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Clinical Investigations

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galenos yayınevi

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· The declaration of transparency from the corresponding author.

· Clinical trial registration, if applicable.

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STARD checklist for the reporting of studies of diagnostic accuracy (Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig LM, et al, for the STARD Group. Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative. Ann Intern Med 2003;138:40-4.) (http://www.stard-statement.org/),

STROBE statement-checklist of items that should be included in reports of observational studies (http://www.strobe-statement.org/),

MOOSE guidelines for meta-analysis and systemic reviews of observational studies (Stroup DF, Berlin JA, Morton SC, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting Meta-analysis of observational Studies in Epidemiology (MOOSE) group. JAMA 2000; 283: 2008-12).

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- A short title of no more than 50 characters, including spaces, for use as a running foot.

- All author name(s), institutional, corporate, or commercial affiliations, and up to two major degree(s).

- Corresponding author's name, address, telephone (including the mobile phone number), fax numbers and e-mail address (the corresponding author will be responsible for all correspondence and other matters relating to the manuscript).

Precis

The precis is a one-sentence synopsis of no more than 30 words that describes the basic findings of the article. Precis sample can be seen below:

'Using a 45 point questionnaire, we have evaluated the trend of Robotic surgery training in the gynecologic surgery fellowship programs across the nation'.

Abstract

All manuscripts should be accompanied by an abstract. All information in the abstract should be consistent with the information in the text, tables, or figures. Avoid use of commercial names in the abstract. Original research reports should have a structured abstract of no more than 250 words, using the following headings:

· Objective: Main question, objective, or hypothesis (single phrase starting with, for example, "To evaluate..." or "To estimate." [never start with "To determine."]).

· Materials and Methods: Study design, participants, outcome measures, and in the case of a negative study, statistical power.

· Results: Measurements expressed in absolute numbers and percentages, and when appropriate indicate relative risks or odds ratios with confidence intervals and level of statistical significance; any results contained in the abstract should also be presented in the body of the manuscript, tables, or figures.

· Conclusion: Directly supported by data, along with clinical implications.

Authors from Turkey or Turkish speaking countries are expected to submit a Turkish abstract including subheadings such as "Amaç, Gereç ve Yöntemler, Bulgular, Sonuç". The abstract of Authors whose native language is not Turkish will be provided free of charge translation services into Turkish language.

A structured abstract is not required with review articles and case reports.

Keywords

Below the abstract provide 3 to 5 keywords. Abbreviations should not be used as keywords. Keywords should be picked from the Medical Subject Headings (MeSH) list (www.nlm.nih.gov/mesh/MBrowser.html).

Turkish abstracts should have keywords "Anahtar Kelimeler" picked from www.atifdizini.com under "Türkiye Bilim Terimleri" link.

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Article type	Abstract Length	Manuscript Word Count*	Maximum Number of Authors	Maximum Number of References$^{\Phi}$
Original Research	250 words	5,500 words (~22 pages)$^{\Psi}$	NA	30
Case report	150 words	2,000 words (~8 pages)	4	8
Systematic review	300 words	6,250 words (~25 pages)	4	60
Current commentary	250 words	3,000 words (~12 pages)	4	12
Procedure and Instruments	200 words	2,000 words (~8 pages)	4	10
Letters	NA	350 words	4	5

*Manuscript length includes all pages in a manuscript (ie, title page, abstract, text, references, tables, boxes, figure legends, and appendixes). $^{\Phi}$Suggested limit. $^{\Psi}$The Introduction should not exceed 250 words. ~approximately; NA, not applicable.

Original researches should have the following sections;

Introduction

State concisely the purpose and rationale for the study and cite only the most pertinent references as background. Avoid a detailed literature review in this section.

Materials and Methods

Describe the research methodology (the patients, experimental animals, material and controls, the methods and procedures utilized, and the statistical method(s) employed) in sufficient detail so that others could duplicate the work. Identify methods of statistical analysis and when appropriate, state the basis (including alpha and beta error estimates) for their selection. Cite any statistical software programs used in the text. Express p values to no more than two decimal places. Indicate your study's power to detect statistical difference.

Address "IRB" issues and participants informed consent as stated above, the complete name of the IRB should be provided in the manuscript. State the generic names of the drugs with the name and country of the manufactures.

Results

Present the detailed findings supported with statistical methods. Figures and tables should supplement, not duplicate the text; presentation of data in either one or the other will suffice. Authors should report

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outcome data as both absolute and relative effects since information presented this way is much more useful for clinicians. Actual numbers and percentages should be given in addition to odds ratios or relative risk. When appropriate, number needed to treat for benefits (NNTb) or harm (NNTh) should be supplied. Emphasize only your important observations; do not compare your observations with those of others. Such comparisons and comments are reserved for the discussion section.

Discussion

Begin with a description of what your study found in relation to the purpose or objectives as stated in the Introduction. State the importance and significance of your findings to clinicians and actual patient care but do not repeat the details given in the Results section. Limit your opinions to those strictly indicated by the facts in your report. Compare your finding with previous studies with explanations in cases where they differ, although a complete review of the literature is not necessary.

Study Limitations

Provide information on the limitations of the study. No new data are to be presented in this section. A final summary is not necessary, as this information should be provided in the abstract and the first paragraph of the Discussion. Although topics that require future research can be mentioned, it is unnecessary to state, "Further research is needed."

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The conclusion of the study should be highlighted. The study's new and important findings should be highlighted and interpreted.

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Authors must indicate whether or not they have a financial relationship with the organization that sponsored the research.

The main text of case reports should be structured with the following subheadings:

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TURKISH JOURNAL OF OBSTETRICS AND GYNECOLOGY

LETTER FROM THE PRESIDENT

Dear Collagues,

I am glad that our journal to reach this satisfactory level with your great contributions. I am also aware of the fact that we have to move forward in order to further progress.

I herein thank in the first place to the editor of the journal and also those who work hard to make the journal worthy of the doctors countrywide.

I especially thank the obstetrics and gynecology community for your support for the TJOD congress which will be held between May 9-13, 2018. We are preparing for our congress with a great excitement and enthusiasm.

I will be pleased to meet and recognize a few new academician collague as it occurs in every congress. I think this congress would be an unique opportunity for our young collagues to express and show themselves, and to enhance their scientific knowledge as well.

In addition, there would be a marvellous ambiance for the young collagues with the most experienced and knowledgeable colleagues of all the major branches of our specialty, to inform all the women about the reproductive health.

I look forward to seeing each and every one of our precious collague in our congress which will be held between May 9-13, 2018.

Best regards,

Ateş Karateke, Prof. MD
President of TJOD

TURKISH JOURNAL OF OBSTETRICS AND GYNECOLOGY

EDITORIAL

Dear Colleagues,

After our inclusion in the PubMed the manuscript submissions to our journal trippled. I would like to thank to all the scientists who gives us the oppurtunity to evaluate their work. The visibility and fulltext download of our articles exceeded 1500 during the first half of September 2017. This year our journal received second highest number of submissions in its history. In 2013 we have received 228 manuscripts and in 2017 we have received 200 manuscripts. The manuscripts submitted are mostly in the field of Perinatology followed by Reproductive Endocrinology and infertility, Gynecological Oncology and Urogynecology.

Our acceptance rate was 26% in 2017. We try to be constructive and provide feedback to the authors about possible sources of bias, methodological flaws, reporting errors and we encourage the authors to write the limitations of their studies. I think this provides an area of scientific discussion among colleagues and improve experience of every one of us even the manuscripts are not accepted. We would like to thank to our 228 reviewers and our Editorial Board for donating their time to our journal just for improvement of science. In 2018 we are planning a series of Systematic Review and Metaanalysis courses for encouraging scientists to evaluate the literatüre and provide a highest level of scientific information for the readers of our journal. Please save the date of first course on 17-18 February 2018. Those who wants to join this team can mail to me.

I wish all the best in the new year.

Eray Çalışkan
Editor in Chief

Clinical Investigation / Araştırma

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Turk J Obstet Gynecol 2018;15:1-7

Emergency peripartum hysterectomy in the Dubai health system: A fifteen year experience

Dubai sağlık sisteminde acil peripartum histerektomi: On beş yıllık tecrübe

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Abstract

Objective: To determine the incidence, demographic data, risk factors, indications, outcome and complications of emergency peripartum hysterectomy (EPH) performed in two major tertiary care hospitals in Dubai, and to compare the results with the literature.

Materials and Methods: The records of all women who underwent EPH from January 2000 to December 2015 in two major tertiary care hospitals in Dubai were accessed and reviewed. Maternal characteristics, hysterectomy indications, outcomes, and postoperative complications were recorded using descriptive statistics to describe the cohort.

Results: There were 79 EPH out of 168.293 deliveries, a rate of 0.47/1000 deliveries. The most common indications for hysterectomy were abnormal placentation (previa and/or accreta) and uterine atony. The majority of hysterectomies were subtotal (70%). The complications were dominated by massive transfusion, urinary tract injuries, one case of maternal death, and one case of neonatal death.

Conclusion: The main indication for EPH was abnormal placentation in scarred uterus and uterine atony. The major method of prevention of EPH is to assess women's risks and to reduce the number of cesarean section deliveries, by limiting the rate of primary cesareans. This is challenging in the United Arab Emirates (UAE) where the culture is for high gravidity and high parity. Recommendations to act to reduce primary and repeated cesareans should be included on the national agenda in UAE.

Keywords: Emergency hysterectomy, peripartum hysterectomy, abnormal placentation, risk factors

Öz

Giriş: Acil peripartum histerektominin (APH) insidansını, demofrafik verisini, risk faktörlerini, endikasyonlarını, sonlanım ve komplikasyonlarını belirlemek için Dubai'de iki büyük üçüncül bakım hastanesinde çalışmalar yapıldı ve sonuçlar literatürle karşılaştırıldı.

Gereç ve Yöntemler: Ocak 2000'den Aralık 2015'e kadar Dubai'deki iki büyük üçüncül bakım hastanesinde APH ameliyatı olan tüm kadınların kayıtlarına erişildi ve gözden geçirildi. Kohortu tanımlamada betimsel istatistik kullanılarak anneye ait özellikler, histerektomi endikasyonları, sonlanım ve postoperatif komplikasyonlar kaydedildi.

Bulgular: 168,293 doğumdan 79'u APH idi (0,47/1000 oranında). Histerektomide en yaygın endikasyonlar anormal plasentasyon (previa ve/veya akreta) ve uterus atonisiydi. Histerektomilerin büyük çoğunluğu subtotaldi (%70). Komplikasyonlara; masif transfüzyon, üriner sistem hasarları, bir anne ölümü ve bir de yenidoğan ölümü olgusu hakimdi.

Sonuç: APH'nin asıl endikasyonu yaralı uterus ve uterus atonisinde anormal plasentasyondu. APH'nin temelde önlenmesi; primer sezaryen oranını sınırlayarak, kadınlardaki risklerin değerlendirilmesi ve sezaryen doğumlarını azaltmaktır. Bu durum, kültürel hayatın yüksek yoğunluk ve yüksek doğum sayısı ile devam ettiği Birleşik Arap Emirlikleri'ni (BAE) zorlamaktadır. Primer ve tekrarlayan sezaryeni azaltmak için harekete geçme tavsiyeleri, BAE yerel gündemine dahil edilmelidir.

Anahtar Kelimeler: Acil histerektomi, peripartum histerektomi, anormal plasentasyon, risk faktörleri

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PRECIS: The purpose of this study was to review the incidence of cesarean sections, risk factors, indications, medical and surgical management, and the outcomes of peripartum hysterectomy over the past 15 years in Dubai.

Introduction

Emergency peripartum hysterectomy (EPH) is an uncommon obstetric procedure performed after 20 weeks of gestation and within twenty-four hours of birth, usually performed as a life-saving measure in cases of intractable obstetric hemorrhage. We could not find any epidemiologic evidence of the incidence rate of EPH in the United Arab Emirates (UAE) and desired to know if it was similar to the estimated 1.5 per 1.000 deliveries that have been reported in countries with modern health care facilities[1]. Dubai won the "Highly Recommended Destination of the Year" award from the Medical Travel Journal in 2016 and is actively becoming a medical tourism destination expecting more than one million medical tourists by 2020[2]. Many of these tourists will be pregnant and we are concerned with the world wide increase in EPH and our need to understand its impact on all patients and the health system in the UAE. We realize that postpartum hemorrhage (PPH) remains a significant threat to maternal outcomes despite technological and pharmacologic advances. Globally, obstetric hemorrhage is still the leading cause of maternal mortality[3] and therefore peripartum hysterectomy poses a challenging complication for obstetricians given the high risk of maternal death and morbidity[4]. We understand that management of EPH is complicated by the need for massive blood transfusions, injury to the urinary tract, coagulopathy, and the need of re-exploration, followed by prolonged hospitalization[5-7]. Throughout the world, the factors necessitating EPH include uterine atony and uterine rupture, but this has been largely overtaken by abnormal placentation, which is now the most common indication for peripartum hysterectomy[3,8,9]. We identified a need to know if similar factors were involved in the UAE so that we might prepare for and include improved conservative methods to treat obstetric hemorrhage that might reduce the incidence and complications of uterine rupture. Abnormal placentation involving placenta previa and the morbidly adherent placenta followed a global rise in cesarean section (CS) rates over the past two decades. We needed to address the rates of CS in medical tourists and our local population. We know that uterine scarring associated with abnormal placentation has been reported to increase the risk of EPH following previous CS[10,11]. Given the socio-economic differences among the Arab countries in the Middle East, the UAE has a constant patient influx from neighboring countries as well as medical tourists. In the UAE, Dubai in particular, one of the two large emirates, stands out as an affordable quality healthcare provider. The modern healthcare infrastructure, physician expertise, and multi-ethnic healthcare providers make Dubai attractive for health tourism. High-risk cases, such as multiple CS and multiparity are commonly handled by the Dubai Health Authority (DHA), a public sector healthcare provider in Dubai with four hospitals and 16 primary health care centers. Dubai public hospitals, governed by the DHA are accredited by the Joint Commission International and serve as teaching hospitals for medical students and residency training[12,13]. A vast majority of the physicians in the DHA network are either trained in the United States or Europe. The DHA mandates a multidisciplinary team approach for the management of women with abnormal placentation, such as the placenta accreta team, which includes radiologists, feto-maternal medicine specialists, urologists, vascular surgeons, gynecologic oncologists and an anesthetist. The neonatal intensive care unit (NICU) has maintained an affiliation with the Oxford Vermont network since 2010. In spite of the modern healthcare system in Dubai, unregistered women without antenatal care and without referrals frequent the DHA maternity hospitals. Although the exact proportion of these women is not known, we expect that they may make up 20% of the total maternity patients. These and other challenges prompted us to investigate the rate of CS, EPH, and associated factors in women attending DHA hospitals. The purpose of this study is to review the incidence of CS, risk factors, indications, medical and surgical management, and the outcomes of peripartum hysterectomy over the past 15 years in DHA maternity hospitals.

Materials and Methods

Study population

This study was approved by the Research Ethics Committee of the DHA (approval number: DSREC-12/2015_15). Consent was not needed for retrospective studies as per the DHA ethics committee. The Latifa Hospital (LH) and Dubai Hospital (DH) are specialized tertiary hospitals for women and children governed by the DHA. Both hospitals serve as referral centers for high-risk, complicated cases within the country and the region. On average, 10.000 deliveries per year occur in both hospitals combined. Both LH and DHs are considered multidisciplinary care centers, defined as institutions with 24-hour in-house obstetrician-gynecologists, anesthesiologists, fully-stocked blood banks, immediate availability of a gynecologic oncologist, vascular surgeon, and a urologist. The study was conducted at these two hospital sites and our design was a retrospective cohort including all pregnancies complicated by EPH over a 15-year period from January 2000 until December 2015. The International Classification of Diseases-9 code was used to extract data from all hysterectomy cases, which included placenta accreta, postpartum hysterectomy, PPH (immediate and delayed), postpartum coagulation defects, and retained placenta or membranes (with or without complications or hemorrhage or both) from the hospital information system by the health informatics department. Peripartum hysterectomy,

defined as a woman who had a hysterectomy for a hemorrhage that was unresponsive to all other treatment modalities served as the inclusion criteria. Deliveries of less than 24 weeks gestation were excluded from the study. Data extraction was independently performed by two extractors and variations in data were subjected to the interrater reliability test.

Demographic data, risk factors, indications, and outcome variables

After the records had been obtained and de-identified, a matrix database was assembled. We identified maternal sociodemographic details, past medical, surgical and obstetrical histories, labor and delivery events, including gestational age, mode of delivery, indications of CS and type of hysterectomy performed. We also reviewed any additional procedures performed, blood loss, blood transfusions, and postoperative complications. PPH was defined as blood loss of 500 mL or more from the genital tract within 24 hours of the birth of a baby. Antepartum hemorrhage was defined as bleeding from or into the genital tract occurring from 24 weeks of pregnancy and before the birth of the baby. Blood loss was estimated by evaluating the woman's hemodynamic status, serial intraoperative hemoglobin by the anesthetist, blood-soaked swabs and linen, and blood collected in the kidney trays. The numbers of units of blood transfused, the pathology reports, and maternal complications including maternal death and urologic, infectious and wound complications were evaluated after the operation. Blood transfusions were calculated by the number of units of fresh frozen plasma and whole blood given during hospitalization. Placenta accreta is defined as the placenta being adherent to the uterine wall without easy separation and included the spectrum of placenta accreta, increta, and percreta[11]. The diagnoses were suspected by ultrasound/magnetic resonance imaging findings and confirmed by histopathologic-evidence of placental invasion into the myometrium, by clinical assessment of abnormal adherence of the placenta, or by evidence of gross placental invasion at the time of surgery. The cases were subjected to chart abstraction for maternal medical, obstetric, and gynecologic history; the timing of diagnosis; antepartum and intrapartum management; maternal postpartum course; and complications occurring within the postpartum period. Delivery was considered "elective" if planned at least 24-hours in advance and performed not urgently because of either documented fetal lung maturity or clinical concerns for risks associated with expectant management such as eventual hemorrhage or labor. The patient (woman) was considered "registered" if she had previous antenatal visits, and "unregistered" if she had no prenatal visits before and seen first time during the admission.

Statistical Analysis

Descriptive statistics were used to describe the cohort. Student's t-test, χ^2 analysis, and Fisher's exact test were used as appropriate. The Mann-Whitney U test was used to compare medians between groups for nonparametric data. SPSS Statistical Software Version 23 (SPSS Inc. Chicago, USA) was used for this purpose.

Results

A total of 168.293 deliveries were performed at LH and DH with 44.376 (26%) CSs and 79 cases of EPH, during the 15-year period. The incidence of EPH was 0.47 per 1000 deliveries (Table 1). The mean age of the patients with EPH was 33.5±4 years (range, 22-41 years), 43 (54%) were UAE nationals, 66 (83.5%) were housewives, all were married, with a mean body mass index of 29.7±6 kg/m^2 (range, 18.9-56.8 kg/m^2). The mean gestational age was 33.7±3.8 weeks (range, 21-41 weeks) with a mean birth weight of 2366±828 g (range, 610-4500 g), and the mean parity was 3.2±1.9 (range, 0-10) for the EPH group. The general characteristics of the women with EPH are presented in Table 2. Cohen's kappa yielded a score of 0.82, indicating acceptable inter-rater reliability (data not shown). All hysterectomies were performed within 24 hours after delivery, and all the women received prophylactic antibiotics. The majority of the hysterectomies (n=55, 70%) where subtotal. The mean number of postoperative hospitalization days was

Table 1. Rate of emergency peripartum hysterectomy by year in the dubai health authority

Year	Total deliveries (n)	Caesarean deliveries [n (% of total deliveries)]	EPH* (n)	Rate of EPH*/ 1000 deliveries
2000	12616	2618 (21)	0	0.00
2001	12569	2750 (22)	3	0.24
2002	13075	2730 (21)	2	0.15
2003	12052	2532 (21)	2	0.17
2004	8986	2261 (25)	2	0.22
2005	9924	2536 (26)	5	0.50
2006	10623	2841 (27)	3	0.28
2007	11619	3364 (29)	7	0.60
2008	10910	3078 (28)	3	0.27
2009	11324	3176 (28)	0	0.00
2010	10276	3077 (30)	6	0.58
2011	9025	2615 (29)	5	0.55
2012	9320	2714 (29)	9	0.97
2013	9091	2568 (28)	11	1.21
2014	8722	2788 (32)	7	0.80
2015	8161	2728 (33)	14	1.72
Total /average	168293	44376 (26)	79	0.47

*EPH: Emergency peripartum hysterectomy

16±14 days (range, 4-66 days), the median operating time was 125 minutes (range, 62-230 minutes), the mean estimated blood loss during PPH was 5.2±3.4 L with a range between 0.5 to 20 L. Out of 79 women, 77 (98%) births were delivered through CS, and two had vaginal deliveries, one of which was complicated by uterine atony and another by uterine rupture. Previous CS (one or more) had been performed in 69 women (87%), 71 (93%) had placenta previa associated or not with placenta accreta. Nineteen (20%) women with EPH had a previous curettage, 16 of which were associated with one or more CS. Abnormal placentation was reported in 71 (93%) patients (Table 2). There was a significant association between many previous CSs and abnormal placentation (p=0.008). Although abnormal placentation has been seen more frequently in multiparous women (72/74), this association was not statistically significant (p=0.614). No association was detected between abnormal placentation and previous uterine procedures (p=0.183). The operative notes and the pathology reports of the uterus and placenta were used to determine the final indication for the procedure. The most common indications were placenta previa with accreta 36 (46%), placenta previa without accreta 35 (44%), uterine atony 23 (29%), and uterine rupture 2 (3%) (Figure 1). Twenty-nine (37%) women had antepartum hemorrhage, whereas 71 (90%) were diagnosed as having placenta previa with or without accreta. There were 76 (96%) multiparous and 3 (4%) primiparous women. Uterine atony was the most common indication for hysterectomy in primiparous, whereas placenta accreta was the most common in multiparas. Fifty-five (70%) of the women had a subtotal abdominal hysterectomy. Uterine atony was the major reason for total hysterectomies (19 out of 23, 83%). Nine of the 35 (26%) women with placenta previa without accreta underwent a total hysterectomy. Thirteen of 36 (36%) women with placenta previa with accreta underwent a total hysterectomy. No difference in the type of hysterectomy was noted when the indication was placenta previa with or without accreta (p=0.34). To prevent EPH, different pharmacologic agents and surgical procedures were used to stop bleeding including uterotonics in 60 (76%), oversewing bleeding points 8 (10%), use of uterine packs or/and balloon tamponade in 62 (78%), B Lynch suture in 4 (5%), uterine artery ligation in 50 (63%), and internal iliac artery ligation in 32 (41%), (Figure 2). The most common intraoperative complication was bladder injury in 18 (23%), followed by ureteric injury 2 (2%). Additional complications included vaginal cuff cellulitis/vault hematoma

Table 2. General characteristics of patients with peripartum hysterectomy

Maternal characteristics	No (n=79)
Type of pregnancy	
Singleton	75 (95%)
Twin	4 (5%)
Previous cesarean delivery	
0	10 (13%)
1 or more	69 (87%)
Previous uterine surgery	
Curettage	19 (20%)
Myomectomy	1 (1%)
Ectopic	3 (4%)
Placentation*	
Normal	5 (7%)
Previa without accreta	35 (46%)
Placenta previa with accreta	36 (47%)
Labour	
Presence of labor	17 (21%)
Induction of labor	4 (5%)
Augmentation of labor	1 (1%)
Reason of admission	
Antepartum hemorrhage	29 (37%)
Elective	28 (35%)
Abdominal pain	7 (9%)
Preterm premature rupture of membranes	6 (8%)
Labor	5 (6%)
Others†	4 (5%)
Comorbidities	
Hypertension	4 (5%)
Diabetes mellitus	16 (20%)
Type of cesarean section	
Elective	39 (49%)
Emergency	40 (51%)
Registered in the hospital	
Yes	64 (81%)
No	15 (19%)

†Postdate, threatened preterm, threatened miscarriage, *Data were available for 76 patients only

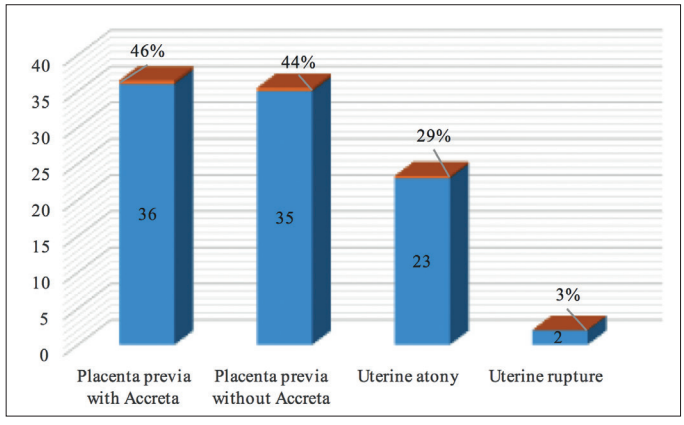

Figure 1. Indications of emergency peripartum hysterectomy in Dubai health authority

6 (8%), disseminated intravascular coagulopathy 5 (6%), pulmonary edema in 2 (2%), deep vein thrombosis 1 (1%), and wound infection 1 (1%); Table 3. All women were actively managed with resuscitation and transfusions, the average numbers of blood and blood products transfused are presented in Table 4. The median number of packed red blood cell units transfused was 8 (range, 0-31). The average maternal length of postoperative stay in hospital was 16±14 days (range, 4-66 days) and the mean length of postoperative stay in the intensive care unit was 2.2±1.9 days (range 1-6 days). We had one case of maternal death (1%): para one woman with triplets who had PPH and hysterectomy and was complicated with disseminated intravascular coagulation. Twenty-two (28%) of delivered neonates were admitted to NICU, with one case of death (1%) (Table 3).

