kanseri görülme sıklığı 40 yaşın altındaki kadınlarda düşük olduğundan, bu açıdan meme kanseri riski fazla anlamlı değildir. Kombine oral kontraseptif kullanıcılarında nadir olgularda iyi huylu, çok nadiren de habis karaciğer tümörleri gözlemlenmiştir. Sınırlı olguda bu tümörler yaşamı tehdit eden batın içi kanamalara yol açar. **Diğerleri:** Böbrek yetmezliği olan hastalarda potasyum atılım kapasitesi sınırlı olabilir. Hipertrigliseridemisi olan ya da bu şekilde bir aile öyküsüne sahip bulunan kadınlarda, kombine oral kontraseptif kullanımıyla pankreatit gelişimi riskinde artış ortaya çıkabilir. Kombine oral kontraseptif alan kadınların çoğunda kan basıncında hafif artış görüldüğü bildirilmesine rağmen, klinik olarak anlamlı artış enderdir. Karaciğer fonksiyonlarında görülen akut ve kronik değişiklikler, kombine oral kontraseptif kullanımının fonksiyon testi değerleri normale dönene dek kesilmesini gerektirebilmektedir. Gebelik sırasında ilk kez ortaya çıkan ya da daha önce seks steroidlerinin kullanıldığı sırada görülmüş olan kolestatik sarılığın nüks etmesi kombine oral kontraseptif kullanımının kesilmesi gerekliliğini göstermektedir. Kombine oral kontraseptif kullanan diyabetik kadınlar dikkatle gözlenmelidir. Crohn hastalığı ve ülseratif kolit kombine oral kontraseptif kullanımı ile ilişkilendirilmiştir. Özellikle gebelik maskesi öyküsü olan kadınlarda daha belirgin olmak üzere kloazma ortaya çıkabilir. Kloazma eğilimi olan kadınlar kombine oral kontraseptif kullanımı esnasında güneşe çıkmaktan ya da ultraviyole ışınlarına maruz kalmaktan kaçınmalıdır. Azalmış etkinlik: Kombine oral kontraseptiflerin etkinliği tablet alımı unutulduğunda (Bkz. Tablet alımı unutulduğunda), mide-barsak bozuklukları olması halinde (Bkz. Mide-barsak bozuklukları durumunda), ya da eş zamanlı ilaç tedavilerinde (Bkz. İlaç Etkileşmeleri) azalabilir. Azalmış siklus kontrolü: Tüm kombine oral kontraseptiflerde, özellikle kullanımın ilk aylarında düzensiz kanamalar (lekelenme veya kırılma kanamaları) gelişebilir. Eğer kanama düzensizliği devam eder veya kanamalar düzenliyken ortaya çıkarsa non-hormonal etkenler göz önüne alınmalı ve malignite veya gebeliğin ekarte edilmesi için kürtajın da dahil olabileceği uygun tanısal girişimlerde bulunulmalıdır. Bazı kadınlarda tablet alınmayan dönemde çekilme kanaması oluşmayabilir. **Yan etkiler/advers etkiler:** Kombine oral kontraseptiflerin kullanımıyla ilişkilendirilen en ciddi yan etkiler "Uyarılar/Önlemler" bölümünde ele alınmıştır. Aşağıdaki diğer yan etkiler kombine oral kontraseptif kullanıcılarında bildirilmiş ve ilişkileri ne doğrulanmış ne de yanlışlığı kanıtlanmıştır. Göz: kontakt lense toleranssızlık; Gastrointestinal sistem: bulantı, kusma, batında ağrı, diyare; İmmun sistem: hipersensitivite; Metabolizma ve beslenme: sıvı retansiyonu, ağırlık artışı, ağırlık azalması; Sinir sistemi: başağrısı, migren, libido azalması, libido artışı, depresif duygu durumu, duygu durum değişiklikleri; Üreme sistemi ve meme: meme hassasiyeti, meme ağrısı, memede hipertrofi, memede akıntı, vaginal akıntı; Cilt ve ciltaltı: döküntü, ürtiker, eritema nodozum, eritema multiforme. **İlaç etkileşmeleri:** Oral kontraseptifler ve diğer ilaçlar arasındaki etkileşimler kırılma kanamalarına ve/veya kontraseptif başarısızlığa yol açabilirler. Aşağıdaki etkileşimler literatürde bildirilmiştir. Hepatik metabolizma: Mikrozomal enzimleri etkileyen ilaçlarla (ör. fenitoin, barbitüratlar, primidon, karbamazepin, rifampisin ve muhtemelen okskarbazepin, topiramat, felbamat, ritanovir, griseofulvin ve "St. John's wort" içeren ürünler) olan etkileşimler, seks hormonlarının klerensinin artması ile sonuçlanabilir. Enterohepatik dolaşımla etkileşmeler: Belirli antibiyotik ajanların (ör. penisilinler, tetrasiklinler) verilmesi durumunda estrogenlerin enterohepatik dolaşımının azalabileceğini ve bunun da etinilestradiol düzeylerini azaltabileceğini savunan klinik raporlar mevcuttur. Kullanım şekli ve dozu: Kullanım: Tabletler paketin üstünde gösterildiği yönde, hergün yaklaşık aynı zamanda bir miktar suyla alınmalıdır. Birbirini izleyen 21 gün boyunca hergün bir tablet alınır. Her bir sonraki pakete 7 günlük, sıklıkla çekilme kanamasının izlendiği, tablet alınmayan dönemi takiben geçilir. Bu kanama genellikle son tabletin alınmasını takiben 2.-3. gün başlar ve bir sonraki pakete başlandığında kesilmemiş olabilir. Tablet alımı unutulduğunda: Eğer kullanıcı tabletini almakta 12 saatten daha az geç kalmışsa, kontraseptif koruyuculuk azalmaz. Hatırlanır hatırlanmaz tablet alınmalı ve bir sonraki tabletler de her zamanki gibi alınmaya devam edilmelidir. Eğer 12 saatten daha fazla gecikme olmuşsa kontraseptif koruyuculuk azalmış olabilir. Mide-barsak bozuklukları durumunda: Şiddetli gastrointestinal bozuklukların olması durumunda emilim tam olmayabilir ve ek kontraseptif önlemler alınmalıdır. **Ticari takdim şekli:** PVC/Aluminyum blister'de 63 (3x21) adet film kaplı tablet. **Ruhsat tarihi:** 20.02.2002, **Ruhsat no:** 111/87, **Ruhsat sahibi:** Bayer Türk Kimya San. Ltd. Şti., Fatih Sultan Mehmet Mah. Balkan Cad. No:53 34770 Ümraniye - İstanbul Tel: (0216) 528 36 00 Faks: (0216) 538 37 40 Reçete ile satılır. 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