Discussion

Although rare in modern obstetrics, EPH is a major surgery and is invariably performed in the presence of life-threatening hemorrhage during or immediately after abdominal or vaginal deliveries[14]. Modern obstetricians employ EPH when all conservative measures have failed to achieve hemostasis during life-threatening hemorrhage. The unplanned nature of the EPH surgery, the need for performing it expeditiously, and the acute loss of blood complicates the performance of EPH. Emergency PPH following a CS was first described by Porro and reported to be used to prevent maternal mortality due to postpartum hemorrhage[6,7]. Dubai, in the UAE, is a metropolitan city with more than 180 nationalities, lifestyle changes, and cultural differences which have transformed Dubai into a major healthcare hub. Our study included 168.293 deliveries performed in two major tertiary care hospitals in Dubai from 2000 to 2015. Both hospitals are tertiary, government-owned, and they serve as referral centers for many community-based hospitals and other nearby cities. Interestingly, despite the constant rise in CS rates (32% in the past four years) in DHA-governed hospitals, the EPH rate has remained low when compared with data from the region and global rates. Our study demonstrates an EPH incidence of 0.47 per 1000 deliveries (Table 1). The global reported impact of EPH varies from 0.24

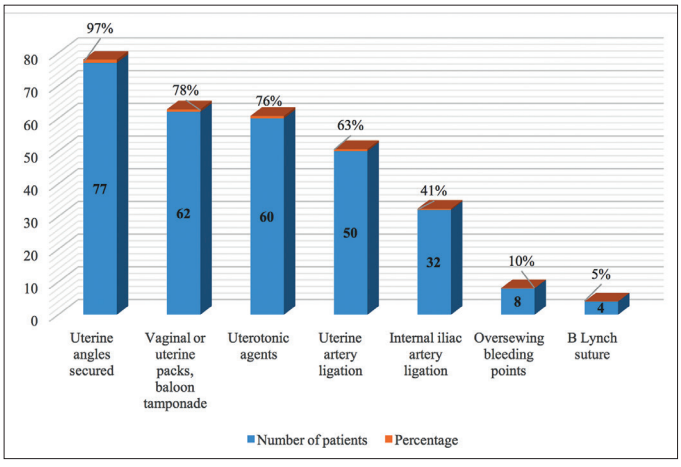

Figure 2. Interventions made prior to emergency peripartum hysterectomy

Table 3. Distribution of mothers according to parameters related to their peripartum hysterectomy

Parameters	No (%) (n=79)
Time of delivery to time of PPH* (minutes)	
<30 min	76 (96%)
>30 min	3 (4%)
Time of PPH* to hysterectomy (minutes)	
<30 min	4 (5%)
>30 min	75 (95%)
Type of hysterectomy	
Total	24 (30%)
Subtotal	55 (70%)
Subsequent laparotomy	
Yes	14 (18%)
No	65 (82%)
Intra-operative complications	
Bowel injury	0
Bladder injury	18 (23%)
Ureteric injury	2 (2%)
Type of anesthesia	
General	68 (86%)
Spinal	11 (14%)
Post-operation complications	
Vaginal cuff cellulitis/vault hematoma	6 (8%)
Coagulopathy	5 (6%)
Pulmonary oedema	2 (2%)
Deep vein thrombosis	1 (1%)
Wound infection	1 (1%)
Average length of post-operation stay in hospital (d)†	
4 or less	2 (3%)
5-8	39 (51%)
9 or more	36 (47%)
X ± SD	12±10.7
Average length of post operation stay in icu (d)†	
4 or less	54 (70%)
5-8	11 (14%)
9 or more	1 (1%)
X ± SD	4.4±14
Maternal mortality	1/79 (1%)
Neonatal ICU admission	22 (28%)
Neonatal mortality	1/79 (1%)

†Data were available for 77 patients only, *Peripartum hysterectomy
SD: Standard deviation, PPH: Postpartum hemorrhage ICU: Intensive care unit

Table 4. Quantity of blood and blood products transfused

Parameters	Mean ± standard deviation	Minimum - maximum
Estimated blood loss (L)	5.2±3.4	0.5-20
Red cells transfusion (unit)	9.1±6.1	0-31
Fresh frozen plasma transfusion (unit)	7.9±6	0-26
Cryo transfusion (unit)	7.8±9.1	0-36
Platelets transfusion (unit)	5.6±7	0-30
Whole blood transfusion (unit)	1.4±4.4	0-30

to 8.9 per 1000 deliveries,[8] ranging from 0.33 (Netherlands), 0.2 (Norway), 0.3 (Ireland), 0.63 (Saudi Arabia) and 1.2 to 2.7 per 1000 deliveries in the United States of America (USA)[14]. A difference in the incidence of EPH is noted following a vaginal delivery and CS, and is reported to be 0.1 to 0.3/1000 in vaginal births[5,14]. The incidence of EPH following CS varies widely between 0.17 and 8.7/1000 deliveries with a global rate of EPH ranging from 0.2 and 2.7 in 1000 deliveries[11]. This differs between countries with the lowest rates of 0.2 (Norway),[5] 0.24 (China),[15] 0.3 (Ireland),[16] 0.36 (Turkey),[17] and in countries with 1.9 (India),[18] 1.39 (Iran),[19] 1 (Kuwait),[20] 0.5 [the Kingdom of Saudi Arabia (KSA)],[21] 0.8 (Canada),[14] and 1.2 to 2.7 (USA)[22] per 1000 deliveries. CS deliveries have been on the rise in the past 20 decades throughout the world[23]. The reason for this significant rise is multifactorial: use of electronic fetal heart monitoring, risks and fear of litigation, payment schemes with cesarean deliveries being paid more, and increased convenience for both obstetricians and women[24] are the main contributing factors. In fact, there is an increased demand of elective CS on maternal request[25]. Presently, we do not have clear data on how many of the CSs in UAE are based on a maternal request, but according to Hamilton et al.[9] worldwide, the maternal request CS rate has been estimated between 12-15%. We have noted that although the total number of deliveries per year was decreasing in Dubai, the number of CSs has risen (Table 1). This increase of CS rate eventually causes an increase in abnormal placentation, and consequently an expected rise in the incidence of EPH[11]. It has been presented that women who had a history of placenta previa with a previous uterine scar had a 16% higher risk of hysterectomy compared with 3.6% in women with unscarred uteri[5,14,21]. In our series, 94% (74/79) of EPH cases were due to abnormal placentation, and there was a significant association between the number of previous CSs and abnormal placentation (p=0.007). Equal percentages were shared between placenta accreta and placenta previa without accreta as an indication for EPH (Table 2). This supports the fact that recognizing and assessing patients at risk and appropriate and timely intervention facilitates, and counseling women at risk enables better EPH outcomes. In this study, 70% of the EPHs were subtotal hysterectomies, and 30% were total, which is in line with a literature review[8]. The main reasons for preferring subtotal hysterectomy are less blood loss, reduction of operating time as well as postoperative complications. No evidence for a difference in the rates of incontinence, constipation, or sexual function between total and subtotal hysterectomy has been reported[26]. The most frequent indications of EPH in modern obstetrics are placenta accreta and uterine atony, respectively,[8] and our observations in the UAE are consistent with this.

Peripartum hysterectomy is associated with high complication rates, including a maternal mortality (range from 0 to 12.5% with a mean of 4.8%),[14] and the death rate was close to 1% 1 (79) in our settings. Despite the high incidence of CSs and multiparity in UAE, the risk for EPH (0.47/1000) was lower in comparison with other countries in the region: 1.9 (India),[18] 1.39 (Iran),[19] 1 (Kuwait),[20] and 0.5 (KSA)[21]. The results of the study are important in many ways and provide the first national estimate for EPH, especially post CS in the UAE. To the best of our knowledge, there have been no other studies conducted in the UAE to assess EPH rates in the context of CS. The data from this study can further enable the implementation of local guidelines to reduce CS, which might decrease the incidence of EPH. Patient participation and counseling can be encouraged to mitigate the risks of EPH in our local hospitals. We can expect to limit the number of CS through the reduction of unnecessary inductions of labor, encouragement of external cephalic version, allowing adequate time for labor initiation in the first stage, and reducing medicalized labor. We believe that patient-payer mechanisms should encourage normal delivery and that auditing indications for CS in public and private hospitals might also reduce the CS rate. It is anticipated that results from our study might be used by countries with a similar culture in the Eastern and Middle Eastern region of the world.

Study Limitations

The present report is the first study to analyze EPH in the UAE. Our primary objective was to elucidate the incidence, indications, and outcomes of EPH in the Emirate of Dubai, UAE. Although our data include a reasonable number of cases, several limitations must be acknowledged. This is a retrospective cohort study where data were abstracted from birth registries and mother's files. While we attempted to select all cases, it is likely that some women experienced complications that were not recorded. Also, despite having similar guidelines, the two hospitals might have differences in their approach to such cases. Analogous to any observational study, the influence of unmeasured biases that may confound the findings cannot be ruled out, although every attempt has been made to limit inter-rater variability.

Conclusion

The primary indication of the EPH was the abnormal placentation in a scarred uterus and uterine atony. The major method of prevention of EPH is to assess women's risks and

to reduce the number of CS deliveries, by limiting the rate of the primary cesareans. This is challenging in the UAE where the culture is associated with high gravidity and high parity. We recommend that the national agenda in the UAE should include public health actions that might assist in the reduction of primary and repeat CSs.

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Ethics

Ethics Committee Approval: This study was approved by Research Ethics Committee of the Dubai Health Authority (approval number: DSREC-12/2015_15).

Informed Consent: Consent was not needed for retrospective studies as per DHA ethics committee.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: M.A.T., N.M.H., Concept: M.A., F.R.C., M.A.T., S.M., Design: M.A.T., M.A., S.M., Data Collection or Processing: S.M., M.O., F.C., Analysis or Interpretation: M.A., F.R.C., M.A.T., N.M.H., Literature Search: M.A., F.R.C., M.A.T., S.M., Writing: M.A.T., M.A., N.M.H., F.R.C., S.M., M.O., F.C.

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The impact of different 75 g oral glucose tolerance test target ranges within normal limits on neonatal outcomes: A validation study

Normal sınırlardaki farklı 75 gr oral glukoz tolerans test hedef aralıklarının neonatal sonuçlar üzerine etkisi: Bir geçerlilik çalışması

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Abstract

Objective: To investigate the impact of different 75 g glucose tolerance test (OGTT) target ranges within normal limits on neonatal outcomes, thus to investigate the validity of 75 g OGTT thresholds.

Materials and Methods: The normal 1-hour and 2-hour ranges of 75 g OGTT levels of 110 pregnant women with no gestational diabetes mellitus (GDM) were further divided into three different sub-groups; for the 1 hour as group 1 (<120 mg/dL), group 2 (120-140 mg/dL), group 3 (>140 mg/dL) and for the 2 hour as group 1 (<120 mg/dL), group 2 (120-135 mg/dL), and group 3 (>135 mg/dL).

Results: For the 1-hour results, there was no statistically significant difference between groups in terms of age, body mass index, multiparity, neonatal hypoglycemia, hyperbilirubinemia, neonatal intensive care unit admission, birth weight, and LGT rates; however, the rate of small-for-gestational-age (SGA) infants was significantly higher in group 2 compared with those in group 3. For the 2-hour results, statistically similar results were found between the groups.

Conclusion: A 2-hour 75 g OGTT has reliable threshold values for GDM screening. However, because there are still adverse neonatal outcomes in women with OGTT results below the current thresholds and the number of SGA fetuses is higher in the glucose range 120-140 mg/dL of the first hour, the validity of the 75 g OGTT thresholds still needs further investigation.

Keywords: Gestational diabetes mellitus, glucose tolerance test, neonatal outcome, screening, pregnancy

Öz

Amaç: Bu çalışma normal sınırlardaki farklı 75 gr oral glukoz tolerans test (OGTT) hedef aralıklarının yenidoğan sonuçlarına etkisini araştırarak 75 gr OGTT eşik değerlerinin geçerliliğini araştırmak için yapıldı.

Gereç ve Yöntemler: Gestasyonel diabetes mellitus (GDM) saptanmayan 110 gebe kadınının normal sınırlardaki 1. saat ve 2. saat 75 gr OGTT değerleri 1. saat için grup 1 (<120 mg/dL), grup 2 (120-140 mg/dL), grup 3 (>140 mg/dL) olarak ve 2. saat için grup 1 (<120 mg), grup 2 (120-135 mg/dL), grup 3 (>135 mg/dL) olarak üç alt gruba ayrıldı.

Bulgular: Birinci saat sonuçlarda yaş, vücut kitle indeksi, multiparite, yenidoğan hipoglisemi, hiperbilirubinemi, yenidoğan yoğun bakım ünitesi ihtiyacı, doğum ağırlığı ve LGT oranları açısından gruplar arasında istatistiksel olarak anlamlı bir fark yoktu; bununla birlikte, grup 2'deki gebelik yaşı için küçük (SGA) bebeklerin oranı grup 3'e göre istatistiksel olarak anlamlı derecede yüksekti. İkinci saat sonuçlar gruplar arasında istatistiksel olarak benzer bulundu.

Sonuç: İki saatlik 75 gr OGTT, GDM taraması için güvenilir eşik değerlerine sahiptir. Bununla birlikte, mevcut eşik OGTT değerleri altında olan kadınlarda olumsuz yenidoğan sonuçlar olması ve birinci saat 120-140 mg/dL glukoz aralığında SGA fetüs sayısının yüksek olması nedeniyle 75 gr OGTT eşik değerlerinin geçerliliği hala araştırmayı gerektirmektedir.

Anahtar Kelimeler: Gestasyonel diabetes mellitus, glukoz tolerans testi, yenidoğan sonuç, tarama, gebelik

PRECIS: A 2-hour 75 g glucose tolerance test has reliable threshold values for gestational diabetes mellitus screening. However, the validity of the 75 g glucose tolerance test thresholds still needs further investigation.

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Introduction

Gestational diabetes, which affects 3% to 6% of all pregnancies, is an important issue that should be handled with specific treatment in addition to routine antenatal care to reduce the risks of maternal and perinatal morbidity[1]. It has been suggested that risks for adverse outcomes differ according to the single or combined thresholds selected[2]. The hyperglycemia and adverse pregnancy outcome (HAPO) study, pointed out the continuously increased risk between maternal glucose levels and adverse pregnancy outcomes even within ranges previously considered to be normal for pregnancy[3]. This study was the cornerstone for the diagnosis of gestational diabetes mellitus (GDM), identified using the 75 g oral glucose tolerance test (OGTT) when any of the following plasma glucose values are exceeded: fasting, ≥5.1 mmol/L (92 mg/dL); 1 h, ≥10 mmol/L (180 mg/dL); and 2 h, ≥8.5 mmol/L (153 mg/dL)[4]. These cut-offs recommended by the International Association of the Diabetes and Pregnancy Study Groups (IADPSG)[5], have been adopted by the World Health Organization (WHO)[6] and the American Diabetes Association (ADA)[4]. As a result of new diagnostic criteria, the increase in the incidence of GDM and use of treatment modalities will be inevitable; however, considering the increasing rates of obesity and diabetes globally, the changes are updated, and recommended to reduce adverse outcomes[4,5,7]. On the contrary, in 2015, the National Institute for Health and Care Excellence (NICE) opted for a higher fasting glucose threshold [fasting ≥5.6 mmol/L (≥101 mg/dL), and/or 2 h ≥7.8 mmol/L (≥140 mg/dL)][8].

The objective of this study was to investigate the validity of 75 g OGTT thresholds by evaluating the impact of different 75 g OGTT target ranges within normal limits on neonatal outcomes because the diagnostic dilemma on the most appropriate test for GDM and its thresholds is still ongoing.

Materials and Methods

This is a cross-sectional study of 110 consecutive pregnant women who attended our outpatient antenatal clinic and were diagnosed as having no GDM using the 75 g OGTT at 24-28 weeks of gestation. A 2 hour 75 g OGTT is performed for screening GDM at 24-28 weeks of gestation as a standard obstetric practice at our institution. The study protocol was approved by the Ethics Committee of Lütfi Kırdar Kartal Training and Research Hospital (89513307/1009/372). Written informed consent was obtained from all subjects before the study. The diagnosis of the GDM was made according to the ADA/IADPSG criteria, when any of the following plasma glucose values were exceeded: fasting, ≥92 mg/dL; 1 h, ≥180 mg/dL; 2 h, ≥153 mg/dL[4,5]. The exclusion criteria included women with GDM, pre-gestational diabetes mellitus (GDM), hypertension, multiple pregnancies, and fetal anomalies. The normal 1 h and 2 h ranges of 75 g OGTT levels of 110 pregnant women were further divided into three different sub-groups; for the 1 h as group 1 (<120 mg/dL), group 2 (120-140 mg/dL), group 3 (>140 mg/dL), and for the 2 h as group 1 (<120 mg/dL), group 2 (120-135 mg/dL), and group 3 (>135 mg/dL). Neonatal outcomes were compared between these new range groups. Neonatal hypoglycemia, hyperbilirubinemia, intensive care unit admission, large-for-gestational-age (LGA) and small-for-gestational-age (SGA) newborns were considered as adverse outcomes. The presence of one or more adverse outcome was determined as an abnormal result.

Statistical Analysis

All data were analyzed using SPSS Statistics for Windows, Version 22 (IBM Corp, Armonk, NY) and p values <0.05 were considered to be statistically significant. Continuous variables

Table 1. Maternal characteristics and neonatal outcomes of groups according to different 75 g oral glucose tolerance test target 1 h ranges within normal limits

	Group 1 (n=50)	Group 2 (n=32)	Group 3 (n=28)	p
OGTT 1 h cut-off (mg/dL)	<120	(120-140)	(>140)	
Age (years)	28.26±5.00	29.31±6.96	31.53±5.24	0.056
BMI (kg/m2)	28.33±3.98	27.57±3.95	28.46±3.89	0.617
Multiparous	32 (64)	19 (59.4)	16 (57.1)	0.819
Neonatal hypoglycemia	5 (10)	1 (3.1)	0 (0)	0.138
Neonatal hyperbilirubinemia	13 (26)	13 (40.6)	9 (32.1)	0.382
NICU admission	7 (14)	5 (15.6)	6 (21.4)	0.690
SGA	8 (16)	11 (34.3)	1 (3.6)	0.007[a]
LGA	5 (10)	5 (15.6)	3 (10.7)	0.727
Birth weight (g)	3296.9±527.5	3305.1±592.5	3437.1±583.8	0.538
Abnormal result	23 (46)	23 (71.9)	13 (46.4)	0.05

Values are expressed as mean ± standard deviation or n (%). OGTT: Oral glucose tolerance test, NICU: Neonatal intensive care unit, SGA: Small for gestational age, LGA: Large for gestational age, BMI: Body mass index, [a]: Group 2 vs group 3 p<0.05

are presented as mean ± standard deviation and categorical variables as numbers and percentages. For the analysis of qualitative data, the chi-square test was used. For the analysis of quantitative data, One-Way ANOVA and Kruskal-Wallis tests were used.

Results

One hundred ten pregnant women without GDM were enrolled in the study. The women were further divided into subgroups according to different ranges of normal 75 g OGTT results to compare neonatal outcomes.

The number and percentage of the subjects were 50 (45.5%), 32 (29%) and 28 (25.5%) for the first hour (Table 1), and 82 (74.5%), 14 (12.7%), and 14 (12.7%) (Table 2) for the second hour, for groups 1-3, respectively. For the 1 h results, there was no statistically significant difference between the groups in terms of age, body mass index (BMI), multiparity, neonatal hypoglycemia, hyperbilirubinemia, intensive care unit admission, birth weight, abnormal results, and LGA rates; however, the rate of SGA infants was statistically significantly higher in group 2 compared with group 3 (Table 1). For the 2 h results, statistically similar results were found between the groups (p>0.05) (Table 2).

Table 2. Maternal characteristics and neonatal outcomes of groups according to different 75 g oral glucose tolerance test target 2 h ranges within normal limits

	Group 1 (n=82)	Group 2 (n=14)	Group 3 (n=14)	p
OGTT 2 h cut-off (mg/dL)	<120	(120-135)	(>135)	
Age (years)	29.17±5.78	28.57±5.85	31.57±5.77	0.308
BMI (kg/m^2)	28.32±4.26	26.70±2.42	28.57±2.90	0.335
Multiparous	50 (61)	8 (57.1)	9 (64.3)	0.927
Neonatal hypoglycemia	3 (3.7)	2 (14.3)	1 (7.1)	0.258
Neonatal hyperbilirubinemia	26 (31.7)	6 (42.9)	3 (21.4)	0.476
NICU admission	13 (15.9)	3 (21.4)	2 (14.3)	0.763
SGA	14 (17.1)	5 (35.7)	1 (7.1)	0.128
LGA	10 (12.2)	1 (7.1)	2 (14.3)	0.824
Birth weight (g)	3328.1±555.8	3141.7±642	3568.2±433	0.128
Abnormal result	43 (52.4)	11 (78.6)	5 (35.7)	0.132

Values are expressed as mean ± standard deviation or n (%). OGTT: Oral glucose tolerance test, NICU: Neonatal intensive care unit, SGA: Small for gestational age, LGA: Large for gestational age, BMI: Body mass index

Discussion

The accurate diagnosis of GDM and prompt and proper precautions to prevent adverse outcomes are crucial for both the mother and the fetus. There are many studies in the literature about the adverse effects of gestational diabetes on pregnancy outcomes[3,9-13]. The initial criteria for the diagnosis was determined more than 40 years ago[14]; however, the ongoing debate about the thresholds of the OGTT is yet to be concluded. The HAPO study, with a large, multinational cohort of 25505 pregnant women, showed a continuous relationship between maternal glycemia and adverse outcomes, with no obvious thresholds at which risks increased[3]. With the results showing a strong and continuous association between adverse outcomes and higher levels of maternal glucose, which are lower than those diagnostic of diabetes, and with the inclusion of a large number of subjects from a broad geographic area of the participating centers; this study changed the concept, and was the basis for the IADPSG new criteria, which was also adopted by WHO and ADA[4,7]. Considering the continuous relationship between glycemia and adverse outcomes, in our study, we investigated different 75 g OGTT target ranges within normal limits on neonatal outcomes and found adverse outcomes even in pregnant women with no GDA. The 2 h results were similar among groups in terms of age, BMI, multiparity, neonatal hypoglycemia, hyperbilirubinemia, intensive care unit admission, birth weight, abnormal result, SGA and LGA rates; however, for the 1 h results, the rate of SGA infants was statistically significantly higher in group 2 (120-140 mg/dL), compared with group 3 (>140 mg/dL). The American College of Obstetricians and Gynecologists reported that the one-step approach would increase the prevalence of GDM and health care costs without evidence for clinical improvements in maternal and neonatal outcomes, and favored the two-step approach[15]. In a recent study, it has been suggested that the one-step method identifies high-risk women at least as well as the two-step method[16]. Identifying subjects at risk and prompt, specific interventions to reduce maternal hyperglycemia can reduce maternal and perinatal morbidity[1,17,18]. In this present study, the 75 g OGTT one-step approach was used, and to minimize the risk the neonatal outcomes, the results were compared between different ranges, within the normal limits of the IADPSG/ADA criteria. In 2015, NICE recommended new diagnostic thresholds for the diagnosis of GDM, with a higher fasting but lower 2 h post-load glucose thresholds of those proposed by the IADPSG[8]. In a study to identify ethnic-specific criteria for the diagnosis of GDM, it was suggested that the United Kingdom NICE might have underestimated the prevalence of gestational diabetes, especially in south Asian women[19]. In another recent study that evaluated neonatal and obstetric outcomes among women who were test positive for the IADPSG criteria but negative for the NICE 2015 criteria, a higher risk for LGA, cesarean delivery, and polyhydramnios was suggested compared with women with negative screening

results and no OGTT. The IADPSG criteria was determined to identify women at substantial risk of complications who would not be identified by the NICE 2015 criteria. As a result, it was reported that according to the NICE criteria, a high-risk group could be unidentified and left untreated depending on the higher fasting threshold, and a low-risk group could be treated instead depending on the lower 2 h threshold.[20]

Study Limitations

The limitation of the study is its small sample size. The validity of the 75 g OGTT thresholds still needs to be investigated and verified by large studies.

Conclusion

This study demonstrates that the 75 g OGTT (IADPSG/ADA) has reliable threshold values for GDM screening as the neonatal outcomes do not differ between the low normal and high normal levels of the first and second-hour test results, and provides evidence that there are still adverse neonatal outcomes in women with OGTT results below the current thresholds. The study also reports a higher number of SGA in the glucose range 120-140 mg/dL of the first hour, which needs further evaluation. As a result, the validity of the 75 g OGTT thresholds still needs to be investigated and verified by large studies.

Ethics

Ethics Committee Approval: The study protocol was approved by the Ethics Committee of Lütfi Kırdar Kartal Training and Research Hospital (approval number: 89513307/1009/372)
Informed Consent: Consent form was filled out by all participants.
Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: S.S., Concept: Z.M.P., Design: Z.M.P., Data Collection or Processing: S.S., Analysis or Interpretation: G.A.İ., Literature Search: S.S., G.A.İ., Writing: G.A.İ.
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Natural cycle versus artificial cycle in frozen-thawed embryo transfer: A randomized prospective trial

Dondurulmuş-çözülmüş embriyo transferinde doğal siklusa karşı yapay siklus: Bir randomize prospektif çalışma

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Abstract

Objective: To investigate whether there was a difference in pregnancy outcomes between modified natural cycle frozen-thawed embryo transfer (NC-FET) cycles and artificial cycles (AC)-FET in women who all had regular menstrual cycles.
Materials and Methods: One hundred seventy patients who met the inclusion criteria and had at least two cryopreserved embryos were included in a prospective randomized controlled trial. Eighty-five patients were randomized based on Bernoulli distribution into the following two groups: 1) Modified NC-FET using human chorionic gonadotropin for ovulation induction and 2) AC-FET, in which endometrial timing was programmed with estrogen and progesterone. The main studied outcome measure was the clinical pregnancy rate per cycle.
Results: No significant differences were found between the two groups with regard to the chemical, clinical, and ongoing pregnancy rates (48.2% vs 45.9%, p>0.05; 38.9% vs 35.3%, p>0.05; and 37.6% vs 34.1%, p>0.05, respectively), as well as the live birth or miscarriage rates per cycle (35.3% vs 31.8%, p>0.05; and 1.2% vs 1.2%, p>0.05, respectively).
Conclusion: These findings suggest that although both FET protocols are equally effective in terms of pregnancy outcomes in women with regular menstrual cycles, NC-FET is more favorable because it requires no medication, has no adverse events, and has a significant cost reduction.
Keywords: Frozen-thawed embryo transfer, artificial cycle, natural cycle, clinical pregnancy rate

Öz

Amaç: Bu çalışmanın amacı; düzenli adet döngüsü olan kadınlarda modifiye doğal siklus dondurulmuş-çözülmüş embriyo transferi (NC-FET) ve yapay siklus (AC)-FET arasında, gebelik sonuçları bakımından bir farklılık olup olmadığını araştırmaktır.
Gereç ve Yöntemler: Dahil etme kriterlerini karşılayan ve en az iki embriyosu dondurularak saklanmış 170 hasta, prospektif randomize kontrollü çalışmaya dahil edildi. Bernoulli dağılımı baz alınarak 85 hasta iki gruba randomize edildi: 1) Ovülasyon indüksiyonu için insan koryonik gonadotropin kullanılan modifiye NC-FET ve 2) Endometriyal zamanlamanın östrojen ve progesteron ile programlandığı AC-FET. Çalışılan temel sonuç ölçeği, siklus başına klinik gebelik oranıydı.
Bulgular: İki grup arasında; kimyasal, klinik ve devam eden gebelik oranları açısından (sırasıyla; %48,2'ye karşı %45,9, p>0,05; %38,9'a karşı %35,3, p>0,05 ve %37,6'ya karşı %34,1, p>0,05) hem de siklus başına canlı doğum ve düşük yapma oranları açısından anlamlı bir farklılık saptanmadı (sırasıyla, %35,3'e karşı %31,8, p>0,05 ve %1,2'ye karşı %1,2, p>0,05).
Sonuç: Bu bulgular, her iki FET protokolünün, düzenli adet döngüsü olan kadınlarda gebelik sonuçları açısından eşit etkili olduğunu kanıtlamasına rağmen, NC-FET daha elverişlidir; çünkü ilaç tedavisi gerektirmez, advers etkileri olmaz ve önemli bir maliyet düşürücüdür.
Anahtar Kelimeler: Dondurulmuş-çözülmüş embriyo transferi, yapay siklus, doğal siklus, klinik gebelik oranı

PRECIS: Modified natural cycles are recommended in frozen–thawed embryo transfer cycles, at least in patients with regular menstrual cycles due to numerous advantages including no medication, no adverse events, and a significant cost reduction.

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Introduction

Many patients have benefited greatly from frozen–thawed embryo transfer (FET) cycles to achieve pregnancy following either successful *in vitro* fertilization (IVF) or failed fresh embryo transfer (ET) cycles[1]. The cost-effective FET cycles improve the cumulative pregnancy rate per oocyte retrieval[2-4]. Additionally, IVF-associated complications such as Hyperstimulation syndrome and multiple births can effectively be prevented by FET[5].

An important factor in improving FET is optimal endometrial receptivity as well as synchronization between embryonic and endometrial developments[6-8]. To achieve this, several methods for endometrium preparation have been proposed. In FET cycles, the transfer of embryos may be timed either in natural cycles after spontaneous ovulation or in artificial hormonally-controlled cycles using sequentially administered exogenous estrogen (E) and progesterone[9-12]. Natural cycle-FET (NC-FET) may be preferable for women with regular menstrual cycles because it requires less medication and has a lower cost for patients. Nevertheless, even in these women, ovulation may not always happen or an unexpected ovulation may occur. Thus, the timing of FET can also be problematic. Furthermore, the predictability and reliability of artificial cycle-FET (AC-FET) cycles have been favored in clinics[1,6]. A recent systematic review and meta-analysis of the literature that compared different protocols for FET reported no differences in the clinical pregnancy rate, ongoing pregnancy rate, and live birth rate[11]. However, some controversy exists because some investigators reported better pregnancy outcomes with AC-FET,[1,2,5,13] whereas the results of some retrospective studies demonstrated the superiority of NC-FET[14,15]. Because there are insufficient well-designed randomized controlled trials (RCTs) to determine which type of cycle regimen is superior in FET cycles[6,12] and considering the existence of conflicting reports in this regard, this study focused on comparing two different protocols for endometrial preparation: modified NC-FET versus AC-FET in women who all had regular menstrual cycles. The chemical, clinical, and ongoing pregnancy rates, miscarriage and live birth rates were compared in these two distinct FET cycles to identify predictive factors and to influence the reproductive outcome.

Materials and Methods

After obtaining institutional review board approval, this prospective randomized clinical trial was approved by the Ethics Committee of Tehran University of Medical Sciences (approval number: IR.TUMS.REC.1394.2051) and written informed consent was obtained from all participants. All women who were aged between 18 and 40 years and had regular menses (25-34 days) and who had at least two cryopreserved embryos derived from intracytoplasmic sperm injection (ICSI) treatment cycles from January 2012 to December 2014 were referred to the infertility clinic of Shariati Hospital (a university teaching hospital) and were enrolled in the study. The period of study was from January 2015 to July 2016. Women with endometriosis, immune diseases, recurrent abortion, donated sperm or oocyte, uterine abnormality, ovarian cyst or previous ovarian surgery, history of previous IVF failure, and any known contraindications or allergy for oral estradiol or progesterone therapy were excluded from participating in the study. In addition, patients were excluded if their clinical history included percutaneous epididymal sperm aspiration or testicular sperm extraction. The enrolled women were divided randomly into two groups to undergo either a modified natural cycle FET (group A) or artificial cycle FET (group B) using computerized software in a 1:1 fashion (Figure 1). The sequence of allocation to the two groups was generated by the aforementioned software and then the treating physicians (n=2) gave treatment based on the allocated chart. A baseline transvaginal ultrasound (Siemens, Sonoline G20) using a 7.5 MH transvaginal probe was performed in all patients by the same attending physician on the 2nd or 3rd days of the menstrual cycle to assess the endometrium and rule out the presence of an ovarian cyst.

The endometrial preparation protocols

Based on the study protocol and inclusion criteria, 85 patients were considered as group A (modified NC-FET) and 85 were classified as group B (AC-FET) and were assigned to receive the related protocol. The demographic characteristics and clinical data of the two groups are given in Table 1.

Modified natural cycle frozen–thawed embryo transfer

An ultrasound examination was performed on days 10 to 12 of the cycle after a spontaneous menses to detect the leading follicle. When at least one dominant follicle reached ≥18 mm in diameter and the thickness of the endometrium was at least 8 mm, a bolus of 10.000 IU of human chorionic gonadotropin

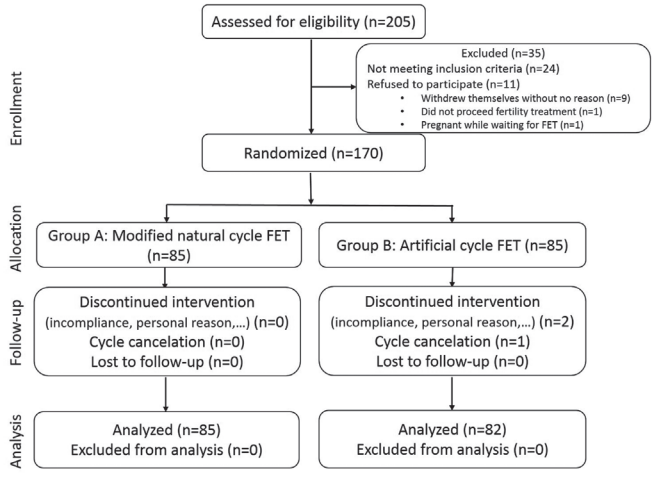

Figure 1. Participant consolitated standards of reparting trials flow diagram

FET: Frozen-thawed embryo transfer

(hCG) (Pregnyl; N.V. Organon, Oss, The Netherlands) was injected intramuscularly for the induction of ovulation and the embryos were thawed and transferred 4 days later.

Artificial cycle frozen–thawed embryo transfer

From the 21st day of the previous cycle, 500 µg/day of buserelin acetate (Suprecur; Hoechst UK Ltd, Hounslow, UK) was subcutaneously injected. Oral estradiol valerate (Progynova, Bayer, Germany) was then administered from day 2 of the next cycle from 2 mg/d to 2 mg/d ×4. The E dosage was adjusted based on the endometrial thickness as assessed using transvaginal ultrasound. After a baseline transvaginal ultrasound, a second ultrasound examination was performed on days 10 to 12 for the endometrial thickness assessment. Four hundred milligrams ×2 daily progesterone vaginal suppositories (Cyclogest, Actavis, Devon, UK) were administered in the following 3 days when the endometrium reached a thickness of 8 mm or maximum. ET was performed after 3 days of progesterone administration. Luteal phase support commenced on the day of ET in all the participants, using 400 mg ×2 daily progesterone vaginal suppositories (Cyclogest, Actavis, Devon, UK). Serum beta-hCG levels were evaluated for all patients 14 to 16 days after ET to confirm biochemical pregnancy. Progestin support continued up to the end of the 12th weeks' gestation if pregnancy was achieved. Vitrification and thawing of the cleavage-stage embryo were implemented using the same method reported in a previous publication[16]. The embryos were thawed on the day of the ET and those that were classified as grade I or grade II (according to cleavage stage, blastomere size and shape, and fragmentation) and had at least 50% intact blastomeres were transferred. The number of transferred embryos per cycle was limited to a maximum of three and was dependent upon the number of previous treatments, the number of embryos frozen in the same straw, and the quality of available embryos. Moreover, similar techniques were used by the two expert physicians who performed ET.

Table 1. Demographic characteristics and clinical data of modified natural cycle frozen-thawed embryo transfer and artificial cycle-frozen-thawed embryo transfer groups

	Modified NC-FET group	AC-FET group	p value*
Number of patients	85	82	
Age (years)[a]	30±6.1	31.4±5.6	NS
Body mass index (kg/m^2)[a,b]	24.2±4.4	25.4±5.8	NS
Duration of infertility[a]	3.8±0.9	3.9±0.9	NS
Type of infertility[c]			NS
Primary	58 (68.2%)	55 (64.7%)	
Secondary	22 (25.9%)	25 (29.4%)	
Causes of infertility			NS
Male factor	32 (37.6%)	26 (30.6%)	
Female factor	29 (34.1%)	23 (27.05%)	
Male and female factors	14 (16.5%)	17 (20%)	
Unexplained	10 (11.8%)	19 (22.35%)	
Basal FSH (mIU/mL)[a]	5.6±3.1	5.3±2.1	NS
Basal LH (mIU/mL)[a]	5.7±2	5.5±2.1	NS

NC-FET: Natural cycle frozen-thawed embryo transfer, AC-FET: Artificial cycle frozen-thawed embryo transfer, NS: Non-significant, [a]: Values are mean ± standard deviation, [b]: Calculated as weight in kilograms divided by the square of height in meters, [c]: Values are number (percentage), *: Independent Student's t-test was used, FSH: Follicle stimulating hormone, LH: Luteinizing hormone

Outcome measures

The primary outcome measure was clinical pregnancy. The secondary outcomes were biochemical pregnancy, ongoing

Table 2. Characteristics and pregnancy outcomes of frozen-thawed embryo transfer cycles

		Modified NC-FET (n=85)	AC-FET (n=82)	p value
Endometrial thickness on hCG injection day (mm)	mean ± SD	8.6±0.6	8.01±0.79	NS
The number of transferred embryos per cycle	mean ± SD	2.85±0.52	2.93±0.57	NS
Embryo grade	(%)			NS
I		48 (60%)	53 (66.25%)	
II		32 (40%)	27 (33.75%)	
Biochemical pregnancy rate per cycle	(%)	41/85 (48.2%)	39/85 (45.9%)	NS
Clinical pregnancy rate per cycle	(%)	33/85 (38.9%)	30/85 (35.3%)	NS
Ongoing pregnancy rate per cycle	(%)	32/85 (37.6%)	29/85 (34.1%)	NS
Live birth rate per cycle	(%)	30/85 (35.3%)	27/85 (31.8%)	NS
Miscarriage rate per cycle	(%)	1/85 (1.2%)	1/85 (1.2%)	NS

NC-FET: Natural cycle frozen-thawed embryo transfer, AC-FET: Artificial cycle frozen-thawed embryo transfer, NS: Non-significant, hCG: Human chorionic gonadotropin, SD: Standard deviation

pregnancy, live birth rate, and miscarriage rate. We applied the term "biochemical pregnancy" to an elevated serum beta-hCG level two weeks after hCG administration. Clinical pregnancy was established by the detection of a fetal heartbeat through transvaginal ultrasound in the 6th week. "ongoing pregnancy" referred to any pregnancy beyond 20 weeks of gestation. The miscarriage rate was measured using transvaginal ultrasonography and a decrease in serum beta-hCG level. All pregnant women were followed up to obtain delivery data. A live birth was defined as the completion of expulsion or extraction of a live baby from its mother.

Statistical Analysis

To have power of 0.8 to detect a 10% difference in clinical pregnancy rates between the study groups with a significance level of 0.05, we required 80 patients in each study group. Data are expressed as mean ± standard deviation for descriptive statistics when normally distributed, otherwise as median and range. All the analyses were performed using the SPSS software (version 17.0 for Windows; SPSS Inc., Chicago, IL, USA). The normality of distribution was checked using the Kolmogorov-Smirnov test. The comparison of the treatment outcomes between the two protocols was performed using the independent Sample t-test and/or chi-square test (or Fisher's exact test if required). The level of significance was $p<0.05$.

Results

A total of 205 women undergoing ICSI who had cryopreserved embryos were evaluated, 170 of whom were randomized to receive either modified NC-FET cycles or AC-FET (Figure 1). There were no statistically significant differences between the two groups terms of age, duration, type, and causes of infertility, day 3 follicle stimulating hormone or body mass index (Table 1). The characteristics and pregnancy outcomes for both cycle types are shown in Table 2. No significant difference was found between the NC-FET and the AC-FET groups regarding the average number of dominant follicles, endometrial thickness, the average number of transferred embryos, and embryo grade. Of the 170 cycles, a total of 63 clinical pregnancies occurred in the NC-FET and the AC-FET groups [33 (38.9%) versus 30 (35.3%) clinical pregnancies; p=0.4, respectively]. As demonstrated in Table 2, there were no significant differences between the two cycle types in terms of chemical, clinical, and ongoing pregnancy rates, miscarriage and live birth rates.

Discussion

This prospective RCT demonstrated that there were no differences in FET outcomes between modified natural and artificial cycles in patients with regular menstrual cycles. In the present study, patients in the modified natural cycle group received supplemental hCG (as a trigger to ovulation) and transvaginal progesterone to offset any probability of poor endogenous luteal phase. Patients with artificial cycles depended entirely on exogenous estradiol and progesterone, with prior gonadotrophin-releasing hormone agonist (GnRHa) down-regulation. The investigation of factors that affect the success of FET has steadily intensified during the past few years in order to transfer fewer embryos and to improve laboratory techniques[12,17-19]. The success of FET is dependent upon the reciprocal interaction between embryo development and the receptive uterus,[5,7] which can be evaluated through endometrial volume, endometrial thickness, and artery blood flow[8]. Numerous biomarkers, including leukemia inhibitory factor, integrin, and homebox A10[20-22] have been proposed as reliable markers of a receptive endometrium. Moreover, in older women, endometrial development in the follicular phase can be negatively affected by age, resulting in a lower pregnancy rate[12]. Adequate endometrial development in FET cycles can be achieved through three frequently used cycle regimens: natural cycles with or without ovulation induction using hCG; hormonally-manipulated artificial cycles using E followed by progesterone to prime the endometrium with/without a GnRHa; and stimulated cycles in which follicular development is supported by follicle-stimulating hormones[15]. However, no consensus has yet been reached regarding the best FET protocol for endometrial preparation[2,15]. Zheng et al.[5] reported that ovulation in hormone replacement treatment (HRT) cycles had a detrimental effect on pregnancy, although HRT increased the possibility of pregnancy. This finding is in line with the results of other large retrospective studies[1,13] that also reported a higher positive pregnancy test rate in the substituted cycle with E and progesterone than in natural cycles with hCG or progesterone. In contrast, some studies reported superior pregnancy outcomes in natural cycles[14,15]. Higher estradiol (E_2) levels may interfere with the window of implantation and cause endometrial receptivity and implantation windows to close earlier[23]. Fritz et al.[24] also suggested that elevated E_2 levels were associated with lower ongoing pregnancy/live birth rates, possibly due to the opposing effect on the endometrium from excess unopposed E_2 exposure. Furthermore, it has recently been reported that natural cycles have a better effect on endometrial transcriptome than artificial cycles in which E has a stronger negative effect than progesterone on the endometrial transcriptome[25]. On the other hand, consistent with our findings, comparable FET outcomes have been suggested by several studies in natural and artificial FET cycles[12,19,26-29]. In addition, a 2017 update of the 2008 Cochrane review also showed no evidence of a difference between the two cycles in rates of live birth or miscarriage rates[12,30]. AC-FET can be more easily scheduled, which leads to a better control of embryo thawing and transfer timing and also decreases cancellation rates compared with NC-FET. This is the result of ovulation suppression and the programmed replacement of exogenous hormones[5]. However, these advantages are somewhat counterbalanced by its possible adverse effects through exposure to exogenous hormones, higher risk of thrombo-embolic events, and providing a higher financial burden on patients, a burden that many are incapable

of overcoming[12,31,32]. Although NC-FET is complicated to plan due to its requirement for more frequent ultrasonographic evaluations of the dominant follicle, the risk of unexpected ovulation and insufficient development of the endometrium, its advantages such as being more patient friendly, convenience, less medication, and lower price cannot be denied[6,28]. Consequently, patients should be given the option of NC-FET in order to maintain autonomy in choosing the cycle protocol.

Study Limitations

Further clinical trials with larger sample sizes are required to illuminate the clinical and biochemical benefits of NC-FET.

Conclusion

In conclusion, based on the results of our study, modified natural cycles should be recommended in FET because they carry numerous advantages and have comparable FET outcomes, it at least in patients with regular menstrual cycles.

Acknowledgements

The authors are grateful to all participants, without whom this work would have been impossible.

Ethics

Ethics Committee Approval: The study was approved by the Ethics Committee of Tehran University of Medical Sciences (approval number: IR.TUMS.REC.1394.2051).

Informed Consent: Consent forms were filled out by all participants.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: L.H., Concept: M.A.H., F.S., A.A., Design: M.A.H., F.S., A.A., M.G.H., Data Collection or Processing: M.S., M.K., Analysis or Interpretation: M.G., M.S.N., M.K., Literature Search: L.H., F.S., Writing: F.S., M.A.H.

Conflict of interest: The authors declare that there are no conflicts of interest that could be perceived as prejudicing the impartially of the research reported.

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Segmental duplication-quantitative fluorescent-polymerase chain reaction: An approach for the diagnosis of Down syndrome in India

Segmental duplikasyon-kantitatif floresan polimeraz zincir reaksiyonu: Hindistan'da Down sendromu tanısına yönelik bir yaklaşım

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Abstract

Objective: Early detection of high-risk pregnancies for Down syndrome (DS) is the main target of offering prenatal diagnosis. Segmental duplication-quantitative fluorescent-polymerase chain reaction (SD-QF-PCR) can be used as an alternative method for prenatal diagnosis of DS. SD-QF-PCR involves SD sequences between the test and control chromosomes to detect aneuploidies. SD are two similar sequences with different fragment lengths, located on two different chromosomes. When these SD regions are amplified, the peak ratio between the two different chromosomes remains as 0.9 to 1.1 and the trisomy 21 results in the ratio of 1.4 to 1.6.

Materials and Methods: In this study, we applied SD-QF-PCR to detect the presence of trisomy 21 in 60 age-matched controls and 60 DS samples. The PCR amplification of SD regions is performed using a single pair of fluorescent-labelled primers, the peak ratio between the two different chromosome regions are evaluated.

Results: All sixty control samples showed the peaks to range from 0.9 to 1.1, which was suggestive of normal samples, and peaks of 65 DS samples ranged from 1.4 to 1.6, which suggested the presence of trisomy 21.

Conclusion: Segmental duplication quantitative fluorescent PCR is a sensitive and rapid aneuploidy detection technique and hence can be used as a standalone test to detect trisomy 21 as well as other aneuploidies.

Keywords: Segmental duplication-quantitative fluorescent-polymerase chain reaction, aneuploidies, trisomy 21

Öz

Amaç: Yüksek riskli hamileliklerde Down sendromunun (DS) erken teşhisi doğum öncesi tanı koymada ana hedeftir. Segmental duplikasyon-kantitatif floresan-polimeraz zincir reaksiyonu (SD-KF-PCR), DS'nin doğum öncesi tanısında alternatif bir yöntem olarak kullanılabilir. SD-KF-PCR, anöploidiyi saptamak için test ve kontrol kromozomları arasındaki SD dizilerini içerir. SD, iki farklı kromozom üzerinde yer alan farklı fragment uzunluğuna sahip iki benzer dizidir. Bu SD bölgeleri amplifiye edildiğinde, iki farklı kromozom arasındaki pik oranı 0,9 ile 1,1 arasında kalır ve trisomi 21; 1,1 ile 1,6 arasında bir oranda sonuç verir.

Gereç ve Yöntemler: Bu çalışmada, trisomi 21'in varlığını belirlemek için 60 kişiden oluşan yaş uyumlu kontrol grubuna ve 60 DS numunesine SD-KF-PCR yöntemi uygulanmıştır. SD bölgelerinin PCR amplifikasyonu, tek bir floresanla işaretli primer çifti kullanılarak yapılır, iki farklı kromozom bölgesi arasındaki pik oranı değerlendirilir.

Bulgular: Altmış kontrol örneğinin tümü, kontrol örnekleri için normal aralık olarak kabul edilen pik oranının 0,9 ile 1,1 olduğunu gösterirken; 65 DS örneğinin pik oranının 1,4 ile 1,6 arasında değişiyor olması trisomi 21'in varlığına işaret eder.

Sonuç: SD-KF-PCR hassas ve hızlı anöploidi belirleme tekniğidir, dolayısıyla diğer anöploidilerin yanı sıra trisomi 21'in ortaya çıkarılmasında bağımsız bir test olarak kullanılabilir.

Anahtar Kelimeler: Segmental duplikasyon-kantitatif floresan-polimeraz zincir reaksiyonu, anöploidi, trisomi 21

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Introduction

Trisomy 21 is the main cause of Down syndrome (DS) and it is associated with various other clinical phenotypes such as Alzheimer's disease, congenital heart diseases, cancers, Hirschsprung's disease, leukemias, epilepsy, sleep disorder, infertility-related issues, and a various nutrient deficiencies. The incidence of trisomy 21 is 1 in 1000 live births; however, it differs among ethnic groups[1]. According to National Down Syndrome Society survey, the life expectancy for individuals with DS is 55 years[2-4]. DS is associated with various characteristic facial features such as hypotonia, craniofacial abnormality, flat facial profile, excessive skin at the nape of neck, hypotonia, hyper flexibility of the joints, dysplasia of the pelvis, anomalous ears, dysplasia of the mid phalanx of fifth finger, and a transverse palmer crease (simian crease) in early infancy[4,5]. Besides these, the other common features include an upward slant to the eye, flat nasal bridge, short neck, abnormally shaped ears, and white spots on the iris of the eye (called Brushfield spots) [6]. Most patients have mild-to-moderate intellectual disability. DS children can be prevented by offering a prenatal diagnosis to high-risk pregnancies. However, the sampling methods, chorionic villus sampling and amniocentesis are associated with a 0.5-1% risk of miscarriage[7]. Soft markers such as small or absent nasal bone, increased thickness of the nuchal fold, and the presence of large ventricles are used to detect the risk of trisomy in ultrasound at 12 to 24 weeks of gestation[6,8]. Cytogenic analysis is widely used as the gold standard method for offering a prenatal diagnosis. However, rapid aneuploidy testing methods such as fluorescent in situ hybridization (FISH), quantitative fluorescence-polymerase chain reaction (QF-PCR), and multiplex probe ligation assay (MLPA) are also routinely used for prenatal diagnosis in the laboratory[4]. A novel technique, segmental duplication-QF-PCR (SD-QF-PCR) was established by Kong et al.[9] which involves SD sequences between test and control chromosomes to detect aneuploidies. SDs are two similar sequences with different fragment lengths, located on two different chromosomes. The method involves amplifying SD regions. When these sequences are amplified using a single pair of fluorescent-labelled primers, the peak ratio between the two different chromosomes remains as 0.9 to 1.1, and trisomy 21 results in the ratio of 1.4 to 1.6[9,10].

Materials and Methods

The study included 60 patients with DS confirmed by karyotype (Figure 1) and 60 control samples after obtaining informed consent. Two milliliters of peripheral venous blood were collected in ethylenediaminetetraacetic acid from Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, India. The study was approved by the institutional ethics committee (Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, India) IEC code: 2014-140-PhD-79. The study was conducted in accordance with the code of Ethics of the World Medical Association (Declaration of Helsinki, 1975 revised in 2000) for experiments in humans. Genomic DNA isolation was performed using the standard phenol-chloroform method followed by PCR amplification using primers obtained from elsewhere [Muthuswamy and Agorwal[10]]. The PCR

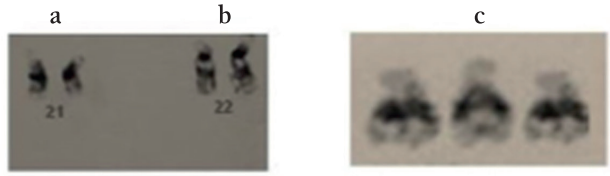

Figure 1. Chromosome as visualized on conventional karyotyping. a) Normal chromosome 21 pair, b) Normal chromosome 22 pair, c) Trisomy 21 showing presence of extra allele

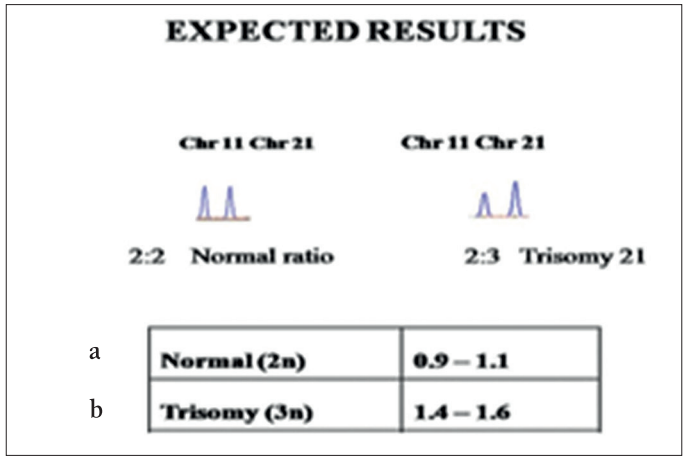

Figure 2. a) The ratios of the resulting peak obtained after normal or Down syndrome individuals, b) Ratios for normal and trisomy

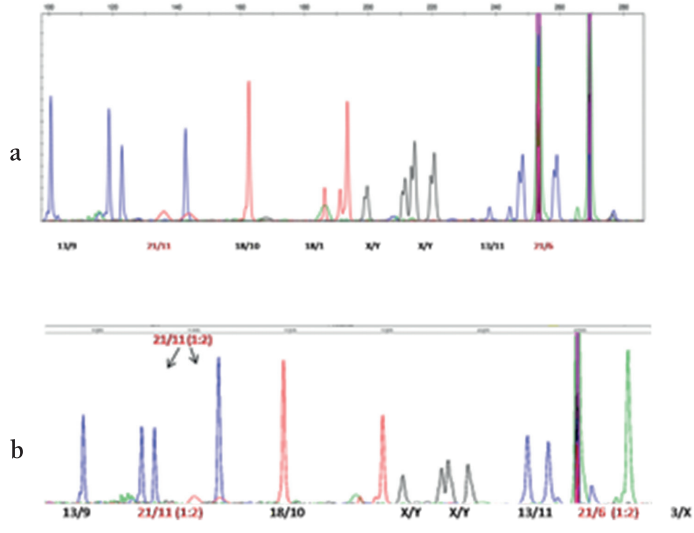

Figure 3. Results of segmental duplication-quantitative fluorescent-polymerase chain reaction. a) Normal individuals showing all normal sized alleles, b) Patients with Down syndrome showing 1:2 peak ratio for markers 21/11 and 21/6, respectively

conditions included initial denaturation at 95 °C for 5 minutes, followed by 35 cycles of 30 seconds at 95 °C, 30 seconds at 60 °C, and 30 seconds at 72 °C, and a final extension step at 72 °C for 10 minutes[9,10]. Amplified PCR products (2 µL) were denatured with 8 µL HiDI and 0.5 µL LIZ at 95 °C for 5 minutes and loaded onto the genetic analyzer (ABI 310 Genetic Analyzer, Applied Biosystems). On the basis of the area acquired by the peak, the relative peak signal ratios were calculated. The expected value for a normal and trisomic sample are 0.9 to 1.1 and 1.4 to 1.6, respectively.

Statistical Analysis

Statistical analysis was not required.

Results

SD-QF-PCR confirmed all 60 patients with DS to be positive for trisomy 21. Figure 2a shows the resulting peak in the case of euploids, the expected value was between 0.9 to 1.1, whereas for the trisomy, the value changes to 1.4 to 1.6, confirming the presence of an extra region. Figure 2b shows the expected value of the ratio for euploid, monosomy, and trisomy samples. Figure 3a shows the electropherogram obtained after SD-QF-PCR for euploid samples showing a normal allele ratio for both the markers, 21/11 and 21/6. Figure 3b shows the electropherogram obtained after SD-QF-PCR for trisomy patient samples showing values between 1.4 to 1.6 for markers 21/11 and 21/6, respectively, confirming the presence of DS.

Discussion

The study aimed to confirm the use of SD-QF-PCR as an alternative method for postnatal diagnosis of DS, as well being usable for prenatal diagnosis. We recruited 60 age-matched controls and 60 DS samples and checked these samples for the presence of trisomy through the amplification of SD regions using a single pair of fluorescent-labelled primers. The peak ratio between the two different chromosome regions were evaluated. For euploid samples, the expected value was found to be between 0.9 and 1.1, and the expected value for trisomy 21 cases was found to be between 1.4 and 1.6. All samples were correctly diagnosed using the SD-QF-PCR method and the accuracy of the markers was found as 100%. SD-QF-PCR offers various advantages over other molecular based methods for both prenatal and post natal diagnosis of DS. Table 1 shows the list of all the techniques used for the diagnosis of Down syndrome. Cytogenetic analysis of metaphase chromosomes is performed on metaphase-stage fetal cells on amniotes creating unique banding patterns on the chromosomes. However, cytogenetic analysis is a time-consuming method and labor intensive[4,9-11]. MLPA can also be employed to evaluate the copy number of DNA sequences and offers a number of advantages such as simplicity of use, cost effectiveness, and requires a very short time for diagnosis. MLPA is divided into four steps: DNA denaturation, hybridization probe ligation, PCR amplification. After PCR amplification, the amplified products are loaded onto the genetic analyzer for capillary electrophoresis. The overnight hybridization step in this method makes MLPA labor intensive. However, MLPA is unable to detect low level mosaicism. The major drawback of MLPA is that it offers mosaicism or maternal cell contamination. MLPA also uses labeled probes, which are quite expensive, thus making this method costly[12,13]. The most widely used method for prenatal diagnosis is FISH, which is performed on interphase nuclei, using chromosome-specific fluorescent-labelled probes. The main drawback of FISH is that it is a low throughput method, involving hybridization of fluorescently-labelled chromosome-specific DNA[4]. In addition, sometimes diffuse signals are seen in the case of interphase chromosome[14-17]. An alternative method, QF-PCR is a short tandem repeat-based marker approach, which is present on chromosome 21, and by using these markers we can detect trisomy in 86.67% of cases with only two markers, and further, using a larger number of markers can increase the reliability of the test. Thus, QF-PCR is a robust, sensitive, and an automated technique that can handle many samples at a time. The main advantage of this techniques is that the diagnosis can be given within 12 hours[4]. Non-disjunction of parental origin can also be detected simultaneously. However, QF-PCR fails to detect mosaicism and various ploidy levels. However, these problems were overcome by the SD-QF-PCR method, in which various ploidy levels and maternal contamination can easily be detected. Thus, SD-QF-PCR is robust and cheaper than the above-mentioned methods. It is much faster approach than all the above-mentioned assays because the diagnosis can be given within 12 hours. The present report confers the use of SD-QF-PCR for rapid detection of aneuploidies for developing countries such as India. SD-QF-PCR can act as a standalone test for the detection of DS as well as other ploidy levels including monosomies. Furthermore, the present study, which was conducted in prenatal samples using genomic DNA from patients with DS, reported the sensitivity of this technique as 100%. Similar studies should also be conducted in prenatal samples from high-risk pregnancies for aneuploidies, which will further establish this technique as an alternative standalone test for the prenatal diagnosis of aneuploidies.

Study Limitations

The present study was conducted in postnatal samples, however, SD-QF-PCR method should also be subjected in prenatal samples of DS as well.

Conclusion

The primary target of prenatal diagnosis is the early detection of high-risk pregnancies for DS. The choice after prenatal diagnosis of DS as to whether a pregnancy should continue a is a complex process because it involves various socio-economic factors. The risk for fetal trisomy can be evaluated on the basis of various factors such as prior family history, maternal age, fetal ultrasound makers, and biochemical tests of maternal serum. Women who are identified as high-risk carriers can receive genetic counseling and other additional tests such as

Table 1. Description of techniques for diagnosis of Down syndrome Ambreen et al.[4]

Method	Description	Advantages	Disadvantages
Cytogenetics analysis	Giemsa banding is performed on fetal cells at metaphase stage on amniocytes (*grown in vitro*) or CVS	Suitable for low income countries where physicians can be presumed to have acquired a high level of diagnostic skill in the absence of laboratory services	Time consuming Resolution of special importance for the detection of structural abnormalities may be quite low as the spontaneous dividing cells are more condensed than those obtained after cell culture *in vitro* In CVS, occurrence of confined placental mosaicism and occurrence of aberrant cells that do not represent the status of fetus. Chances of giving a false positive and false negative result
Fluorescence *in situ* hybridization	FISH involves hybridization of selected chromosome-specific DNA sequences that have been labeled with fluorescent dye to chromosome preparation. The fluorescent-labeled sequences stick to corresponding DNA of chromosome and can be visualized under microscope	Uses smaller probes thus the signals appear to be more distinct as dots Uses higher number of interphase nuclei for analysis, so the problem of any suspected mosaicism is resolved	Sometimes diffuse signals are obtained because it uses chromosome at interphase stage which appears less condense than those of metaphase Time consuming since it involves preparation of slides, fluorescent microscopy and spot counting (~30 min per sample is expected). Maternal and fetal XX is not distinguished by FISH
Quantitative fluorescent-polymerase chain reaction	Involves amplification and detection of STR using fluorescently labeled primers. The product is thus visualized and quantified as peak areas of respective length using an automated DNA sequencer with Gene Scan software	Highly reliable and reproducible. Chances of getting false negative and false positive cases are rare. Faster approach because it can give the diagnosis within 24 hours	Poses a challenge in the case of mosaicism. While testing sex chromosome abnormalities samples from normal XX female may show homozygous QF-PCR pattern indistinguishable from those produced by sample with single X as in Turner syndrome
Paralogous sequence quantification	A PCR-based method for the detection of targeted chromosome number abnormalities, based on the use of paralogous genes. Paralogous sequences have high degree of sequence identity but accumulate nucleotide substitution in a locus specific manner. These differences are called as paralogous sequence mismatches, which can be quantified using pyrosequencing	The first-generation design of the test requires 10 separate PCR reactions per sample, which significantly reduces the sample throughput and increases the probability of handling errors It can handle 30-40 samples per day and report results in less than 48 hours	Expensive when compared with others. Required a skilled bioinformatics analysis
Multiplex probe ligation assay	MLPA is based on hybridization and PCR. Divided into 4 phases: DNA denaturation, hybridization of probe to the complementary target sequence, probe ligation and PCR amplification of ligated probe. These amplified products are analyzed through capillary electrophoresis	Very short time for diagnosis (2-4 days) Relatively low costs	Unable to exclude low level placental and true mosaicism
Next generation sequencing	Clonally amplified DNA templates are sequenced in a massively parallel. It provides a digital quantitative information, in that each sequence is read is a countable "sequence tag" representing an individual clonal DNA template or a single DNA molecule	The current time for sample processing, sequencing, and data interpretation in experienced hands is 5 to 8	The cost of sequencing is approximately $700 –$1000 per sample. Complex data analysis

Table 1. Continue

Segmental duplication- quantitative fluorescent-polymerase chain reaction	Segmental duplications are two similar sequences with different fragment lengths, located on two different chromosomes maintaining the original ratio between the two different chromosomes upon amplification using a single pair of fluorescent primers. The PCR amplified products of different sizes are analyzed through capillary electrophoresis and the trisomy 21 are determined based on the relative dose between the two chromosomes in a single reaction. This method is called as segmental duplication quantitative fluorescent PCR	Unlike other methods, can easily detect all ploidy levels and maternal contamination Robust and cheaper than above mentioned methods Faster approach as the diagnosis can be given in 12 hours	Mosaicism cannot be detected

CVS: Chorionic villus sampling FISH: Fluorescence in situ hybridization QF-PCR: Quantitative fluorescent-polymerase chain reaction STR: Short tandem repeat MLPA: Multiplex probe ligation assay

cytogenic analysis and other molecular methods (FISH, QF-PCR, and MLPA) can be employed. However, these methods have different disadvantages which were overcome by the novel SD-QF-PCR method. SD-QF-PCR is an automated, rapid, reliable, sensitive, and robust technique, and can be used for the diagnosis of various ploidy levels in a clinical setting.

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Ethics

Ethics Committee Approval: The study was approved by the institutional ethics committee (Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, India) IEC code: 2014-140-PhD-79.

Informed Consent: Consent form was filled out by all participants.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: A.A., Concept: S.A., Design: S.A., A.A., Data Collection or Processing: A.A., Analysis or Interpretation: A.A., Literature Search: A.A., Writing: A.A.

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Do Syrian refugees have increased risk for worser pregnancy outcomes? Results of a tertiary center in İstanbul

Suriyeli mültecilerin daha kötü gebelik sonuçları açısından artmış riskleri var mıdır? İstanbul'da tersiyer bir merkezin sonuçları

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Abstract

Objective: To compare obstetric and perinatal outcomes of Syrian refugee pregnants and Turkish counterparts who gave birth at a tertiary center in İstanbul.

Materials and Methods: A retrospective study including the birth records of 704 Syrian refugees and 744 Turkish pregnant women between January 2016 and May 2017 were analyzed. Demographic data, obstetric and neonatal outcomes were compared. The primary aims of this study were to evaluate the pregnancy outcomes and cesarean rates between the groups. The secondary outcomes were the use of antenatal vitamin supplementation, hemoglobin-hematocrit values, and maternal complications.

Results: Our results showed that the use of folic acid and iron supplementation rates during pregnancy were similar between the groups (folic acid supplementation 8.1% vs 6.5%, p=0.264; iron supplementation 20.7% vs 19.6%, p=0.125; respectively for Turkish women and Syrian refugees). Cesarean rates were significantly higher for Turkish patients than in Syrian refugees (42.7% vs 32.7%; p<0.05). Gestational age at delivery was significantly higher among Turkish women when compared with Syrian refugees (37.7±2.3 vs 36.4±2.3 weeks, p<0.05), but there was no significant difference regarding the birtweights' of the newborns (3134 g vs 3066 g for Turkish women and Syrian refugees, respectively, p=0.105). Although obstetric complications were seen more often in Syrian refugees, it did not reach statistical difference (9.7% vs 8.1%, respectively, p=0.285).

Conclusion: Syrian refugees use antenatal vitamin supplementations at similar rates to Turkish citizens and obstetric and perinatal outcomes are similar between the groups.

Keywords: Syrian refugees, cesarean delivery, obstetric outcome, maternal complication, perinatal outcome

Öz

Amaç: İstanbul'da tersiyer bir merkezde doğum yapan Suriyeli mülteci gebeler ile Türk gebelerin obstetrik ve perinatal sonuçlarının karşılaştırılması.

Gereç ve Yöntemler: Ocak 2016 ve Mayıs 2017 tarihleri arasında doğum yapan 704 Suriyeli mülteci ve 744 Türk gebenin doğum kayıtları retrospektif olarak değerlendirildi. Demografik data, obstetrik ve neonatal sonuçlar karşılaştırıldı. Primer hedefler gruplar arasında gebelik sonuçlarının ve sezaryen oranlarının değerlendirilmesi idi. Sekonder hedefler antenatal vitamin desteği kullanımı, hemoglobin-hematokrit sonuçları ve maternal komplikasyonların değerlendirilmesi idi.

Bulgular: Antenatal dönemde folik asit ve demir desteği kullanım oranlarını benzer olarak bulduk (Folik asit kullanımı 8,1% vs 6,5%, p=0,264; demir kullanımı 20,7% vs 19,6%, p=0,125; Türk ve Suriyeli gebeler için sırasıyla). Sezaryenla doğum oranı Türk hastalarda Suriye gebelere göre istatistiksel olarak anlamlı derecede daha yüksekti (42,7% vs 32,7%; p<0,05). Doğumda gebelik haftası Türk gebelerde Suriyelilere göre istatistiksel olarak daha fazla idi (37,7±2,3 vs 36,4±2.3 weeks, p<0,05) ancak yenidoğanların doğum kiloları arasında fark yoktu (Türk ve Suriyeli yenidoğanlar için sırasıyla 3134 gr vs 3066 gr, p=0,105). Obstetrik komplikasyonlar Suriyeli gebelerde daha fazla görülmüş olsa da, aradaki fark istatistiksel olarak anlamlı değildi (sırasıyla 9,7% vs 8,1%, p=0,285).

Sonuç: Suriyeli mülteciler antenatal vitamin desteğini Türklerle benzer oranlarda kullanmaktadır ve obstetrik ve perinatal sonuçlar iki grup arasında benzer bulunmuştur.

Anahtar Kelimeler: Suriyeli mülteci, sezaryen, obstetrik sonuçlar, maternal komplikaston, perinatal sonuçlar

PRECIS: We retrospectively analyzed the obstetric and perinatal outcomes and mode of delivery for Syrian refugees compared with Turkish citizens. Our data showed similar pregnancy outcomes among Syrian refugee women with Turkish women.

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Introduction

The war startedin Syria, resulted with immigration of Syrians to neighbouring countries such as Jordan, Lebanon, and Turkey. Millions of refugees entered Turkey, some refugees started to live in camps near the Syrian border but most were scattered around Turkey; some rented apartments, some started living with relatives or friends. Such a constrained movement naturally caused many severe health problems for the refugees but also negatively affected reproductive health and antenatal care for pregnant refugees[1]. İstanbul is one of the cities to which refugees immigrated intensely. According to the records of the Turkish Government Disaster and Emergency Management Agency, the number of Syrian refugees reached 2.7 million by March 2016, with approximetely 600.000 residing in İstanbul, thousands of whom were women of reproductive age[2]. Refugees at childbearing age and pregnant refugees face many difficulties, dealing with changing family dynamics, and assimilating into a new society while fearing for their safety[3,4]. They also have no medical insurance and consequently have problems reaching medical support in some countries. The Turkish government has provided free healthcare for Syrian refugees, so they can access medical treatment and support just like Turkish citizens without paying any money, including the drugs prescribed. Accordingly, there is no obstruction for Syrian refugees to obtain health care or medication in Turkey. Nevertheless, it is reported in the literature from different countries that the prevalence of poor reproductive health outcomes and antenatal complications such as preterm labour, low birthweight (LBW), increased incidence of cesarean sections (CS), bleeding during delivery, and increased puerperal infections are seen more often in refugee populations[5-7]. The aim of the current study was to compare the obstetric and perinatal outcomes of Syrian refugee women and their Turkish counterparts who gave birth at a tertiary center in İstanbul.

Materials and Methods

A retrospective study between January 2016 and May 2017 at Süleymaniye Maternity Research and Training Hospital was planned. The birth records of 705 Syrian refugee and 750 Turkish pregnant women were retrospectively analyzed. One Syrian refugee and 6 Turkish women were excluded because the they had missing data. Finally, the records of 704 Syrian refugees and 744 Turkish pregnant women were included. The study was approved by the Süleymaniye Maternity Research and Training Hospital Local Ethics Committee (approval number: 2017/E 4737). Demographic data including maternal age, gravidy, complete blood count, presence of gestational diabetes mellitus (GDM) or preeclampsia were obtained. Smoking status and use of folic acid and iron supplementation during the patients' pregnancies were noted. Gestational age at delivery, mode of delivery, the newborn's 1st and 5th minute Appearance, Pulse, Grimace response, Activity, Respiration (APGAR) scores, birth weight, and if necessary, admission to neonatal intensive care unit (NICU) status was established. Complications developed during delivery, requirement of blood transfusion, and length of hospitalization of the mother were noted. All data was extracted from our hospital's database system. Patients with systemic disorders such as pre-existing diabetes mellitus (type 1 or type 2), autoimmune disorders, acute or chronic active infections, heart diseases, and hematologic disorders were excluded from both groups. Gestational age was calculated according to the patients' last menstrual period (LMP). For patients who did not know their LMP, gestational age was calculated according to the first trimester ultrasound of the patient from her file.

Statistical Analysis

Descriptive statistics are stated as percentage, mean ± standard deviation, median, frequency, ratio, minimum and and maximum. The Mann-Whitney U test was used for independent quantitative data. The chi-square test was used for independent qualitative data, if the chi-square test was not relevant, Fisher's exact test was used. Logistic regression was performed to identify independent factors associated with the mode of cesarean delivery for the Turkish women and Syrian refugees. The distribution of variables was analyzed using the Kolmogorov-Smirnov test. The SPSS software program version 22.0 was used for statistical analyses. $P<0.05$ was considered statistically significant.

Results

The mean age for Syrian mothers was 23±4.3 years and 22.9±4.7 years for the Turkish mothers, and this was not statistically different. There was no difference between the groups in terms of gravidy and body mass index (BMI) results (Table 1). Although the rate of multiple pregnancies and hepatitis B surface antigen positivity was more frequent in the refugee population than in the Turkish subjects, this difference did not reach statistical significance. Hemoglobin and hematocrit values were also similar between the Syrian and Turkish patients (11.5±1.5 g/dL vs 11.6±1.6 g/dL; 33.1±7.3% vs 33.9±6.2%, respectively; $p>0.05$). Cigarette smoking was significantly higher among Turkish women than in the Syrians (14% vs 2%, respectively; $p<0.05$). The use of folic acid and iron supplementation during pregnancy was similar between the groups (Table 1). GDM was significantly higher for the Turkish population but preeclampsia rates were similar between the groups.

Gestational age at delivery was significantly higher among Turkish women when compared with the Syrian refugees (37.7±2.3 vs 36.4±2.3 weeks, respectively; $p<0.05$), but there was no significant difference for the birtweights' of the newborns (Table 2). The comparison of deliveries, neonatal outcomes, and obstetric complications is shown in Table 2. When the route of delivery was analyzed, cesarean rates were significantly higher for Turkish patients than for Syrian refugees (42.7% vs 32.7%, respectively; $p<0.05$). The main reason for CS was previous cesarean for both groups. The second most common indication for CS was nonprogressive labor for Turkish women

(27.2%) and breech presentation for Syrian women (24.3%). Additionally, we perfomed logistic regression analysis for the parameters that increased cesarean delivery probability for both groups and found that increasing age, presence of preeclampsia, and multiple pregnancies increased cesarean delivery rates, and conversely, as BMI decreased, cesarean rates also decreasd (Table 3). Admission to the NICU and fetal anomaly rates for the newborn were similar between the groups and the most common symptom for NICU admission was respiratory distress. First and 5th minute APGAR scores were also similar between both groups. Length of hospitalization was significantly shorter among Syrian refugees (1.6±0.8 vs 1.8±1.6 days, respectively; p<0.05). One of the main endpoints of this study was to compare the complications that developed during delivery, and although obstetric complications were seen more often in Syrian refugees, it did not reach statistical significance (8.1% vs 9.7%; p=0.28). The most common maternal complication was postpartum hemorrage and blood transfusion for both Syrian refugees and Turkish citizens (4.6% vs 4.4%, respectively; p>0.05). The second most common maternal complication was 3rd degree perineal laceration for Turkish women (1.4%) and maternal infection for Syrian refugees (2%).

Table 1. Demographic and clinic characteristics of both groups

	Turkish			Syrian			
	Mean ± SD	n%	Median	Mean ± SD	n%	Median	p
Age	22.9±4.7		24.0	23.0±4.3		23.0	0.746
Gravidy	1.8±1.2		1.0	1.9±1.4		1.0	0.643
BMI	27.0±2.8		27.0	26.9±3.0		27.4	0.127
Hb	11.6±1.6		11.7	11.5±1.5		11.8	0.867
Hct	33.9±6.2		35.1	33.1±7.3		35.0	0.386
HbsAg+	29.0	3.8%		31.0	4.3%		0.837
Multiple pregnancy	10.0	1.3%		10.0	1.4%		0.901
GDM	36	4.8%		6	0.9%		0.000
Preeclampia	24	3.2%		18	2.6%		0.448
Smoking status	104	14.0%		14	2.0%		0.000
Folic acid supplementation	60	8.1%		46	6.5%		0.264
Iron supplementation	154	20.7%		138	19.6%		0.125

Hb: Hemoglobin, Hct: Hematocrit, HbsAg: Hepatitis B surface antigen, GDM: Gestational diabetes mellitus, BMI: Body moss index, SD: Standard deviation

Table 2. Comparison of obstetric and neonatal outcomes

		Turkish			Syrian			p
		Mean ± SD	n-%	Median	Mean ± SD	n-%	Median	
Gestational age at birth		37.7±2.3		38.0	36.4±2.3		36.5	**<0.000**
Birth weight		3134±566		3150	3066±553		3125	0.105
APGAR 1		7.6±1.3		8.0	7.5±1.5		8.0	0.962
APGAR 5		8.6±1.1		9.0	8.5±1.4		9.0	0.857
Length of hospitalization		1.8±1.6		2.0	1.6±0.8		1.0	<0.000
Type of birth	Vaginal	426	57.3%		474	67.3%		<0.000
	Cesarean	318	42.7%		230	32.7%		
NICU admission		156	21.0%		138	19.6%		0.519
Fetal anomaly		9	1.2%		8	1.1%		0.273
Obstetric complication		60	8.1%		68	9.7%		0.285

SD: Standard deviation, NICU: Neonatal intensive care unit, APGAR: Appearance, Pulse, Grimace response, Activity, Respiration score

Table 3. Factors independently associated with cesarean delivery including both groups

	OR	% 95 Confidence interval	p
Age	1.03	1.00-1.05	0.037
BMI	0.96	0.93-1.00	0.038
Preeclampsia	2.24	1.21-93.62	0.011
Multiple pregnancy	15.25	3.52->100	<0.000

BMI: Body mass index, OR: Odds ratio

Discussion

Syrian civil war caused displacement of millions of Syrians and the number of Syrian refugees giving birth in Turkey is increasing over time. Women in conflict areas may experience poorer pregnancy outcomes, including increased fetal mortality[8], low birth weight[9], premature labor, antenatal complications, and an increase in puerperal infections[5] compared with pre-conflict levels. Several studies from Lebanon, Jordan, and Turkey reported higher cesarean rates for Syrian refugees than their own citizens, but different from them, we found higher cesarean rates for Turkish citizens[1,7,10]. Higher medico-legal anxiety related with Turkish pregnant women may be one of the reasons for such a condition. Similar to Alnuaimi et al.[1] our data also supports that previous cesarean was the most common cause of another cesarean. The second most common indication for cesarean delivery was nonprogressive labor for Turkish women and breech presentation for Syrian women. Although the cesarean rate for breech presentation seems to be high, when it is corrected for all Syrian deliveries, the cesarean rate for breech presentation was 7.9% (56/704). Patients who are known to need cesarean for breech presentation are referred to our hospital from other government and private hospitals because we are a reference hospital. These data support that the most important point to decrease cesarean rates is to decrease primary CS rates. A study similar to ours was performed by Demirci et al.[11] and they analyzed birth characteristics of Syrian refugees and Turkish citizens in Turkey in 2015. They reported lower hemoglobin values, lower length of hospitalization, and lower birthweight for the newborn of Syrian refugees compared with Turkish women. On the other hand, they reported higher cesarean delivery rates and higher GDM rates for Turkish citizens when compared with Syrian refugees. All of these findings were similar to our study, except for hemoglobin values. Our study demonstrated similar hemoglobin and hematocrit levels betwen Syrian refugees and Turkish citizens. Use of iron supplementation was similar between the groups in our study population, so this may account for the similar hemoglobin-hematocrit values. Kandasamy et al.[3] reported similar GDM and preeclampsia rates between refugee women and control patients. In our study, the rate of GDM was significantly higher among Turkish citizens but preeclampsia rates were similar between the groups. Our clinical experience shows that there is higher compliance between Turkish citizens to glucose tolerance tests to diagnose GDM during antenatal period and this may cause the result of higher GDM diagnoses for Turkish pregnant women. On the other hand, preeclampsia is a diagnosis made by doctors to hospitalized patients according to their clinical and laboratory results. Reese Masterson et al.[6] reported that the rate of preterm delivery, low birth weight, and bleeding during delivery occurred more frequently in Syrian refugee women when compared with control patients. Although Reese Masterson et al.[6] and Büyüktiryaki et al.[7] reported higher rates of LBW neonates for Syrian refugees compared with Lebanese and Turkish citizens, the reults of Erenel et al.[12] and our results do not support this finding because we found no difference for the newborns' birthweights. Iron deficiency may increase the risk of LBW[13] but our hemoglobin and hematocrit results showed no iron deficiency for refugees. Moreover, their iron supplementation was similar to the Turkish pregnant population so this may be a possible explanation for the normal-weight newborns. The APGAR scores of Syrian newborn babies were not lower than their Turkish counterparts and NICU admission rates were similar in our study; these data are also different from some other reports[1,14]. These similar APGAR scores and NICU admission rates may be because the newborns' weights and congenital anomaly rates were similar between the groups in our study. The length of hospitalization was longer for Turkish citizens than Syrian refugees. This could be attributed to the higher rate of cesarean delivery because length of hospitalization is higher in cesarean deliveries.

Study Limitations

This study also has some limitations. First, it was a retropective study so the data that we could obtain was limited to what we could find in the records of the patients. It would be better if we could obtain much more data about the newborn's NICU period. Furthermore, this study was performed at a tertiary center in İstanbul and the results may be different for refugees living in camps and for places where there limitations for refugees to reach health care providers. Previous studies of refugees from different countries identified the cost of health care and security concerns as barriers to women seeking health care but this is not valid for Turkey because our country provides health care to refugees without any limitation and free of charge. A government report noted that 90% of Syrian refugees in camps and 60% of those outside of camps used health services in Turkey and were satisfied with them[15].

Conclusion

In conlusion, as we started to collect the data, we expected that morbidity among Syrian refugee pregnant women and infants would be more common than Turkish citizens. However, according to our results, although there were some differences between the groups according to some headings, pregnancy outcomes among Syrian refugee women appears to be similar to

those of Turkish citizens. The policy of the Turkish government about Syrian refugees may have a positive effect on these results, but an international contribution is essential to minimize the effect of the Syrian crisis both for Turkey and also for the sake of the refugees.

Ethics

Ethics Committee Approval: The study was approved by the Süleymaniye Maternity Training and Research Hospital Local Ethics Committee (approval number: 2017/E 4737).

Informed Consent: Retrospective study.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Concept: E.S.G., O.S., Design: E.S.G., Data Collection or Processing: O.S., G.İ., Analysis or Interpretation: F.F.V., Literature Search: G.İ., E.S.G., Writing: E.S.G., F.F.V.

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Are the skin scar characteristics and closure of the parietal peritoneum associated with pelvic adhesions?

Deri skarı özellikleri ile parietal peritonun kapatılması pelvik adhezyonlar ile ilişkili mi?

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Abstract

Objective: To assess whether the abdominal scar characteristics and closure of the peritoneum were associated with pelvic adhesions.

Materials and Methods: Patients who had undergone cesarean section between December 2015 and February 2016 were assessed prospectively in terms of age, gravida, body mass index, number of living children, number of cesarean sections, time passed since the last cesarean section, closure status of the peritoneum in the last cesarean section, presence of other diseases, smoking status, location of incision in the abdomen (medial, pfannenstiel) scar dimensions (length, width), scar status with respect to skin (hypertrophic, flat, depressive), scar color [color change/no color change (hyperpigmented/hypopigmented)], adhesion of bowel-omentum-uterus, omentum-anterior abdominal wall, uterus-anterior abdominal wall, uterus-bladder, bladder-anterior abdominal wall, fixed uterus, and uterus-omentum-anterior abdominal wall in abdominal exploration.

Results: One hundred five pregnant women who had undergone previous ceserean section surgery by the same physician, were at least in their 30th gestational week, had surgery notes about their previous operation, and had no chronic diseases were included in the study. Age, gravida, body mass index, number of children, number of cesarean sections, time passed since the previous cesarean section, closure/non-closure of peritoneum in the previous cesarean section, and smoking status had no effect on pelvic adhesions. Intraabdominal adhesion was not found to be associated with scar length [odds ratio (OR): 1.54, 95% confidence interval (CI): 1.1-2.2; p=0.02], depressive scar (OR: 9.3, 95% CI: 3.2-27.2; p<0.001), or hypopigmented scar [OR: 0.01, 95% CI: 0.003-0.11; p<0.001].

Conclusion: Adhesions following surgical operations are of great importance due to complications for the patient, complications in relaparotomy, and high costs. Depressive and hypopigmented abdominal scars may be associated with pelvic adhesions. We believe that closure or non-closure of the parietal peritoneum is not associated with pelvic adhesions.

Keywords: Skin scar, pelvic adhesion, closure of parietal peritoneum

Öz

Amaç: Batın skar özelliklerinin ve periton kapatılmasının pelvik adezyon ile ilişkisinin olup-olmadığının değerlendirilmesi.

Gereç ve Yöntemler: Bu çalışma 2015 Aralık-2016 Şubat tarihleri arasında, daha önce gebelik nedeniyle sezaryen olan hastaların yaşları, gravidaları, vücut kitle indeksi, yaşayan çocuk sayısı, olduğu sezaryen sayısı, son operasyondan sonra geçen süre, bir önceki operasyonda periton kapatılıp kapatılmadığı, ek hastalığının olup olmadığı, sigara içip içmediği, skarın batındaki insizyon yeri (mediyal, pfannenstiyel gibi), skarın boyutu (uzunluğu, genişliği) skarın deri seviyesine (hipertrofik, düz, depresif) göre durumu, skarın rengi [renk değişikliği yok/var (hiper-hipopigmente)], batın eksplerasyonda barsak-omentum-uterus, omentum-batın ön duvarı, uterus-batın ön duvarı, uterus-mesane, mesane batın ön duvarı, fikse uterus ve uterus-omentum-batın ön duvarı yapışıklarına bakılarak prospektif olarak değerlendirildi.

Bulgular: Aynı hekimler tarafından operasyonu yapılan ve en az bir kere sezaryen operasyonu olan 30 hafta ve üzeri gebeliği olan önceki ameliyatı hakkında ameliyat notu olan ve kronik hastalığı olmayan 105 gebe çalışmaya alındı. Pelvik adezyon üzerinde hastaların yaşları, gravidaları, vücut kitle indeksi, çocuk sayısı, olduğu sezaryen sayısı, son operasyondan sonra geçen süre, bir önceki operasyonda periton kapatılıp kapatılmadığı ve sigara içip içmediği ilişkisi bulunamadı. Batın içi yapışıklığı sezeryan scar uzunluğu ile [odds oranı (OR): 1,54, %95 güven aralığı (CI): 1,1; 2,2; p=0,02], depresif skar ile (OR: 9,3, %95 CI: 3,2; 27,2; p<0,001), ve hipopigmente skar ile ilişkili bulunmuştur (OR: 0,01, %95 CI: 0,003-0,11; p<0,001).

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Sonuç: Cerrahi operasyonlar sonrası adezyon hem hasta açısından oluşturduğu komplikasyonlar, hem de relaparatominin getirdiği komplikasyonlar ve yüksek maliyetler nedeniyle oldukça önem taşımaktadır. Batın skarının deprese ve hipopigmente olması pelvik adezyonlar ile ilişkili olabilir. Pariyetal peritonun kapatılıp- kapatılmamasının pelvik adezyonla ilişkisinin olmadığı görüşündeyiz.
Anahtar Kelimeler: Deri skarı, pelvik adezyon, pariyetal peritonun kapatılması

PRECIS: Although depressive and hypopigmented abdominal scars are associated with pelvic adhesion, there is no association between peritoneal closure and pelvic adhesions.

Introduction

In spite of advancements in surgical techniques and the emergence of substances that prevent adhesions, pelvic adhesions continue to be a problem for the patient and the physician. As in every surgical branch, pelvic adhesions also cause many long-term problems in gynecologic and obstetric operations. Major problems caused by pelvic adhesions include various complications such as organ damage in future operations, intestinal obstruction, and chronic pelvic pain[1]. The aim of this study was to prospectively investigate whether surgical scar characteristics and closure/non-closure of the peritoneum in the previous operation were associated with pelvic adhesions.

Materials and Methods

Pregnant women at the 30th gestational week or over who underwent cesarean section (CS) between December 2015 and February 2016 in the Van İpekyolu Maternity and Children's Hospital and Yüzüncü Yıl University Faculty of Medicine, Department of Gynecology and Obstetrics, were included in the study. The study was approved by the University of Health Sciences, Van Training and Research Hospital Local Ethics Committee (approval number: 21.04.2015-2015/3). Informed consent forms were filled out by all participants. The participants had previously undergone at least one other CS by the same surgeon. The scar location in the previous CS, scar dimensions (length, width), scar's status with respect to skin (hypertrophic, flat, depressive), scar color [color change/no color change (hyperpigmented/hypopigmented)] were noted retrospectively for the participants. Whether the parietal peritoneum was closed and pelvic adhesions during the operation were examined using the records of the previous CS. Those who did not have surgical operation notes, received scar revision in the previous operation, had chronic diseases (diabetes mellitus, inflammatory bowel diseases, Familial Mediterranean fever, endometriosis), had chronic steroid use, and low-molecular-weight heparin use were excluded from the study.

In compliance with the literature, 4 different scores were used to assess abdominal adhesions in the most recent CS. No adhesions was scored as 0, filmy adhesions needing blunt dissection were scored as 1, strong adhesions requiring sharp dissection were scored as 2, and very strong vascularized adhesions that required sharp dissection and hardly preventable damage were scored as 3[2].

Cesarean technique

The most current and the previous cesarean operations performed by the surgeons in the study involved cutting the skin and the subcutaneous tissue transversely at 2 cm above the pubic bone (Pfannenstiel incision) under general and spinal anesthesia, then blunt dissecting the rectus sheath with a finger, and entering the abdomen. After cutting the lower segment of the uterus transversely (Kehr incision), the infant was delivered. The uterus incision was closed in a continuous interlocking manner in a single layer and the endometrium, myometrium, and perimetrium layers facing each other (entering from the perimetrium and exiting from endometrium boundary; entering from endometrium boundary and exiting from perimetrium boundary) using absorbable 1-0 Vicryl suture (Ethicon, Piscataway, NJ). The abdomen was cleaned (amnion fluid and clots were removed), and checked for bleeding. The peritoneum was closed with 2-0 Monocryl (not closed in some cases). The rectus fascia was closed in a continuous manner using a 1-0 Vicryl suture (Ethicon, Piscataway, NJ). After the subcutaneous bleeding inspection, the skin was closed with a 2-0 Monocryl suture subcutaneously. Prophylactic antibiotic was given intraoperatively to all patients after delivery of the fetus (urgent, elective) as 1 g intravenous cefazolin sodium.

Statistical Analysis

Descriptive data are presented as mean ± standard deviation, median, and ratio. The non-parametric Mann-Whitney U test was used to compare data between the groups. $p<0.05$ was considered to be statistically significant. SPSS 22.0 was used for data analysis. Logistic regression analysis was used to determine significant predictors of intraabdominal adhesions. In the logistic regression, intraabdominal adhesion scores were used as dependent variables, and age, gestational week, body mass index (BMI), smoking, parity, the number of previous CS, time passed since the previous CS, the presence of peritonization in the previous CS, and skin scar (length, width, color, pigmentation) were used as independent variables. The data were stored in STATA 13.0 (Stata Corporation, Texas, USA) and the entire analysis was performed using this system. $P<0.05$ was accepted as the level of statistical significance.

Results

The demographic characteristics of the patients including age, gestational week, BMI, number of CSs, and time passed since the previous CS can be seen in Table 1 as mean values and standard deviation. Age, gestational week, BMI, smoking, parity, the number of previous CSs, time passed since the previous CS, and the presence of peritonization in the previous CS had no effect on the probability of intraabdominal adhesions (Table 2). There was a significant association between scar length [odds ratio (OR): 1.54, 95% confidence interval (CI): 1.1-2.2; p=0.02], depressed scar (OR: 9.3, 95% CI: 3.2-27.2; p<0.001), hypopigmented scar (OR: 0.01, 95% CI: 0.003-0.11; p<0.001) and the amount of intraabdominal adhesions. No association was found between scar color and the amount of intraabdominal adhesions.

Table 1. Mean values of the demographic characteristics of the participants

	Mean ± SD (n=105)
Age	27.18±5.40
Gestational week	35.64±2.41
BMI (kg/cm^2)	29.70±4.28
Number of CSs	1.33±0.53
Time passed since the previous CS	2.43±1.30

The values are expressed as mean ± standard deviation
CS: Cesarean sections, BMI: Body mass index, SD: Standard deviation

Table 2. Association between the amount of intraabdominal adhesion and its possible predictors

	OR (95% CI)	p values
Age (years)	1.06 (0.98-1.14)	0.09
Parity	1.42 (0.95-2.12)	0.09
Gestational week	0.98 (0.84-1.15)	0.89
BMI (kg/cm^2)	1.02 (0.93-1.12)	0.58
Smoking	0.58 (0.24-1.42)	0.23
Number of previous CSs	1.09 (0.52-2.26)	0.80
Time passed since the previous CS, years	1.25 (0.92-1.71)	0.14
Peritonization in the previous CS	0.74 (0.34-1.61)	0.45
Scar width (cm)	0.67 (0.25-1.78)	0.43
Scar length (cm)	1.54 (1.1-2.2)	0.02
Scar color	0.85 (0.38-1.90)	0.71
Scar pigmentation		
Hypopigmentation	(0.003-0.11)	<0.001
Hyperpigmentation	0.48 (0.15-1.54)	0.22
Scar height		<0.001
Depressed	9.3 (3.2-27.2)	0.65
Hypertrophic	0.75 (0.21-2.65)	0.84
Irregular	1.2 (0.2-7.44)	

OR: Odds ratio, CI: Confidence interval, CS: Cesarean sections, BMI: Body mass index

Discussion

The incidence of adhesions following surgical operations is reported to be 93%, which is a very high level[3]. Such adhesions occur in one tenth or more of the patient's abdominal cavity[4]. Adhesions following surgical operations may lead to infertility, organ damage due to adhesions in future operations, intestinal obstruction, and chronic pelvic pain[5]. Higher numbers of CSs received by patients leads to more frequent organ damage in following relaparotomies. The annual cost incurred due to complications caused by pelvic adhesions is estimated to be 1.2 million dollars in the United States[6]. Independent from bleeding, the physiopathology of intraabdominal adhesions following surgical operations is reported to be induced by fibrin clots due to fibrinogen activated by the tissue factor or more specifically, the "fibrin gel matrix"[7]. Fibrinogen emerging from surfaces damaged during the surgical operation is a soluble protein, which forms fibrin monomers by reacting with thrombin and polymerizes. Fibrin polymers must be removed when they emerge because they are initially soluble. If they remain for prolonged periods, they contact with certain coagulation factors such as Factor XIIIa, become insoluble, and form a fibrin gel matrix[8]. Damage in the peritoneum associated with trauma and ischemia induces a quick response and the damaged regions are closed by neutrophils within four hours. Complete healing after constant reactions occurs within approximately one week[9]. As can be understood from the information given above, the formation of adhesion begins with the release of tissue factors. For this reason, the size of the scar may be associated with intraabdominal adhesions. We found a relationship between cesarean incision and intraabdominal adhesion (OR: 1.54, 95% CI: 1.1-2.2; p=0.02).

The hypothesis that peritoneal fibrinolytic activity plays an important role in the pathophysiology of the dissociation of adhesions has been suggested[7]. Tissue plasminogen activator (tPA) in mesothelial cells is a significant natural defense against the formation of adhesions following surgical operations. Active fibrinolysis enzymes, which emerge from inactive plasminogen via tPA, turn the fibrin gel matrix into fibrin destruction products that have no effect on the formation of adhesions[7]. If local fibrinolysis is sufficient, fibrinous adhesions are lysed. If it is not sufficient, connective tissue formation and adhesion development may occur[9]. It was observed in many studies that closure of the parietal peritoneum increased adhesions in gynecologic operations,[10] general surgical operations,[11] and animal experiments[12]. Based on the above data, the parietal peritoneum is routinely closed in gynecologic and obstetric operations[10,13]. However, some other studies reported that,

unlike other abdominal operations, closure of the peritoneum decreased pelvic adhesions in pregnant women[14]. However, with respect to the significance of the fibrinolytic activity in the pathophysiology explained above, amnion fluid was found to show significant fibrinolytic activity after the 37th gestational week[15]. Myers and Bennett[16] and Roset et al.[17] reported that the closure of the parietal peritoneum reduced adhesions in pregnant women. In the present study, we found intraabdominal adhesions were not affected by whether the parietal peritoneum was closed (OR: 0.74, 95% CI: 0.34-1.61, p=0.45). The healing phases of skin scars includes the inflammatory phase (including the injury and prevents infection), the proliferative phase (granulation of macrophages, proliferative degeneration, and characterized by epithelial tissue), and the remodeling phase (regulation of the extracellular matrix), which is a long process. Considering the significant points of the molecular biology behind the healing of scars, the factor that is effective at the molecular level is transforming growth factor-beta (TGF-β)[18]. In adults, TGF-β and receptors are observed to be evidently active in scar tissue and involved in scar formation at the site of injury. However, TGF-β expression is temporary in the fetus and does not form scar tissue[19]. Additionally, fibroblasts were observed to synthesize proteins involved in continuous TGF-β signal transduction in both hypertrophic scars and keloids[20-24]. The number of studies on the relationship between scar tissue and intraabdominal adhesions is limited. However, Salim et al.[25] found that, among all abdominal scar characteristics, only depressive scars were associated with an increase in number and severity of evident adhesion incidence. Also, the incidence of frozen pelvis was found to increase by almost 12 times in women with depressive scars compared with those without depressive scars. Similar to many other researchers, Ferreira et al.[26] reported that hormonal, immunologic, genetic, and tissue growth factors played significant roles in scar development. Nissen et al.[27] showed that filmy intraabdominal adhesions, excessive fibrovascular structures, and depressive scars led to hypertrophic scars and might be affected by tensile strength. In this study, we found that hypopigmentation and depression of scars were associated in with intraabdominal adhesions (p<0.001, p<0.001).

It was reported in a previous study that there was no significant difference between women who underwent CS only once and women who underwent CS two or three times in terms of intraabdominal adhesion incidence[25]. According to other studies, no difference was reported between women with multiple abdominal incisions and women with a single abdominal incision in terms of intraabdominal adhesions[28,29]. Similarly, we observed no statically significant difference between women with different numbers of CSs in terms of abdominal adhesions (OR: 1.09, 95% CI: 0.52-2.26; p=0.80).

Study Limitations

A limitation of our study is the small number of patients. Another limitation is that we did not emphasize whether the first cesarean operation of patients was performed as an emergency or electively.

Conclusion

Adhesions following surgical operations are of great importance due to complications for the patient (e.g., infertility, chronic pelvic pain), and complications in relaparotomy, and high costs. Depressive and hypopigmented abdominal scars provide important information for preoperative prediction of pelvic adhesions. We found that closure or non-closure of the parietal peritoneum was not associated with pelvic adhesions. We believe that more comprehensive studies are required to explain the effect of factors involved in pelvic adhesions.

Ethics

Ethics Committee Approval: The study was approved by The Republic of Turkey Ministry of Health Van Training and Research Hospital Local Ethics Committee (approval number: 21.04.2015-2015/3).

Informed Consent: Consent form was filled out by all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: N.Ç., E.E., Concept: N.Ç., Design: N.Ç., E.E., Data Collection or Processing: E.E., G.G.E., N.A., Analysis or Interpretation: N.Ç., E.E., R.Y., Literature Search: N.Ç., E.E., N.A., Writing: N.Ç.

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Post-cesarean scar endometriosis

Post-sezaryen skar endometriozis

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Abstract

Objective: Endometriosis is seen in women during their reproductive period, where stromal tissue and functional endometrial glands of the uterus are observed outside the uterine cavity. In this study, we aimed to identify the clinical characteristics of our patients who underwent surgery with scar endometriosis and to discuss the surgical results in light of the literature.

Materials and Methods: A total of 24 patients who underwent surgery and diagnosed as having endometriosis as the result of a pathologic examination were retrospectively evaluated.

Results: The mean age of the patients was 31 years. Thirteen presented to general surgery and 11 presented to gynecology outpatient clinics. The pain was cyclical in 19 patients. There was history of cesarean section in 9 patients, twice in 12, and 3 times in three patients. The mean diameter was 39.1 mm on ultrasound, and 37.5 mm on magnetic resonance imaging. Endometriosis was on the left side of the incisions in 13, whereas it was on the right in 11. The mean weight of the lesions was 61.6 grams.

Conclusion: The occurrence of endometriosis is supported by the iatrogenic implantation theory. In the event of a mass in the abdominal wall, previous obstetric and gynecologic operations and a history of a painful mass during menstruation periods must be questioned. In the treatment of scar endometriosis, excision is required by obtaining secure margins. If diagnosis can be established preoperatively, unnecessary surgeries can prevented.

Keywords: Cesarean, endometriosis, scar

Öz

Amaç: Endometriozis reprodüktif dönemdeki kadınlarda fonksiyonel endometriyal gland ve stromal dokunun uterus kavitesi dışında görülmesidir. Bu çalışmada skar endometriozis nedeniyle opere edilen hastalarımızın klinik özelliklerini tanımlamayı ve cerrahi sonuçlarını literatür ışığında tartışmayı amaçladık.

Gereç ve Yöntemler: Patolojik inceleme sonucu endometriozis tanısı alan ve opere edilen 24 hasta retrospektif olarak incelenmiştir.

Bulgular: Çalışmaya alınan hastaların yaş ortalaması 31 olup 13'ü genel cerrahi polikliniğine 11'i ise jinekoloji polikliniğine başvurmuştur. On dokuz hastada siklik ağrı vardı. Dokuz hastada 1 kez, 12 hastada 2 kez ve 3 hastada 3 kez sezaryen öyküsü vardı. Ultrasonografide lezyonların çapının ortalaması 39,1 mm, manyetik rezonans görüntülemede 37,5 mm idi. On üç hastada insizyonun sol tarafında, 11 hastada sağ tarafında endometriozis saptandı. Lezyonların ortalama ağırlığı 61,6 gramdı.

Sonuç: Endometriozisin oluşumu iatrojenik implantasyon teorisi ile desteklenmektedir. Batın duvarında kitle şikayeti ile gelen olgularda öncesinde obstetrik ve jinekolojik operasyon, menstruasyon dönemlerinde artan ağrılı kitle öyküsü iyice sorgulanmalıdır. Skar endometriozisinin tedavisinde güvenli marj sınırı elde edilerek eksizyon gerekmektedir. Skar endometrioziste teşhis önceden konulabilirse gereğinden daha az veya fazla ameliyat yapılması engellenmiş olacaktır.

Anahtar Kelimeler: Sezaryen, endometirozis, skar

PRECIS: Scar endometriosis is a condition seen in women during their reproductive period, excision is required by obtaining secure margins in the treatment.

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Introduction

Endometriosis is a condition seen in women during their reproductive period in which both the stromal tissue and the functional endometrial glands are observed outside the uterine cavity. It mostly occurs through iatrogenic seeding in the wake of obstetric and gynecologic surgeries. Patients generally present to general surgery clinics[1]. Although endometriosis is often found in the pelvic cavity, it may also show localization outside the pelvic region, such as the heart, lungs, liver, kidneys, central nervous system, and the abdominal wall. Even though the endometrium is found in areas outside its normal localization, endometriotic foci still contain normal endometrial tissue. For this reason, they perform a menstrual cycle as an organ functioning within itself. During menstrual periods, thickening, destruction, and menstrual bleeding also occur almost always in these areas, just as in the endometrium. Perhaps the only difference of scar endometriosis from the endometrial tissue inlaid within the uterus is that it fails to drain the blood formed there.

Though several theories have been reported as to its formation, the theory of direct implantation is the most recognized[2]. Ectopic endometriosis foci do not generally show the tendency to become malignant[3]. Various broad series in the literature have addressed why the incidence of the disease in question is rare[4]. In this study, we aimed to identify the clinical characteristics of our patients who underwent surgery due to scar endometriosis and to discuss the surgical results in light of the literature.

Materials and Methods

Data of 29 patients who underwent surgery in the Clinic of General Surgery Haseki Training and Research Hospital between January 2012 and June 2016 with preoperative diagnoses of scar endometriosis, which were confirmed by pathology, were retrospectively examined.

Of the 29 patients, five were excluded from the study due to a lack of abdominal magnetic resonance imaging (MRI). All the demographic data, symptoms at the time of presentation, imaging reports including both ultrasonography (USG) and MRI, and pathology reports were retrospectively reviewed. The study was approved by the Haseki Training and Research Hospital Local Ethics Committee (approval number: 367). Informed consent was obtained from all subjects.

Statistical Analysis

Data concerning demographic and clinical characteristics were analyzed using descriptive methods (means, minimum-maximum). The statistical software used was SPSS for Windows, version 15.0 (SPSS Inc., Chicago, IL, USA).

Results

The mean age of the patients was 31 years (range, 21-40 years). Thirteen (54.2%) patients had presented to the general surgery outpatient clinic and 11 (45.8%) had presented to the gynecology clinic. Twenty-one (87.5%) patients had a painful mass in their previous surgery area, and three (12.5%) had pain only in their previous surgery area. The pain was cyclical in 19 (79.2%) of the patients, whereas it was non-cyclical in six (20.8%). The mean duration of symptoms was 19.8 months (range, 9-31 months). The number of previous ceserean sections in the subjects were as follows: one section in nine patients (37.5%), two in 12 patients (50%), and three in three patients (12.5%). The mean greatest diameter of the endometriotic masses was 39.1 mm (range, 21-54 mm) on USG, and 37.5 mm (range, 21-55 mm) on MRI.

Endometriosis was detected on the left side of the incisions of 13 patients (54.2%), whereas it was found on the right side of the incisions of 11 (45.8%) patients. A solid heterogeneous mass detected in 22 (91.6%) patients, an incisional hernia was detected in one (4.2%), and a mass sporadically containing a solid area suggestive of an abscess was detected in one (4.2%). Three (12.5%) patients underwent surgery due to incisional hernia, whereas 21 (87.5%) patients underwent surgery due to the pre-diagnoses of a tumour on the anterior abdominal wall (Figure 1). During the operations of patients with pre-diagnoses of incisional hernia, a hernia sac and masses adhered to the hernia sac were detected. The masses along with the hernia sac were excised. The defects formed as the result of excising the mass were sutured primarily and then repaired. As the result of the pathology examination, it was reported that all lesions were accordant with endometriosis and that the mean weight of the excised lesions was 61.6 g (range, 46-73 g). None of the patients had any post-operative complications. The patients' mean hospitalizaiton period was 2 days (range, 1-5 days). The mean follow-up period was 22 months (range, 6-51 months), and no recurrence was detected in any patients (Table 1).

Discussion

Endometrioma is defined as endometriosis that forms a mass with a smooth boundary. Although scar endometriosisis can be seen after cesarean surgeries, it may also develop after hysterectomy, hysterotomy, tubal surgeries, appendectomy, trocar-site, amniocentesis, and episiotomy[5-7]. There have been

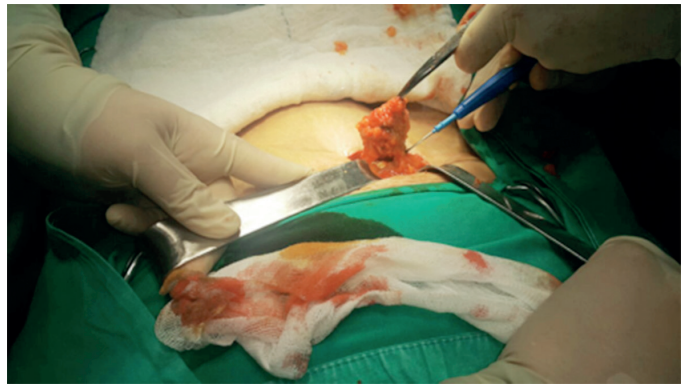

Figure 1. Excision of scar endometriosis

many theories put forward in terms of its etiopathogenesis. Scar endometriosis is accepted to be formed through the iatrogenic auto-transplantation of endometrial cells during surgery[8-10]. It can be seen in the lungs, liver, kidneys, ureters, central nervous system, abdominal scar tissues, and in the extremities, apart from in pelvic organs[2,4]. Even though scar endometriosis may occur months[11] and even years after gynecologic surgery, the mean occurrence period is 30 months.

Elabsi et al.[12] reported an abdominal wall endometrioma that occurred in the wake of a cesarean surgery performed 22 years previously. In our series, the postsurgical period of the patients was 19.8 months (range, 9-31 months) on average.

The incidence of scar endometriosis has been reported to be between 0.03% and 1.7%[13]. The most frequent finding is cyclical or non-cyclical painful mass[14,15]. At the onset of symptoms, patients are often diagnosed as having inguinal hernia, incisional hernia, and abdominal wall tumors, after which they may be exposed to unnecessary interventions. Accordingly, failure to perform the necessary treatment, or any delay in performing the treatment may also cause emotional and physical stress in patients[16]. During the definitive diagnosis; lipoma, granuloma, sebaceous cyst, neuroma, hernia, hematoma, lymphadenopathy, lymphoma, desmoid tumors and sarcomas on the abdominal wall must also be considered[2,4,17]. In our study, 13 (54.2%) patients presented to general surgery clinics and 11 (45.8%) presented to gynecology outpatient clinics.

Andolf et al.[18] in their prospective study in which 578.785 patients were incorporated, detected endometriosis in 749 of 130.305 (0.6%) patients who had given birth through cesarean section. In their study, it was reported that there

Table 1. Patient's demographics

	Age	Symptom	Pain type	C/S (months)	Pathology (cm)	Diameter USG (mm)	MR (mm)	Weight (g)	Symptom duration (months)	Prediagnosis	Admission
1	21	PM	C	45	2*3	26*13	31*21	56	12	SE	GS
2	26	PM	C	46	3*2	32*26	28*21	55	23	SE	GS
3	33	PM	C	70	4*3	35*31	36*34	62	28	SE	GYN
4	40	PM	NC	68	4*3	42*35	37*27	63	18	IH	GS
5	32	PM	C	52	4*3.5	43*41	41*31	65	13	IH	GYN
6	35	PM	NC	76	4.5*3	51*32	42*31	63	23	IH	GS
7	36	P	C	45	3*3	29*26	21*25	59	14	SE	GYN
8	37	PM	C	56	3*2	21*11	27*19	56	27	SE	GYN
9	37	PM	C	62	5*4	48*41	46*38	70	31	SE	GYN
10	25	PM	C	35	3*3	34*32	28*27	64	26	SE	GS
11	32	PM	C	46	4*4	43*41	38*35	68	10	SE	GS
12	35	PM	C	56	3*1	35*17	29*12	54	9	SE	GS
13	31	PM	NC	48	5*4	54*42	48*39	73	21	SE	GS
14	35	PM	C	59	4.5*4.2	51*47	49*43	68	14	SE	GS
15	25	PM	C	38	4*3	43*32	39*26	67	16	SE	GS
16	34	PM	C	47	5*4	53*45	47*35	70	18	SE	GS
17	27	PM	NC	38	2*2	23*22	21*20	46	23	SE	GS
18	24	P	C	32	4*3	43*38	41*32	54	21	SE	GS
19	29	PM	C	46	3*3	30*26	35*30	56	21	SE	GYN
20	33	PM	C	37	3*4	35*34	32*32	66	17	SE	GYN
21	36	PM	NC	39	4*4	45*38	45*40	67	18	SE	GYN
22	28	PM	C	54	3*5	48*25	55*30	59	29	SE	GYN
23	34	PM	C	49	2*5	45*22	50*25	61	27	SE	GYN
24	24	PM	C	32	3*1	30*15	30*18	57	15	SE	GYN

PM: Painful mass, P: Pain, C: Cyclic, NC: Noncyclic, C/S: Cesarean, USG: Ultrasonography, MR: Magnetic resonance, SE: Scar endometriosis, IH: Incisional hernia, GYN: Gynecology, GS: General surgery

was no difference in terms of the risk of the development of endometriosis between those who underwent cesarean once and those who had a history of more than one cesarean; however, it was also reported that the risk of endometriosis in those who gave birth through cesarean section was twice as much when compared with those who had vaginal birth. In our series, there was a history of cesarean section in all patients, none had had vaginal births.

In the evaluation of the mass, USG, computed tomography, and MRI are not examinations that establish the final diagnosis, but provide information about the location of the mass as well as its size and volume. USG and MRI are often the preferable methods for diagnosis (Figure 2-4). Differences in size in radiologic imaging may vary depending on the day of the menstrual cycle, the ratio of stromal and glandular elements, the amount of bleeding, and the inflammatory response in the peripheral tissue. Though hypoechoic, a vascularized nodule is seen in USG, it can also be seen as cystic, polycystic or heterogeneous echo. The advantage of MRI over USG is its ability to distinguish masses that imitate endometriosis on the abdominal wall.

In general, biopsy is not required because anamnesis, physical examination, and imaging methods are adequate in the diagnosis of endometriosis. Biopsy is only performed under conditions in which malignancy is suspected. The diagnosis-establishing value of fine needle aspiration biopsy is low. Nevertheless, it is still recommended in scar endometriosis due to its convenience. For a final diagnosis, an accurate pathologic analysis is required. The pathologic detection of glandular epithelial cells, spindle or

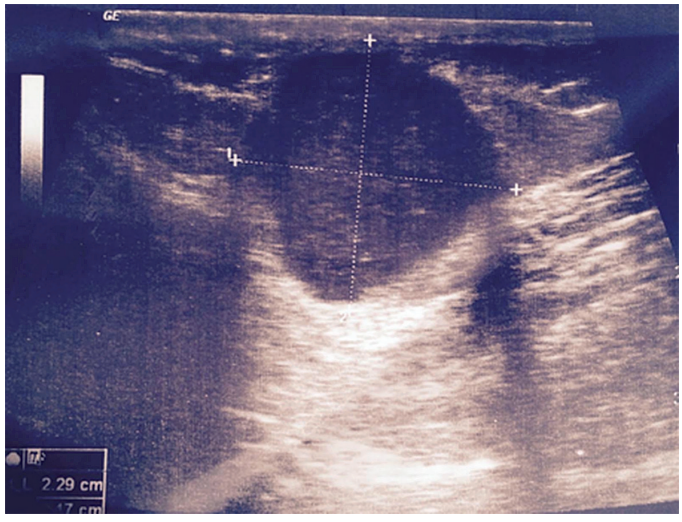

Figure 2. Ultrasonography

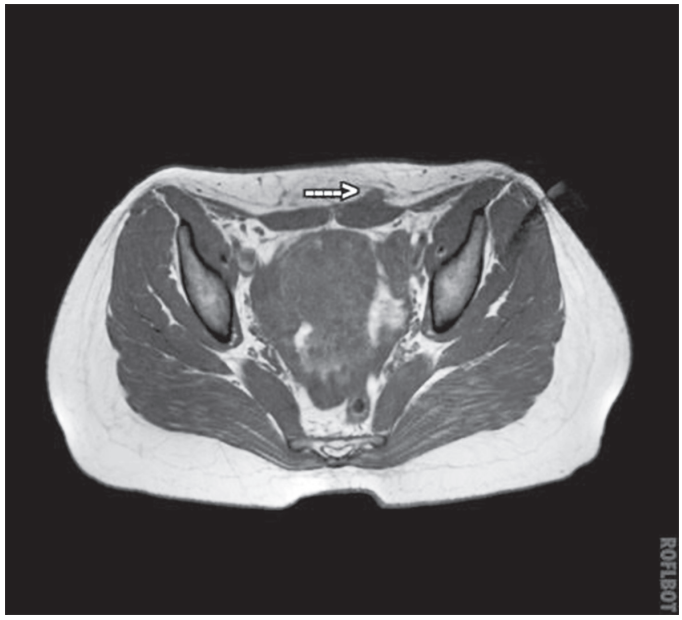

Figure 3. Magnetic resonance image axial

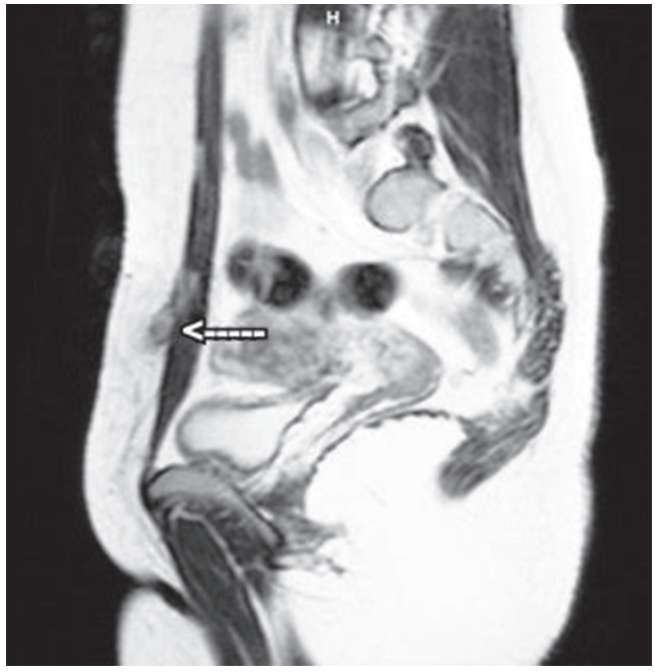

Figure 4. Magnetic resonance image coronar

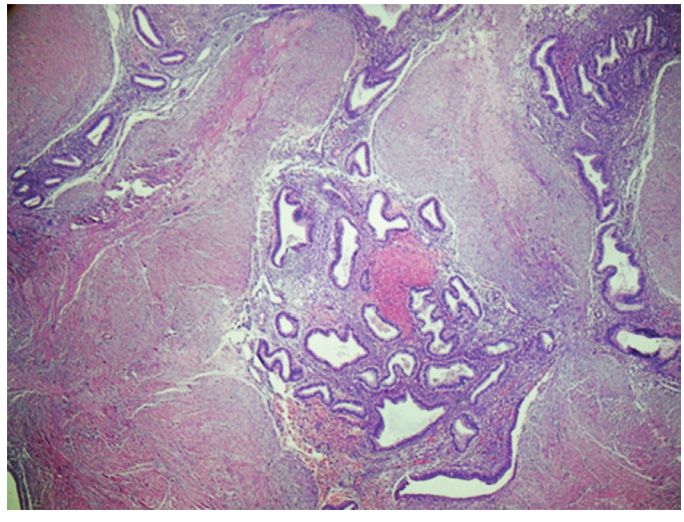

Figure 5. The pathologic detection of glandular epithelial cells, oval stromal cells

oval stromal cells, and hemosiderin-laden macrophages allows for establishing a diagnosis[19,20] (Figure 5). On the other hand, because incisional biopsy will cause endometriosis to spread even further, some studies have advised against performing this procedure[2-4,21]. In our study, neither incisional biopsy nor fine needle aspiration was performed.

Apart from the fact that there are two types of treatment options in pelvic cases, which involve medical and surgical methods, neither of them is an effective method of treatment on its own. However, surgery in scar endometriosis, as is also seen in our surgical series, is the gold standard treatment approach. Medical treatment, on the other hand, must be reserved for patients who cannot undergo surgery.

Surgical excision with at least a 1 cm margin boundary should be performed to prevent recurrence in surgical treatment, in addition, some part of the neighbouring structures, such as fascia or muscle, also needs to be excisedl. Thus, the recurrence of endometriosis in the wound area will be prevented by means of the transplantation of microscopic endometrial tissue residuals. In the event that the invasion depth into structures of the abdominal wall causes large defects after surgery, a repair with synthetic materials should be performed[22,23]. In our case series, a large excision was performed in 5 patients because the masses had invaded the peritoneal surface, and the large defect area formed on the abdominal wall after the operation was supported with Prolene mesh. If no residual tissue is left inside in scar endometriosis, no additional treatment is required. The recurrence rates after total excision is quite low. In our series, no recurrence was seen during follow-up.

Study Limitations

The small patient population and the retrospective nature of the study are our limitations.

Conclusion

The occurrence of endometriosis whose etiopathogenesis has not yet been fully explained is supported by most authors through the theory of iatrogenic implantation. In the patients who visit hospital with symptoms of a mass on the abdominal wall, previous obstetric and gynecologic operations as well as the medical history of a painful mass becoming increasingly severe during menstruation periods must be questioned in full. In the treatment of scar endometriosis, excision is required by obtaining a secure marginal boundary. If diagnosis can be established in advance in scar endometriosis, then the performance of unnecessary surgeries will be prevented. Studies in broad series are needed to be conducted on such diseases that are rarely observed.

Ethics

Ethics Committee Approval: The study was approved by the Haseki Training and Research Hospital Local Ethics Committee (approval number: 367).

Informed Consent: Consent form was completed by all participants.

Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: D.Y., C.T., O.D., T.D., Concept: D.Y., C.T., T.D., R.N.B., Design: C.T., T.D., A.H., M.T., R.N.B., Data Collection or Processing: C.T., O.D., A.H., M.A., M.T., Analysis or Interpretation: C.T., M.A., R.N.B., Literature Search: D.Y., C.T., O.D., A.H., M.A., M.T., Writing: D.Y., C.T., T.D.

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

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The effect of the pooling method on the live birth rate in poor ovarian responders according to the Bologna criteria

Bologna kriterlerine uyan zayıf cevaplı kadınlarda havuz yönteminin gebelik sonuçlarına etkisi

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Abstract

Objective: Pooling is an alternative method to achieve *in vitro* fertilization outcomes. This study was to investigate the effect of pooling method on pregnancy outcomes in poor responder patients according to Bologna criteria.

Materials and Methods: Two hundred-fifty five poor responder patients were enrolled in this study. Pooling embryo transfer (ET) group had 110 and fresh ET group had 145 patients.

Results: Although, age was similar between both treatment groups (p=0.31), antral follicle count (p<0.001), total number of retrieved oocyte (p<0.001), total metaphase II oocyte count (p<0.001), number of stimulation cycles (p<0.001), were significantly different between the groups. The day of ET were similiar between two groups (p=0.72) but the number of ET procedure was significantly higher in pooling ET group compared to fresh ET (p<0.001). Positive pregnancy test [35/110 (32%) vs 53/145 (37%)] (p=0.43) and clinical pregnacy rates [31/110 (28%) vs 49/145 (34%)] (p=0.33) were similar between groups, whereas, implantation [31/191 (16%) vs 49/198 (25%)] (p=0.03) and live birth rates [15/110 (14%) vs 36/145 (25%)] (p=0.04) were significantly higher in fresh ET group. Despite that, abortion rates were significantly higher in pooling ET group [16/31 (52%) vs 13/49 (27%)] (p=0.04). Binary logistic regression analyese has revealed no effect of variables on live birth rates.

Conclusion: Even though, pooling strategy seems to have a slight positive effect on pregnancy outcomes, there is no benefical effect on live birth rates. Furthermore, this strategy is increasing the abortion rates in parallel with clinical pregnancy rates.

Keywords: Poor responder, frozen embryo transfer, *in vitro* fertilization

Öz

Amaç: *İn vitro* fertilizasyon sonuçlarının elde edilmesinde havuz yöntemi bir alternatiftir. Bu çalışma, Bologna kriterlerine göre kötü yanıt veren hastalarda havuz yönteminin gebelik sonuçları üzerine etkisini araştırmak amacıyla yapılmıştır.

Gereç ve Yöntemler: Bu çalışmaya iki yüz elli beş zayıf cevaplı hasta alındı. Havuz embriyo transfer (ET) grubunda 110, taze ET grubunda 145 hasta vardı.

Bulguar: Her iki tedavi grubu arasında yaş benzer olmakla birlikte (p=0,34), antral folikül sayısı (p<0,001), toplanan toplam oosit sayısı (p<0,001), toplam metafaz II oosit sayısı (p<0,001), stimülasyon siklus sayıları (p<0,001) gruplar arasında anlamlı olarak farklıydı. ET'nin günü iki grup arasında benzerdi (p=0,72), ancak ET işlemi, taze ET'ye kıyasla havuz ET grubunda anlamlı derecede yüksekti (p<0,001). Pozitif gebelik testi ve klinik gebelik oranları gruplar arasında benzer iken [(35/110'a (%32) karşın 53/145 (%37) p=0,43), (31/110'a (%28) karşın 49/145 (%34) p=0,33)], implantasyon [31/191 (%16) ile 49/198 (%25)] (p=0,03) ve canlı doğum oranları [15/110 (%14) vs 36/145 (%25)] (p=0,04), taze ET grubunda anlamlı derecede yüksekti. Buna rağmen havuz ET grubunda düşük oranları anlamlı derecede yüksekti [16/31'e (%52) karşın 13/49 (%27)] (p=0,04). İkili lojistik regresyon analizi, değişkenlerin canlı doğum oranları üzerinde etkisi olmadığını ortaya koydu.

Sonuç: Havuz stratejisinin gebelik sonuçları üzerinde hafif bir pozitif etkisi olduğu görülse de, canlı doğum oranları üzerinde hiçbir olumlu etkisi yoktur. Ayrıca, bu strateji klinik gebelik oranlarına paralel olarak düşük oranlarını arttırmaktadır.

Anahtar Kelimeler: Zayıf overyan yanıt, dondurulmuş embriyo transferi, *in vitro* fertilizasyon

PRECIS: Pooling does not increase live birth rates among poor responder patients.

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Introduction

There is ongoing debate about the management of poor responder women in *in vitro* fertilisation (IVF) centres. Although they receive an increased gonadotropin dose compared with normoresponders, fewer oocytes are eligible for the procedure, and thus, the pregnancy outcomes are lower[1-3]. Therefore, physicians have focussed on other methods of increasing pregnancy rates in poor responder women. Several treatment options, such as oestrogen use in the luteal phase[4], adding a recombinant luteinizing hormone preparation during stimulation[5], and pre-treatment with growth hormone[6] and androgen[1] have been investigated. Yet, lower pregnancy rates are still reported in poor responders compared with normoresponder women. In recent years, the cryopreservation of embryos has become an essential component of treatment with assisted reproductive technology, and due to the technological developments in the embryology arm, frozen/thawed embryo transfer (FET) has been offered as an alternative to physicians. Two methods are commonly used-slow freezing and vitrification. Recently, Sites et al.[7] reported significantly lower live birth rates with slow freezing compared with vitrification (25% vs 71%) and fresh embryo transfer (ET) (ET; 25% vs 70%)[8]. FET has become an alternative method to fresh ET in normoresponder women, but there is no consensus about the use of FET in poor responder patients. The number of retrieved oocytes is correlated with the birth rate[9]. Management options are scarce in poor responder women because of the lower oocyte numbers and suboptimal oocyte maturation[10]. Increasing the embryo yield via an accumulation from consecutive stimulation cycles may be a new approach to overcome poor outcomes. Accumulated embryos from consecutive stimulation cycles are frozen and hidden by vitrification, and ET is performed by thawing the entire cohort after reaching the proper number. Theoretically, similar pregnancy and delivery rates to those of normoresponder patients may be achieved. There are many different definitions of poor responders in the literature[11-13]. Most recently, poor responders were defined as detailed by Ferraretti et al.[14]. We consider that sufficient pregnancies can be achieved if enough oocytes are retrieved in consecutive cycles. Thus, the aim of this study was to investigate the effect of embryo collection on the pregnancy, clinical pregnancy and live birth rates in poor responder women, as defined according to the Bologna criteria[14].

Materials and Methods

The study was performed at the Bahçeci Fulya IVF Centre. All patients who underwent ET procedures were screened using electronic records from August 2010 to January 2014. Ethics committee approval was not required for this study because it involved retrospective data analysis. Nevertheless, a consent form was signed by all participants, and clinical investigation commission approval was received. We declare that we have no financial or personal relationships with other people or organisations that could inappropriately influence our work; there is no professional or other personal interest of any nature or kind related to any product, service and/or company that could be construed as influencing the research. To generate homogeneous study groups and show the power of ET rather than transfer cancellation, we excluded women aged ≥46 years at the time of ET; those who had used neoadjuvant therapy, such as dehydroepiandrosterone and growth hormone, before the procedure; those with controlled ovarian stimulation (COS) regimes other than the letrozole/antagonist protocol for the fresh ET arm; those with notification of difficult ET and use of a different catheter apart from a soft catheter by the performing physician; and those undergoing a second FET from a remaining pool. Patients who met the Bologna criteria as described by Ferraretti et al.[14] were included in the study. To ensure a similar endometrial receptivity between the groups, serum oestradiol (E2) and progesterone (P) levels were determined on the human chorionic gonadotropin (hCG) day of the fresh ET cycle and day 15 of the endometrial preparation cycle in the pooling ET arm. Those with a P level >1.5 ng/mL on the hCG day were excluded from the study.

Stimulation cycles ending with a preimplantation genetic diagnosis (PGD) for aneuploidy screening or another situation were excluded from both treatment arms. All patients used their own oocytes because egg donation is illegal in Turkey. The live birth rate was considered the primary outcome. The clinical pregnancy and miscarriage rates were considered secondary outcomes.

Pooling methods

There was no restriction on the stimulation protocol among the participants recruited for the pooling group. However, for the final oocyte maturation, a fixed 250 µg of recombinant hCG (Ovitrelle, Serono, Turkey) was used subcutaneously for all stimulated cycles. All the embryos were generated by intracytoplasmic sperm injection (ICSI) and vitrified afterwards. At least two COS/ICSI cycles were performed. Embryos recruited from the last cycle were also vitrified. The embryo(s) obtained for each cycle were kept in culture until the blastocyst stage in women undergoing blastocyst ET. Embryo(s) that reached the blastocyst stage were frozen. In women undergoing ET at the cleavage stage, the obtained embryo(s) that reached the cleavage stage were frozen. The whole cohort was thawed on the appropriate day, if vitrified, of the developmental stage and selected for transfer as the best-quality embryos according to the morphologic assessment criteria described below.

Endometrial preparation

Transdermal E2 hemihydrate patches (Climara Forte, Bayer, İstanbul, Turkey), which were preferred to prime the endometrium, were started on day 3 of menstruation at a dosage of 100 mcg/day for the first 4 days, 200 mcg/day for the next 4 days, and 300 mcg/day for the last 4 days. The serum P level and endometrial thickness were checked afterwards.

Vaginal P gel (Crinone 8%, 90 mg, MerckSerono, Bedfordshire, UK) was initiated once per day if the P level was <1.5 ng/mL, endometrial thickness >8 mm, and a triple-line appearance was evident. Transdermal patches at a dosage of 300 mcg/day and vaginal P gel were maintained until the pregnancy test. After a positive test result patients continued to apply the transdermal patches with the same dosage, but the vaginal P was increased to twice per day and continued up to the 10th week of pregnancy.

The flexible letrozole/Antagonist protocol

Combination therapy with letrozole (Femara 2.5 mg, Novartis, İstanbul, Turkey) tablets twice per day and 150-450 IU of subcutaneous human menopausal gonadotropin (hMG; Merional, IBSA Institut Biochimique SA, Lamone, Switzerland) injections were started on day 3 of the present cycle. Serial sonographic examinations and serum E2 level measurements were evaluated during the course of follicular development. The gonadotropin-releasing hormone antagonist (Cetrotide, Serono, Turkey) was added at a dosage of 0.25 mg/day when the leading follicle reached 12-14 mm in size and continued up to final triggering. One ampoule of recombinant hCG (Ovitrelle, Serono, Turkey) was administered as soon as the leading follicle reached a mean diameter of 18 mm. Ovum puncture was performed after 36 hours from recombinant hCG injection by transvaginal-ultrasound-guided needle aspiration under general anaesthesia. Cleavage stage embryos or blastocyst transfers were performed afterwards.

Luteal support

Luteal phase support was initiated on the day of the oocyte pickup procedure for the fresh ET arm and day 15 for the pooling ET arm, as described by Bulent Urman et al.[15].

The vitrification and embryo thawing protocol

We used our own solutions for embryo vitrification and thawing procedures. The embryos in the cleavage stage were placed for 6-8 minutes and the blastocyst for 10-12 minutes in equilibration solution at room temperature. Afterwards, they were kept in the vitrification solution for 40 seconds just before being transferred into liquid nitrogen. The thawing process was started with the removal of the cyrovials from the liquid nitrogen and keeping the embryos in the first thawing solution at 37 °C for 60 seconds and then in the second solution for 180 seconds at room temperature. Following this, they were transferred into the culture solution to be put in the incubator.

Embryo and blastocyst morphology

Cleavage-stage embryos were evaluated according to Hardarson et al.[16] description. The morphologic assessment of the blastocysts was performed by means of a staging algorithm, as described by Gardner et al.[17].

Pregnancy definitions

Serum hCG measurements were evaluated 9 days after blastocyst transfer and 12 days after cleavage stage ET. A value of β hCG >5 mIU/mL was accepted as positive. Clinical pregnancy was defined as an intrauterine sac envisioned by transvaginal sonography at 7 weeks of gestation; the implantation rate was obtained by dividing the number of gestational sacs into the number of transferred embryos[18]. Pregnancies that ended before the 24th week of gestation were included in the abortion group. The abortion rate was obtained by dividing the number of pregnancy losses into the number of clinical pregnancies[19]. Live birth was defined as the birth of one or more infants with a gestational age of ≥24 weeks[20]. Live birth rates per patient and per transferred embryo were calculated separately by dividing the total number of births occurring at a gestational age of ≥24 weeks into the whole cohort and the number of transferred embryos, respectively.

Statistical Analysis

The distribution of the variables was assessed using a histogram, the Kolmogorov-Smirnov and One Sample tests. In this study, data are presented in terms of median, minimum, maximum, frequency and percentage. The Mann-Whitney U test was used for quantitative variables thought to be effective on live births. The chi-square test was used to compare categorical variables. p values <0.05 were considered statistically significant. Logistic regression analysis was used for variables thought to be effective on live birth outcomes. The analyses were performed using the Statistical Package for the Social Sciences version 21.0.

Results

One hundred ten patients for the pooling ET arm and 146 patients for the fresh ET arm were included in the study. In the fresh ET arm, one patient was excluded because of ectopic pregnancy. The demographic characteristics of both groups are displayed in Table 1. The age was similar in both treatment arms (p=0.31), but the antral follicle count (antral fdlicle count; p=0.001), total number of retrieved oocytes (p=0.001), total number of metaphase II (MII) oocytes (p=0.001), total gonadotropin dose (p=0.001), number of stimulation cycles (p=0.001), and cost of treatment (p=0.001) were significantly different. The day of ET was similar between the groups, but the number of transferred embryos was significantly higher in the pooling ET arm. The p values were 0.72 and 0.001, respectively (Table 1). In the pooling ET arm of the study, two stimulation cycles for 49 women, three cycles for 30 women, four cycles for 15 women, five cycles for 8 women, six cycles for 6 women, and seven cycles for 2 women were performed (Figure 1). In total, 338 stimulated cycles were performed in 110 women in the pooling ET arm. The protocols used in the stimulated cycles were as follows: the letrozole/antagonist protocol in 163 cycles, gonadotropin/antagonist protocol in 89 cycles, modified natural protocol in 70 cycles, hybrid protocol in 13 cycles, microdose flare-up protocol in 2 cycles, and long protocol in 1 cycle (Figure 2).

In total, 495 oocytes were collected, 399 of which were MII oocytes. The mean oocyte number and MII oocyte number per one cycle were 1.59±0.69 and 1.3±0.63, respectively. In

the fresh ET group, 145 stimulated cycles (flexible letrozole/antagonist) were applied to 145 women. At the end of these cycles, 332 oocytes were collected, 276 of which were MII oocytes. The mean oocyte number and MII oocyte number per one cycle were 2.29±0.75 and 1.9±0.75 (p<0.01), respectively. The pregnancy outcomes for both groups are displayed in Table 2. The positive test result and clinical pregnancy outcomes were similar between both arms (p=0.43 and 0.33, respectively). The implantation rate, live birth rate per patient, and live birth rate per transferred embryo were found to be significantly higher in the fresh ET arm (p=0.03, 0.04, and 0.003, respectively). The abortion rate was observed to be significantly higher in the pooling ET arm (p=0.04). Although binary comparisons revealed that the type of treatment and women's age were effective variables in relation to live births, the total number of oocytes, total number of MII oocytes, number of transferred embryos, and day of ET were found to be ineffective variables (p=0.02, <0.001, 0.63, 0.95, 0.23, and 0.07, respectively) (Table 3). Logistic regression analyses were used to evaluate variables that affected the live birth rates, such as age, total number of oocytes, total number of MII oocytes, number of cycles, type of treatment, number of transferred embryos, and day of ET. None of these variables were identified as risk factors for live birth outcomes. Although the age and type of treatment were different in the binary comparisons, no difference was found in the logistic regression analyses.

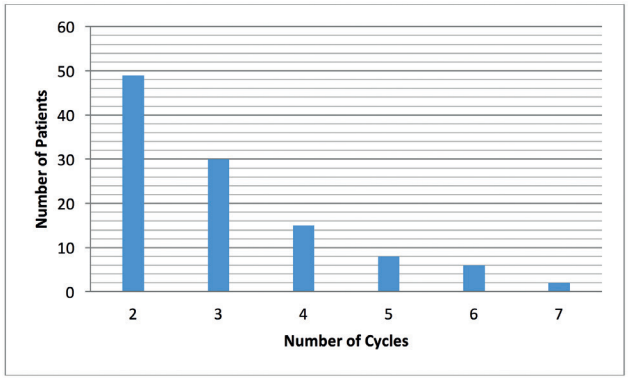

Figure 1. Stimulation cycles of the pooling embryo transfer arm

Two stimulation cycles for 49 women, three cycles for 30 women, four cycles for 15 women, five cycles for 8 women, six cycles for 6 women and seven cycles for 2 women were performed

Discussion

In the present study, we determined no favourable effect of pooled ET for live birth rates. Cobo et al.[21] performed oocyte pooling on 724 poor responder patients in their study. Subsequently, they hey thawed all the oocytes and performed ICSI fertilisation. The live birth rate was higher in the pooling arm

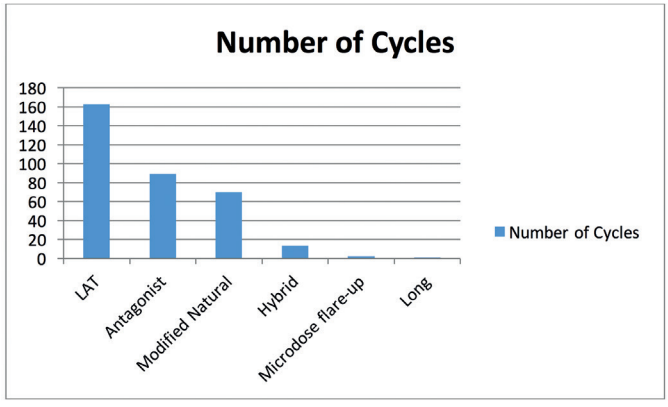

Figure 2. Stimulation protocols of the pooling embryo transfer arm

The LAT protocol was used in 163 cycles, gonadotropin/antagonist protocol in 89 cycles, modified natural protocol in 70 cycles, hybrid protocol in 13 cycles, microdose flare-up protocol in 2 cycles and long protocol in 1 cycle

LAT: Letrozole/antagonist protocol

Table 1. Demographic parameters of the patients

	Pooling ET	Fresh ET	p value
Age	38.2±0.3	37.6±0.3	0.31
AFC	1.8±0.72	2.3±0.84	<0.001*
Total number of cycles	3±1.29	1	<0.001*
TRO	4.5±1.9	2.2±0.75	<0.001*
MII	3.6±1.6	1.9±0.75	<0.001*
Total dose of gonadotropin	5062.5 (min: 750, max: 22650)	2925 (min: 300, max: 7200)	<0.001*
ET days	2 (min: 2, max: 5)	2 (min: 2 max: 5)	0.72
Number of transferred embryos	1.7±0.44	1.3±0.48	<0.001*
Cost of treatment ($)	4926±1691	2209	<0.001*

Age and day of ET were similar in both treatment arms, but AFC, total number of oocytes, MII oocytes, total gonadotropin dosage, total number of stimulated cycles, cost of treatment and number of transferred embryos were significantly different.
TRO: Total number of retrieved oocytes, MII: Metaphase II, AFC: Antral follicle count, ET: Embryo transfer
Dose of gonadotropin: IU/mL, Min: Minimum, Max: Maximum, *: p<0.05 was accepted as significant

(36.4% vs 23.7%), and the authors reported similar outcomes for patients aged ≥40 years (15.8% vs 7.1%). Furthermore, another study suggested that oocyte or embryo accumulation might be useful for specific conditions, such as cystic fibrosis and X-linked microtubular myopathy[22]. Unfortunately, we could not determine any positive effect of embryo accumulation in our study. However, the patient selection criteria in this study were different from those used in previous works. Cobo et al.[21] used the poor responder criteria described previously by Surrey and Schoolcraft[23], whereas Chatziparasidou et al.[22] included poor responders according to low AFC levels (AFC <7) and candidates for PGD. In contrast, we used the Bologna Criteria to identify poor responders[14]; this may have caused a lower follicle pool in our study group, and therefore, a worse oocyte quality than in other studies.

Aneuploidy is mostly related to the non-disjunction of chromosomes during the first meiotic division[24]. The presence of aneuploidy indicates poor quality oocytes. Maternal age is the most determinant factor regarding oocyte aneuploidy[25]. A low ovarian response during COS is related to the depletion of the follicular pool and displays ovarian aging[24]. Setti et al.[26] found similar aneuploidy and abortion rates among 80 poor and normoresponder patients aged >35 years undergoing ICSI/PGD. However, several studies have associated higher abortion rates with a poor ovarian response[24,27]. In addition, this rising pattern has been documented for all age groups in which ≤3 oocytes have been retrieved. Sunkara et al.[24] reported their abortion rate as 20% in women from whom 1-3 oocytes were retrieved, whereas the rate was 13.1% in the ≥15 oocytes group. There is a close relationship between the oocyte number and abortion rate. The foetal aneuploidy rate rises in accordance with a diminishing follicular pool[27]. Furthermore, the abortion rate increases with maternal aging[24]. In our study, the abortion rate for the pooling ET group was 52% (16/31), whereas it was 27% (13/49) for the fresh ET group. Although the total oocyte number was higher in the pooling ET arm (4.5±1.9 vs 2.2±0.75), 3±1.29 COS cycles on average were conducted to collect them. Nevertheless, the increasing number of total

Table 2. Pregnancy outcomes for both groups

	Pooling ET	Fresh ET	p value
Positive pregnancy test per patient	35/110 (32%)	53/145 (37%)	0.43
Implantation rate	31/191 (16%)	49/198 (25%)	0.03*
Clinical pregnancy per patient	31/110 (28%)	49/145 (34%)	0.33
Live birth per patient	15/110 (14%)	36/145 (25%)	0.04*
Live birth per transferred embryo	15/191 (8%)	36/198 (18%)	0.003*
Miscarriage	16/31 (52%)	13/49 (27%)	0.04*

Although the positive test results and clinical pregnancy rates were similar, the implantation, abortion, and live birth rates were found to be significantly different between groups. In the pooling ET arm, the abortion rate was higher and live birth rate lower, both per transferred embryo and per patient.
ET: Embryo transfer, Min: Minimum, Max: Maximum, *p<0.05 was accepted as significant

Table 3. Variables affecting the live birth rates are displayed

		Negative	Live birth	p value
Type of treatment	Pooling ET	95 (86.4%)	15 (13.6%)	0.02*
	Fresh ET	109 (75%)	36 (25%)	
Number of transferred embryos	1	93 (77%)	28 (23%)	0.23
	2	111 (83%)	23 (17%)	
ET days	2	134 (53%)	32 (13%)	0.07
	3	59 (23%)	11 (4%)	
	4	5 (2%)	3 (1%)	
	5	6 (2.4%)	5 (2%)	
Age (years)		39±3.84	35±4.29	<0.001*
TRO		3 (min: 1 max: 13)	3 (min: 1, max: 9)	0.08
MII		2 (min: 0, max: 10)	2 (min: 1, max: 7)	0.53

Type of treatment, age and peak E2 values seemed to affect the live birth rates, but the total number of oocytes, total number of MII oocytes, day of ET and number of transferred embryos did not. ET: Embryo transfer, MII: Metaphase II, TRO: Total number of retrieved oocytes, Min: Minimum, Max: Maximum, *: p<0.05 was accepted as significant

oocytes did not decrease the aneuploidy rates depending on maternal aging; hence, more aneuploidic embryos may have been generated in the pooling ET arm. Therefore, it is reasonable to judge the oocyte factor as relating to increased abortion rates. There is a positive correlation between the total number of retrieved oocytes and live birth rates in both poor and normoresponder women[9,28]. Schimberni et al.[29] reported a 20.3% pregnancy rate per patient and 14.3% abortion rate between poor responder women aged 36-39 years with a single ET. Similar results were reported by Ata et al.[30] in poor responder women aged 38.2±4.9 years when the researchers followed the natural cycle and picked up one oocyte. Branigan and Estes[31] reported 27% implantation and 29.4% clinical pregnancy rates in poor responder women aged under 40 years, with 2.1 oocytes on average. The pregnancy rates in this study were given per ET. Cycle cancellations were not included for either treatment arm. Therefore, a higher pregnancy rate was obtained than reported in the literature[32-34]. In our study, the number of retrieved oocytes per one cycle was lower in the pooling ET arm compared with the fresh ET cycles (1.59±0.69 vs 2.29±0.75, p<0.01). Based on this finding, a worse follicular pool in the pooling ET arm compared with the fresh ET arm may have caused a worse oocyte quality. In our study, the positive pregnancy rates were 32% (35/110) in the pooling ET arm versus 37% (53/145) in the fresh ET arm (p=0.43). The abortion rates were 52% (16/31) and 27% (13/49), respectively (p=0.04). Fewer oocytes were collected per one cycle in the pooling ET arm compared with the fresh ET arm. This may be the reason for the clinically poor pregnancy outcomes in poor responders, and increased rates may be related to the embryo accumulation method.

Study Limitations

Our study has some limitations. This was a retrospective study, and the groups were not randomised. There may have been bias in the patient selection. Women who were expected to exhibit lower IVF success after the clinical evaluation may have been moved to the pooling ET arm. This may have caused higher abortion and lower live birth rates in the pooling ET arm. The length of infertility did not exclude confounding factors causing infertility, such as additional disorders, because this was a retrospective study. Women were not randomised and offered alternative ET methods, such as pooling and fresh ET.

Conclusion

Although the pooling ET method may have a mild positive effect on clinical pregnancy rates, no additional effect was determined for live birth rates. In addition, the abortion rate increased in accordance with the clinical pregnancy rate; abortion may induce anxiety and depression in patients[35]. Further prospective, randomised, controlled studies are needed to investigate the effects of pooling ET on live births.

Ethics

Ethics Committee Approval: Ethics committee approval was not required for this study because it involved retrospective data analysis.

Informed Consent: Consent form was signed by all participants.
Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: E.Y., M.B., S.Ç., D.C.Ç., K.B., Concept: S.Ç., Design: S.Ç., M.B., Data Collection or Processing: D.C.Ç., Analysis or Interpretation: K.B., R.A., S.P., S.Ç., Literature Search: S.Ç., Writing: S.Ç., N.E.T.

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Social stigma and familial attitudes related to infertility

İnfertilite nedenli sosyal dışlanma ve aile tutumu

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Abstract

Objective: To determine the perceived social stigma and familial attitides and perception of sexuality in infertile couples attending infertility clinics.
Materials and Methods: Infertile couples attending infertility clinics between the years of 2014 and 2015 were requested to complete detailed evaluation forms including questions related to the social stigma on their infertility, their familial attitudes, and perception of sexuality. Any partner of the infertile couple accepting to enroll in the study was given the evaluation forms. Their scores related to answers and demographics, and parameters related to infertility were analyzed.
Results: A total 598 partners of infertile couples enrolled in the study, 58% represented 177 couples. Their infertility was primary in 98.3% and the duration of marriage and infertility was 9.81±5.58 and 9.76±5.53 years, respectively. The perception of social exclusion was present in 38% (p<0.001) of infertile couple, which was more significantly pronounced in female partners (p=0.013). Fifteen percent of the infertile couples thought themselves as isolated in public and losing value in public (p<0.001). However, sixty percent of infertile couples thought that they would achieve a notable place in community after having a baby (p<0.001). Infertility was accepted as a reason of divorce in only 13% of infertile couples (p<0.001). The majority of perticipnats, irrespective of sex, rejected that infertile women or men lost sexual appeal (86%; p<0.001).
Conclusions: There is significant effect of infertility on familial attitudes and perception of sexuality of infertile couples. Unfortunately, there is significant negative social stigma on infertile couples.
Keywords: Infertility, intra-cytoplasmic sperm injection, familial attitudes, social stigma

Öz

Amaç: İnfertilite kliniğine başvuran çiftlerde algılanan sosyal dışlanma, aile tutum ve cinsellik algısının saptanması amaçlandı.
Gereç ve Yöntemler: 2014 ve 2015 yılları arasında infertilite kliniklerine başvuran infertil çiftlerden, infertilite ile ilişkili sosyal dışlanma, ailelerinin tutumu ve cinsellik algıları ile ilgili sorular içeren değerlendirme formalarını doldurmaları istendi. İnfertil çiftlerden çalışmaya katılmayı kabul eden her bireye değerlendirme formu verildi. İnfertilite ile ilişkili parametreler, demografi bilgileri ve soru formlarından elde edilen skorları değerlendirildi.
Bulgular: Toplamda 598 infertil kişi çalışma içerisinde yer aldı, katılan bu kişilerin %58'i 177 çifti temsil etmekte idi. Primer infertilite %98,3 olup evlilik ve infertilite süreleri sırası ile 9,81±5,58 ve 9,76±5,53 yıl idi. Sosyal dışlanma algısı %38'inde (p<0,001) mevcut olup bu algı kadın partnerlerde daha belirgin idi (p=0,013). İnfertil kişilerin %15'i kendisinin toplumda izole edilmiş ve toplum içinde değer kaybettiğini ifade etmekte idi (p<0,001). Ancak, infertil kişilerin %60'ı bebekten sonra toplumda saygın bir yer edineceğini düşünmekte idi (p<0,001). İnfertil kişilerin sadece %13'ü infertiliteyi boşanma nedeni olarak kabul etmekte idi (p<0,001). Büyük çoğunluğu infertil kadın veya erkeğin cinsel cazibesini kaybetmeyeceği görüşündeydi (%86; p<0,001).
Sonuç: İnfertilitenin sosyal dışlanma, aile tutumu ve cinsellik algısı açısından belirgin olumsuz etkileri olabilmektedir.
Anahtar Kelimeler: İnfertilite, intrasitoplazmik sperm enjeksiyonu, aile tutumu, sosyal dışlanma

PRECIS: Infertility itself has negative effect on couples in terms of social stigma and familial attitudes, as well as sexuality.

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Introduction

Infertility is a worldwide health issue with approximately 49 million couples affected in 2010[1]. Within the last two decades, there has not been a significant change in the rates of infertility worldwide, regardless of populational growth, despite the development of technical and medical improvements in diagnostic and surgical techniques as well as summated experience on this subject[1]. In all communities worldwide, infertility is an important issue for both sexes because it is an instinctive biologic behavior to have an offspring, but also an important issue to be a family as a part of a community. However, infertility has recently gained attention to its psychological aspects such as depression and sexual dysfunction, and the negative effects on partner relationship with varying severities and rates[2-8]. Therefore, it is also an important issue for medical caregivers working in infertility, to deal with and determine the psychological aspects of infertility. In this prospective questionnaire and interview-based clinical study, we aimed to determine the effect of infertility on familial attitudes, the perception of sexuality of infertile couples, as well as the effect of the infertility-driven social stigma on infertile couples attending infertility clinics. In addition, we aimed to underline the necessity for psychological support for the psychological aspects of infertility beyond its medical management.

Materials and Methods

A prospective study using questionnaire forms conducted in a referral centers for assisted reproduction was conducted after approval of the local ethics committee. After obtaining written informed consent, infertile couples attending infertility clinics between the years of 2014 and 2015 were requested to complete detailed evaluation forms including questions related to the social stigma on their infertility, their familial attitudes, and perception of sexuality together with the Golombok-Rust Inventory of Sexual Satisfaction, Rosenberg's Self-Esteem Scale, and Beck's Depression Inventory[9-11]. Any partner of the infertile couple accepting to enroll in the study was given the questionnaire forms. Their scores related to answers and demographics, and parameters related to infertility were analyzed.

Statistical Analysis

The related statistical comparisons of groups were performed with the ANOVA test and chi-square test where appropriate. Correlation analyses were performed using Pearson's correlation. Statistical analyses were performed using SPSS statistics software (SPSS Statistics for Windows, Version 17.0; SPSS Inc., Chicago, U.S.A). p was set as <0.05 for significance.

Results

A total 598 partners (380 females and 218 male partners) of infertile couples enrolled in the study, 344 (58%) of which represented 177 couples. Their infertility was primary in 98.3% and the duration of marriage and infertility was 9.81±5.58 and 9.76±5.53 years, respectively. In 52%, ≥1 sessions of antiretroviral therapy were applied and pregnancy was achieved in 6% of them previously. Educational status was high school level or higher in 25%. The mean age of the male partners was 35.46±6.26 years and the mean age of the female partners was 32.07±5.44 years. The mean body mass indexes were 26.25±3.23 kg/m^2 and 25.42±3.35 kg/m^2 for the male and female partners, respectively. The patients mostly (94%) attended the infertility clinics with their spouses. When informed about the presence of infertility, 46% tended to inform their spouses initially. When compared for sex, male participants tended to inform at first their spouses significantly more compared with the female participants (53% vs 43%, p=0.007) and the ratio of female participants who at first informed their mothers was higher compared with male participants (34% vs 27%, p=0.007). Almost half of the infertile participants (44%) tended to hide this from the community in which they lived. Non-medical applications were applied in 86%, most of which were referral to a herbalist for officinal plants (30%) or so-called "healing water" (12%).

The perception of social exclusion was present in 38% (p<0.001) of infertile participants, more significantly in female partners (43% in females; 29% in males) (p=0.013). Only fifteen percent of the infertile participants, irrespective of sex, thought that the infertile woman was a second-class person (p<0.001), which was more significantly pronounced among females (19% in females; 10% in males) (p=0.003). On the other hand, in cases of male infertility, this rate dropped to 10% without any significant sex difference. Fifteen percent of the infertile participants thought themselves as isolated in public and losing value (p<0.001). However, 60% of the infertile participants though that they would achieve a notable place in community after having a baby (p<0.001).

The community was informed about someone's infertility by infertile individuals or their spouses in 58%, and members of the community tended to spread this information as frequently as 42% (p>0.05), reflecting the importance that the community gives to the issue of infertility. The community members' attitudes were mostly negative to infertile females compared to males (57% vs 37%; p=0.001). Men tended to give higher scores for their wives' wish for children than for theirs' compared with vice versa (p=0.025) (Table 1). Correlations of parameters related to social stigma showed some significant correlations among each other (Table 2).

Most of the infertile participants (72%) mentioned that they would feel similar if their spouses rather than themselves were diagnosed as infertile (p<0.001). Most of them neither supported nor disagreed with adopting a child. Approximately one third (35%) thought that having a child was a must for being a family (p<0.001). Almost half of the infertile particioants did not think that reproduction was the most important mission and more than two-thirds (68%) disagreed that the most important duty of a woman was to give birth (p<0.001).

Infertility was accepted as a reason of divorce in only 13% of infertile participants (p<0.001), which was more significantly pronounced in female partners (17% in females; 7% in males) (p=0.002). The infertile participants mostly did not wish to have not to been married (89%: p<0.001). However, this rate decreased, albeit still high, to 74% (p<0.001) concerning their thoughts about their spouses' choice, which was statistically more evident in females (67%) compared with male ones (78%) (p=0.014). The majority of the infertile participants, either men or women, rejected the perception of infertility as being a sign of male impotence (86%; p<0.001). Likewise, the majority, irrespective of sex, rejected that infertile women or men lost sexual appeal (86%; p<0.001).

Discussion

Infertility is an important worldwide health issue with no significant change in its prevalence within the last two decades[1]. Childbearing is an important issue on a personal basis and for the community, which in turn causes unfavorable psychological aspects such as depression and sexual dysfunction, and negative effects on partner relationships with varying severities and rates[2-8]. Surprisingly, depression itself is also a predictor of treatment outcome in artificial reproduction techniques[12]. Likewise, stress-related alterations in the hypothalamic-pituitary-gonadal axis has been suggested to result in changes in sexual behavior and in changes in gonadotrophins levels, which in turn may explain the inter-relationship of sexuality, infertility, and stress[13]. Therefore, it is important deal with psychological aspects of infertility to break this possible vicious cycle in infertility treatment. To be success in this aim, the first step is to outline all aspects of personal psychology and partner relationships as well as the social stigma. A recent systematic review suggested incongruent results due to different objectives and methodologies, the lack of specific questionnaires to assess sexual function, and uncontrolled social and relationship variables interfering sexual functions, which made it difficult to establish the impact of infertility on the sexual function of infertile couples[14]. Another systematic review also suggested that infertility had a negative effect on the psychological well-being and sexual relationships of couples, but the evidence was inconclusive for the effect on familial attitudes and quality of life[15]. Therefore, further multicentered studies are warranted to clarify these points. In our study, the behaviours of couples tended to differ even starting from the diagnosis of infertility. That is, infertile men tended to share this initially with their spouses more frequently compared with infertile women. That seems to be related to the higher rates of fear for social exclusion in infertile women and decreased self-esteem resulting from infertility. Regardless of sex, almost half of the infertile couples tended to hide their status of infertility from the commity with the fear of social stigma. In addition, it is confirmed that infertility is an important issue in the community because the community

Table 1. Attitudes of infertile couples and their parents regarding having a baby (scores 0 to 10; 10 indicating the highest rank)

	Importance of having a baby	His/her wish for a baby	His/her spouse's wish for a baby*	His/her parents' wish for a baby	His/her spouse's parents' wish for a baby
Male	9.33±1.92	9.25±2.08	9.58±1.74*	9.24±2.13	9.25±2.20
Female	9.34±1.67	9.15±2.46	9.14±2.35*	8.88±2.67	8.81±2.76
Total	9.34±4.98	9.19±2.33	9.30±2.16	9.01±2.49	8.97±2.58

*: p=0.025

Table 2. Correlations of age, period of infertility, and attitudes of infertile couples and their parents regarding having a baby

	Age	Period of Infertility	His/her wish for a baby	Importance of having a baby	His/her parents' wish for a baby	His/her spouse's parents' wish for a baby	His/her spouse's wish for a baby
Age	1	0.618**	-0.071	-0.043	-0.046	0.014	0.031
Period of Infertility	0.618**	1	-0.007	-0.032	0.075	0.093	0.114*
His/her wish for a baby	-0.071	-0.007	1	0.285**	0.633**	0.621**	0.624**
Importance of having a baby	-0.043	-0.032	0.285**	1	0.336**	0.351**	0.316**
His/her parents' wish for a baby	-0.046	0.075	0.633**	0.336**	1	0.833**	0.708**
His/her spouse's parents' wish for a baby	0.014	0.093	0.621**	0.351**	0.833**	1	0.724**
His/her spouse's wish for a baby	0.031	0.114*	0.624**	0.316**	0.708**	0.724**	1

*: Correlation is significant at the 0.05 level (2-tailed)
**: Correlation is significant at the 0.01 level (2-tailed)

itself spread and evaluated the knowledge of an individual's infertility as a negative property of that person or couple. Ironically, social support has been suggested to be important for self-esteem and quality of life in infertile couples[16]. In this study, it is seen that scores reported for wishing to have a baby were very high for partners of infertile couples and their parents. In the correlation analyses, we found that the desire to have a child showed a weak but positive correlation with advancing infertility period. Correlations of scores reported for wishing to have a baby of infertile couples with those of their parents showed a very strong positive correlation, further indicating the importance of a baby and the possible negative pressure on infertile couples. Interestingly, scores of perceived partners' wishes of having a baby were reported as low by female partners compared with male partners, which further indicates the presence of more social pressure on infertile female partners. In a similar manner, infertile women tended to accept infertility as a reason for getting divorced more compared with infertile men (17% vs 7%), and almost one-third of infertile women wished not to have been married due to low self-esteem or blame related to infertility, whereas this rate was significantly lower in men (22%). In the present study, we found a negative perception of sexual dysfunction or negative effects on perception of sexual appeal irrespective of sex in infertile couples, though at a lower rate. Nevertheless, these results further confirm that psychological counseling is a need, which can provide valuable assistance in accordance with infertility managements of infertile couples[17].

Study Limitations

This study needs to be confirmed by further studies with larger numbers of patients.

Conclusion

There is a significant effect of infertility on familial attitudes and perception of sexuality of infertile couples.

Ethics

Ethics Committee Approval: A prospective study using questionnaire forms conducted in a referral centers for assisted reproduction was conducted after approval of the local ethics committee.

Informed Consent: Written informed consent.
Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: R.N.E., A.P., B.K., D.Ö., K.S., E.Ç., Concept: E.Ç., Design: E.Ç., Data Collection or Processing: R.N.E., A.P., B.K., D.Ö., K.S., E.Ç., Analysis or Interpretation: R.N.E., A.P., B.K., D.Ö., K.S., E.Ç., Literature Search: R.N.E., E.Ç., Writing: R.N.E., E.Ç.,
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Tumor necrosis factor alfa and interleukin 1 alfa induced phosphorylation and degradation of inhibitory kappa B alpha are regulated by estradiol in endometrial cells

Endometriyal hücrelerdeki inhibitör kappa B alfa'nın tümör nekroz faktörü ve interlökin 1 indüklenmiş fosforilasyonunu estradiol ile ayarlanır

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Abstract

Objective: When bound to the inhibitory kappa B (IκB) protein, the transcription factor nuclear factor kappa B (NF-κB) remains inactively in the cytoplasm. Activated NF-κB upregulates the gene expression of many chemokines including monocyte chemoattractant protein-1 and interleukin (IL)-8. We hypothesized that estrogen may regulate IκB phosphorylation and degradation thus influencing NF-κB-dependent gene expression. Regulation of chemokines by estrogen is different in uterine endometrial cells when compared to ectopic endometrial cells of endometriosis.

Materials and Methods: We investigated the *in vivo* expression of IκB in normal endometrium and in eutopic and ectopic endometrium of women with endometriosis. We then studied in cultured endometrial cells to assess the effects of estradiol on IκB and NF-κB function.

Results: Normal endometrium from mid-late proliferative phase revealed the strongest IκB immunoreactivity throughout the cycle ($p<0.05$). When compared to paired homologous eutopic endometrium, ectopic endometrium revealed significantly less immunoreactivity for IκB ($p<0.05$). Moreover, estradiol induced a decrease in tumor necrosis factor-and IL-1-induced IκB phosphorylation, and also decreased the levels of active-NF-κB ($p<0.05$).

Conclusion: Our results support the conclusion that one pathway for estradiol-mediated NF-κB inhibition occurs through the down-regulation of IκB phosphorylation. We propose that the estradiol-induced regulation of IκB and consequent reduction in active-NF-κB may affect inflammatory responses in human endometrial cells.

Keywords: Inhibitory kappa Bα, nuclear factor kappa B, estradiol, endometrium, tumor necrosis factor-α

Öz

Amaç: İnhibitör kappa B (IκB) protein bağlandığı zaman, transkripsiyon faktörü nükleer faktör kappa B (NF-κB) sitoplazmada aktive olamaz. Aktif NF-κB, monosit kemoatraktan proteini-1 ve interlökin (IL)-8 dahil olmak üzere pek çok kemokinin gen salınımını artırır. Biz östrojenin IκB-α fosforilasyonunu ve yıkımını düzenleyerek NF-κB'ye bağımlı genlerin salgılanmasını etkileyebileceğini varsaydık. Östrojen tarafından kemokinlerin düzenlenmesi uterus içindeki endometriyal hücrelerde, endometrioziste görülen uterus dışındaki endometriyal hücreler karşılaştırıldığında farklıdır.

Gereç ve Yöntemler: Normal endometriyum, endometrioziz de uterus içindeki ve dışındaki endometriyal hücrelerden *in vivo* IκB salgılanmasını araştırdık. Ayrıca estradiolün IκB ve NF-κB fonksiyonu üzerindeki etkisini değerlendirmek için endometriyal hücre kültüründe çalıştık.

Bulgular: Normal endometriyum bütün menstrual siklus boyunca orta-geç proliferatif fazda güçlü IκB immünreaktivite gözlemlendi ($p<0,05$). Eşleştirilmiş homolog uterus içi ve dışındaki endometriyum karşılaştırıldığında, uterus dışındaki endometriyumda anlamlı olarak daha düşük bir immünoreaktivite tespit edildi ($p<0,05$). Dahası, estradiol, tümör nekroz faktörü-α ve IL-1 ile indüklenen IκB fosforilasyonunda bir düşüşe neden olmuştur ve ayrıca aktif-NF-κB düzeylerini de azaltmıştır ($p<0,05$). Bulgularımız estradiol aracılı NF-κB inhibisyonu için bir yolun IκB fosforilasyonun aşağı regülasyonu yoluyla ortaya çıktığı sonucunu desteklemektedir.

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Sonuç: Estradiol ile indüklenen IκB regülasyonunun ve dolayısıyla aktif NF-κB'deki azalmanın insan endometriyal hücrelerindeki enflamatuvar yanıtları etkileyebileceğini varsaymaktayız.
Anahtar Kelimeler: İnhibitör kappa Bα, nükleer faktör kappa B, östrojen, endometriyum, tümör nekroz faktörü-α

PRECIS: We have assessed that the estradiol-stimulated regulation of inhibitory kappa Bα and subsequent decrease in nucleor factor faktör kappa B affects inflammatory reactions in human endometrial cells.

Introduction

Immunologic-endocrine interactions mediate and participate in complex physiologic processes that occur within the uterus throughout the menstrual cycle and pregnancy, and are also important to the pathophysiology of endometriosis[1-3]. One of the molecular signaling pathways that may be regulated by the endocrine system, which also participates in the regulation of inflammation, is the nuclear factor kappa B (NF-κB) signaling cascade[4-6]. NF-κB is a transcription factor that is kept in an inactive state in the cytosol while bound to the inhibitory kappa B (IκB) protein[7,8]. First described in B cells, NF-κB was subsequently recognized as a nuclear and cytoplasmic protein that is found in multiple cell types[9]. In many cells, NF-κB positively regulates the expression of a number of genes including those of cytokines, cell adhesion molecules, complement factors, anti-apoptotic factors, and immunoreactions[10-12]. The IκB protein family is composed of 35-70 kDa proteins that are localized in the cytoplasm and inhibit the activation of NF-κB. This protein family includes IκBα, IκBβ, IκBγ, IκB-R, B-cell leukemia-3, p105/p50, p100/52 and the Drosophila melanogaster proteins Cactus and Relish. IκBα and IκBβ preferentially interact with NF-κB dimers composed of proteins p65 and p50, and regulate NF-κB function by converting the heterodimer structure to a trimer that is incapable of binding DNA[13-15]. Tumor necrosis factor-alpha (TNF-α) and interleukin (IL)-1 induce the phosphorylation and subsequent degradation of IκBα. This, in turn, results in the activation and relocation of NF-κB to the nucleus, leading to NF-κB-mediated transcription of responsive genes[16,17]. Ligand binding to most, if not all, of the inflammatory cytokine receptors activates intracellular signaling molecules that engender the activation of NF-κB. Activation of such signaling molecules results in a transient activation of IκB kinase (IKK) and a transient phosphorylation of IκBα (phospho-IκBα). Often, phospho-IκBα peaks 2-15 min after stimulation with the cytokine, and is followed by a rapid acceleration of IκBα degradation. Often, IκBα levels may subsequently increase in the cytosol over the following 2-6 h, in response to NF-κB-mediated upregulation of the IκB promotor[11,13,18]. Several proteins and molecules that activate NF-κB signaling have been described. IL-1 and TNF-α are two principal cytokines that promote IκBα degradation and NF-κB activation. Although these cytokines bind to specific receptors to activate different intracellular second messengers, downstream signals merge with the activation of the same target, namely IKK[19-21]. Estrogen influences the growth, differentiation, and function of many target cells by genomic and non-genomic pathways. Although the genomic effects of estrogen are mediated via estrogen receptors (ERs) and occur over a period of hours or days, the non-genomic effects occur within minutes[22-24]. Previous studies have shown that estrogen down-regulates the expression of many cytokines such as IL-1, TNF-α, IL-6 and regulated-upon activation, normal T-cell-expressed and secreted (RANTES), which are regulated by NF-κB in various cell types[25-27]. Previously, we have shown that estrogen inhibits monocyte chemotactic protein-1 expression in human endometrial stromal cells (ESCs)[28]. Moreover, in response to estrogen, chemokine-mediated regulation of endometrial cells obtained from women with endometriosis is distinct from that observed in normal endometrial cells[29-31]. An estrogen-dependent disease, endometriosis develops outside of the uterus and is characterized by a proinflammatory peritoneal environment[32,33]. Thus, there may be differential regulation of NFκB signaling by estrogen and by cytokines such as TNF-α and IL-l in endometriotic cells as compared with normal endometrial cells. In endometriotic cells, there appears to be synergy between the effects of E_2 and IL-1/TNF-α, whereas these molecules appear to function antagonistically in normal endometrial cells. We hypothesized that estrogen might regulate IκBα phosphorylation and degradation *in vivo* and *in vitro* in normal endometrium and in eutopic and ectopic endometrium of women with endometriosis, thus influencing NFκB-dependent gene expression. First, we investigated the *in vivo* expression of IκBα in normal endometrium and in eutopic and ectopic endometrium of women with endometriosis. We then investigated the modulation of IκBα by E_2 in TNF-α– and IL-1α-treated endometrial stromal and glandular cells, *in vitro*, using Western blot analysis and immunocytochemistry.

Materials and Methods

Tissue collection

Endometrial tissues were obtained from human uteri after hysterectomy conducted for benign diseases excluding endometrial disease, and from endometrial biopsies. Approval for this study was granted by the Human Investigation Committee of Yale University (HIC#22334) and written informed consent was obtained from each patient prior to surgery. The mean age of the patients was 36 years (range, 30-45 years).

For immunohistochemistry, normal cyclic endometrium (n=12) of women without endometriosis, and eutopic and ectopic endometrium pairs of women with endometriosis (n=6) were

collected, and paraffin blocks were routinely prepared and cut at 5-7 mm. For the endometrial cells used in culture, the diagnoses of the patients were leiomyomata uteri or voluntary sterilization by tubal ligation (n=5). The day of the menstrual cycle was established from the patient's menstrual history and was verified through histologic examination of the endometrium. The tissues were placed in Hank's balanced salt solution and transported to the laboratory for separation and culture of endometrial stromal and glandular cells. Each experimental setup was repeated on at least three occasions using cells obtained from different patients.

Isolation and culture of human endometrial stromal and glandular cells

Endometrial tissues were separated and conserved in a monolayer culture, as described previously[34]. The isolated endometrial cells were separated by filtration through a wire sieve (73 μm diameter pore, Sigma). The endometrial glands (largely undispersed) were retained by the sieve, whereas the dispersed stromal cells passed through the sieve into the filtrate. The stromal cells were plated in plastic flasks (75 cm^2, Falcon, Franklin Lakes, NJ), maintained at 37 °C in a humidified atmosphere (5% CO_2 in air), and allowed to replicate to confluence. Thereafter, the stromal cells were passed by standard methods of trypsinization, plated in culture dishes (100 mm diameter), and allowed to replicate to confluence. ESCs after the first passage were characterized as described previously[34] and were found to contain 0-7% epithelial cells, no detectable endothelial cells, and 0.2% macrophages. Experiments were commenced 1-3 days after the cells reached confluence. The confluent cells were treated with serum-free, phenol red-free media for 24 h before treatment with test agents. Stromal cells reached confluence in 7-10 days.

Experiments with glandular cells were performed using a well-differentiated endometrial adenocarcinoma cell line (Ishikawa cell) provided to us by Dr. R. Hochberg (Department of Obstetrics and Gynecology, Yale University, New Haven, CT) from a frozen stock. Thawed cells were maintained in T75 flasks (BD Biosciences, Franklin Lakes, NJ) until passage. The cells were treated with serum-free phenol red-free media for 24 h before treatment with test agents. Cells were treated with E_2 (Sigma) for 3-90 min and immunocytochemistry and Western blot analysis were performed as described.

Immunohistochemistry and immunocytochemistry

Endometrial tissue sections from normal, eutopic, and ectopic endometrium were deparaffinized and washed with phosphate buffered saline (PBS). Thereafter, sections were twice microwaved in citric acid buffer (0.1 M, pH: 6) and thoroughly rinsed in PBS. The same steps used for immunocytochemistry (described below) were followed. ESCs were grown to pre-confluence on four-chamber slides. Following treatment, the chamber slides were fixed in 4% paraformaldehyde for 20 min. After several washes with distilled water and then with PBS (pH 7.4) (three times 10 min each), endogenous peroxidase activity was quenched by 3% H_2O_2 (0.6 mL H_2O_2 and 5.4 mL methanol) for 10 min and the slides were then rinsed in PBS-tween. Slides were then incubated with rabbit anti-IκBα polyclonal antibody (Cell signaling Technology, Beverly, MA) for 60 min at room temperature. In negative control slides, normal rabbit immunoglobulin G (IgG) was used as a control instead of primary antibody. After several rinses in PBS, goat biotinylated anti-rabbit IgG (Vector Laboratories, Burlingame, CA) was applied for 30 min. After several rinses with PBS, the slides were incubated with streptavidin-peroxidase complex for 30 min (Vector Laboratories). The slides were then rinsed several times in PBS and incubated with 3-amino-9-ethyl-carbazole (Vector Laboratories) for 10 min. The slides were lightly counterstained with hematoxylin prior to permanent mounting. Immunocytochemical staining intensity was ranked between 0 (absent) to 3 (most intense). For each slide, an HSCORE value was derived by summing the percentages of cell staining at each intensity multiplied by the weighted intensity of the staining [HSCORE=Σ P_i (i+1), where i is the intensity scores and P_i is the corresponding percentage of the cells]. In each slide, five randomly selected areas were assessed microscopically using 50× magnification. Two investigators who were blinded to the treatments analyzed each slide for intensity. The averages for the scores of both investigators are presented.

IkBa and phospho-IkBa Western blot analysis

Total protein from endometrial cells was extracted in a lysis buffer composed of 50 mM hydroxyethyl piperazineethanesulfonic, pH: 7.4; 150 mM NaCl; 10% glycerol, 1% Triton X-100, 1.5 mM $MgCl_2$-$6H_2O$; 1 mM EGTA; 100 mM NaF, 10 mM sodium pyrophosphate and protease inhibitors, 1 mM Na_3VO_4, 10 μg/mL leupeptin, 10 μg/mL aprotinin; and 4 mM phenylmethylsulfonyl fluoride. The protein concentration was determined by a detergent-compatible protein assay (Bio-Rad Laboratories, Hercules, CA). Protein lysates (20 μg) were loaded and separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis with 10% Tris-Hydrogen chloride Ready Gels (Bio-Rad Laboratories) and electroblotted onto nitrocellulose membrane (Bio-Rad Laboratories). Equal loading of proteins in each lane was confirmed by staining the membrane with Ponceau 2S (Sigma). The membrane was incubated with 5% nonfat dry milk in tris-buffered saline-tween (TBS-T) buffer (0.05% tween-20 in PBS, pH 7.4) for 1 h to reduce nonspecific binding of antibody. The membrane was probed with rabbit anti-IκBα and rabbit anti-phospho-IκBα (Ser32) antibodies (Cell Signaling Technology) overnight to quantitate total and phospho-IκBα forms. After washing with TBS-T, blots were incubated for 1 h with peroxidase labeled anti-rabbit IgG (Vector Laboratories) diluted at 1:10000. Membranes were washed with TBS-T and the immunoblots were developed using chemiluminescent kit following the manufacturer's instructions. (NEN Life Science, Boston, MA). The signal was normalized by dividing the arbitrary densitometry units for phospho-IκBα to the

amount of total IκBα for each band. The signals were quantified by using a laser densitometer (Molecular Dynamics, Sunnyvale, CA) to analyze the autoradiographic bands.

Preparation of nuclear extracts and the active-NF-κB assay

To quantify the amount of active NF-κB, which binds to NFkB response element sites on gene promotors, an enzyme-linked immunosorbent (ELISA) plate covered with NF-κB binding consensus sequence oligonucleotide (5'-GGGACTTTCC-3') was used in combination with nuclear extracts from our cultured cells. Two different primary antibodies against NF-κB each recognize either an epitope on p65 or on p50 that is accessible only after dissociation of IκB from NF-κB, indicating the activation of cytoplasmic NF-κB. An horseradish peroxidase-conjugated secondary antibody provides a colorimetric readout that is quantitated using spectrophotometry (450 nm). As a positive control for activated NF-κB, nuclear extracts from HeLA cells were used. To monitor the specificity of the assay, both wild type and mutated consensus oligonucleotides were employed in each reaction. Nuclear extracts from endometrial cells grown to confluence in 60 mm plates were obtained using a nuclear extraction kit (Active Motif, Carlsbad, CA). Briefly, cells were washed with ice-cold PBS and protease/phosphatase inhibitors, removed from the dish by scraping with a cell lifter and transferred to pre-chilled tubes. Cell suspensions were centrifuged at 4 °C for 5 min at 500 rpm. Pellets were resuspended in hypotonic buffer and incubated for 15 min on ice, detergent was added, and the cells were centrifuged at 4 °C for 30 seconds at 14.000 ×g. The pellet was resuspended in a lysis buffer and incubated for 30 min on ice on a rocking platform. The suspension was centrifuged at 4 °C for 10 min at 14.000 ×g and the supernatant (nuclear fraction) was aliquoted and frozen at -80 °C. Nuclear fractions were quantitated using a Coomassie protein assay (Pierce; Rockford, IL) as per the manufacturer's protocol. Four micrograms of nuclear extract sample were loaded into each well and assayed according to the manufacturer's directions (Active Motif) using a microplate reader. Quantification of the NF-κB p50 subunit was expressed as mean absorbance (λ) per sample.

Statistical Analysis

IκBα immunocytochemistry scores and Western blot results were normally distributed as assessed using the Kolmogorov-Smirnov test. Analysis of variance (ANOVA) and post hoc Tukey test for pair-wise comparisons were used in statistical analysis. $p<0.05$ was considered to be significant. Statistical calculations were performed using Sigma stat for Windows, version 2.0 (Jandel Scientific Corporation, San Rafael, CA).

Results

Expression of IκBα in normal endometrium, and in eutopic and ectopic endometrium from women with endometriosis

Eutopic endometrial stromal and glandular cells from women without endometriosis express immunoreactive IκBα (Figure 1). The antibody used for immunohistochemistry recognizes both phosphorylated and unphosphorylated forms of IκBα. In normal endometrium, glandular cells reveal stronger immunoreactivity for IκBα compared with stromal cells throughout the menstrual cycle. Stronger immunoreactivity was detected in samples of mid-late proliferative endometrium compared with late secretory and early proliferative phase samples ($p<0.05$) (Figure 1, Table 1). When proliferative phase and secretory phase immunoreactivity for IκB were compared, the proliferative phase showed a trend for stronger immunoreactivity although this difference did not reach statistical significance. Eutopic and ectopic endometrium from women with endometriosis also revealed immunoreactivity for IκBα. When the eutopic endometrium from women with endometriosis was compared with the endometrium of women without endometriosis, no significant difference was observed in staining intensity, although eutopic endometrial cells of women with endometriosis showed a trend towards decreased immunoreactivity for IκBα ($p=0.1$) (Figure 1, Table 2). On the other hand, when compared with eutopic endometrium, homologous ectopic endometrium revealed significantly less immunoreactivity for IκBα ($p<0.05$) (Figure 1, Table 2).

Estradiol-regulated expression of IκB in endometrial cells as assessed using immunocytochemistry

ESCs grown on four-chamber slides were placed in serum-free, phenol red-free media for 24 h, and were then treated for 15 min with fresh serum-free, phenol red-free media as control, with TNF-α (2 ng/mL), or estradiol (10^{-8} M) combined with TNF-α (2 ng/mL) for 15 min. Slides were stained with rabbit anti-IκBα antibody. Cells treated with TNF-α alone showed a very weak immunoreactivity for IκBα when compared with the control (Figure 2a, b). On the other hand, cells treated with TNF-α combined with E_2 displayed a stronger IκBα immunoreactivity than those treated with TNF-α alone ($p<0.05$) (Figure 2b, c). We also compared cells maintained for 24 h in serum-free phenol red-free media for 24 h, with or without E_2 (10^{-8} M), followed by TNF-α (2 ng/mL) treatment for an additional 15 min. TNF-α–stimulated IκBα immunoreactivity was stronger in cells pre-treated with E_2 compared with those pre-treated with serum-free media alone ($p<0.05$) (Figure 2d-f).

Regulation of IκBα expression and phosphorylation in endometrial cells as assessed using Western blot analysis

We sought to understand whether the increased IκBα immunoreactivity observed in cells treated with both TNF-α and E_2 was associated with a phosphorylation and subsequent degradation of IκBα. After 24 h of incubation with serum-free, phenol red-free media, ESCs were treated with media alone (control), E_2 10^{-8} M alone, TNF-α 2 ng/mL alone, or with E_2 10^{-8} M combined with TNF-α 2 ng/mL for 3, 6, 12, 30, and 60 min. Total protein was extracted and levels of total IκBα and phospho-IκBα were measured using Western blot analysis. Control and E_2-treated cells showed similar levels of IκBα

throughout the treatment period. On the other hand, treatment with TNF-α resulted in a time-dependent decrease in IκBα levels compared with the control. Moreover, this treatment caused a time-dependent increase in phospho-IκBα levels with a peak between 6 and 12 min of treatment. Meanwhile,

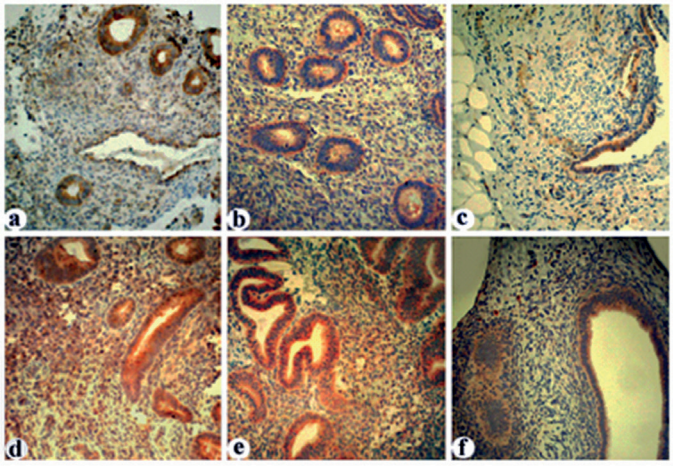

Figure 1. Inhibitory kappa B (IκBα) immunoreactivity in human normal (a, d), eutopic (b, e) and ectopic (c, f) endometrial tissues. IκBα immunoreactivity in proliferative (a, c) and secretory phase (d, f) tissue samples are seen. Stronger immunoreactivity in endometrial glands and stromal cells in normal endometrium are observed when compared with ectopic endometrial and stromal cells. (a-f x40)

Table 1. Inhibitory kappa B immunoreactivity in various cell types of human endometrium throughout the cycle. Early proliferative (n=2), late proliferative (n=4), early secretory (n=4) and late secretory (n=2)

	Early proliferative	Late proliferative	Early secretory	Late secretory
Glandular cells	+	++/+++	++	+/++
Stromal cells	-/+	++	+/++	+
Endothelial cells	+	++	+/++	+/++

Table 2. Inhibitory kappa B immunoreactivity in various cell types of normal, eutopic and ectopic endometrium. Menstrual cycle matched normal endometrium (n=6), eutopic and ectopic pairs of endometriotic endometrium samples (n=6)

	Normal endometrium	Eutopic endometrium	Ectopic endometrium
Glandular cells	++/+++	+/++/+++	+
Stromal cells	+/++	-/+/++	-/+
Endothelial cells	++	+/++	-/+

E_2 combined with TNF-α treatment showed markedly higher levels of IκBα when compared with TNF-α alone (Figure 3). When groups were compared in terms of phospho-IκBα levels, control and E_2-treated cells revealed the lowest levels of phospho-IκBα throughout the treatment periods. However, in cells treated with TNF-α, co-treatment with E_2 induced higher IκBα levels and lower phospho-IκBα levels during the first 12 minutes of treatments ($p<0.05$) (Figure 3). Following 60 min of treatment, IκBα levels were still higher in cells co-treated with E_2 compared with cells treated with TNF-α alone (Figure 4a). Interestingly, in glandular cells, longer treatment with E_2 with TNF-α (90 min) resulted in a significantly higher level of IκBα compared with other treatments, including the control

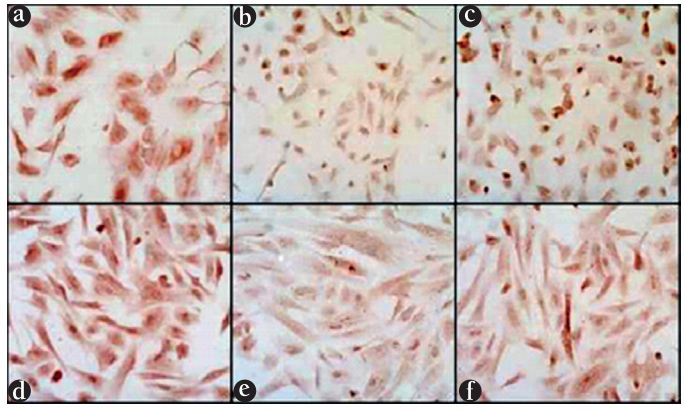

Figure 2. Inhibitory kappa B (IκBα) immunoreactivity in endometrial stromal cells treated with estradiol and tumor necrosis factor-alpha (TNF-α.) Endometrial stromal cells were treated for 12 min with vehicle (control) (a), TNF-α (2 ng/mL) (b), or estradiol (10^{-8} M) combined with TNF-α (c), and were immunostained for IκBα. Cells treated with estradiol combined with TNF-α showed stronger immunoreactivity for IκBα than cells treated with TNF-α alone. Endometrial stromal cells were pretreated with vehicle (control) (d, e) or estradiol (f) for 24 h prior to stimulation with TNF-α (e, f) for 15 min. Following stimulation with TNF-α cells pretreated with estradiol for 24 h (f) showed stronger immunoreactivity for IκBα than cells that were not pretreated with estradiol (e)

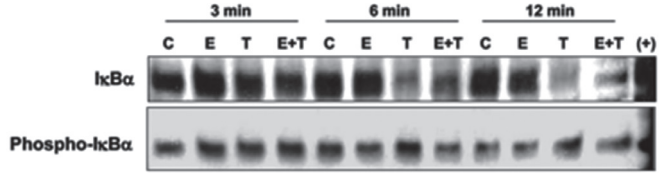

Figure 3. Regulation of inhibitory kappa B (IκBα) in endometrial stromal cells by estradiol and tumor necrosis factor-alpha (TNF-α). Endometrial stromal cells treated with estradiol (E_2; 10^{-8} M), TNF-α (mg/mL) alone, or estradiol with TNF-α (E_2+T), or vehicle (C; control) were analyzed for IκBα and its phosphorylated form following 3-12 min treatment. Estradiol treatment suppressed partially the TNF-α-induced IκBα degradation at 6 and 12 min. (+: positive control from TNF-α -induced HeLa cell extracts)

Phosphorylation of IκBα: Inhibitory kappa B alpha

group (Figure 4b). Glandular cells treated with E_2 plus TNF-α demonstrated higher phospho-IκBα levels when compared with cells treated with TNF-α alone ($p<0.05$).

As observed using immunoblotting, the effect of E_2 on IκBα was more pronounced when glandular cells were pre-treated with E_2 for 24 h prior to TNF-α treatment (Figure 5). To determine whether the effect of E_2 on IκBα phosphorylation was specific to the TNF-α signaling cascade, we also explored the effect of estrogen on IL-1α-mediated activation of NF-κB. Cells were treated with E_2 (10^{-8} M), IL-1α (2 ng/mL), E_2 plus IL-1α, or vehicle alone (control). E_2 induced lower phospho-IκBα and higher IκBα levels in IL-1α-treated cells as compared with cells treated with IL-1α alone (Figure 6).

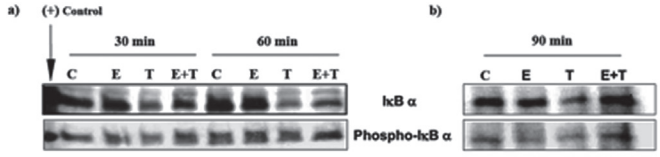

Figure 4. Regulation of inhibitory kappa B-alpha (IκBα) in endometrial stromal cells by estradiol and tumor necrosis factor-alpha (TNF-α). Endometrial stromal cells were treated with estradiol (E_2); 10^{-8} M, TNF-α (T; 1 mg/mL), estradiol in addition to TNF-α (E_2+T), or vehicle (C; control) for 30-60 min. Estradiol has a partial opposing effect on TNF-α–induced IκBα phosphorylation and degradation at both time points (a). Endometrial glandular cells were treated in a similar manner for 90 min, and similar effects were observed (b)

Phospho-IκBα: Phosphorylation of inhibitory kappa B-alpha

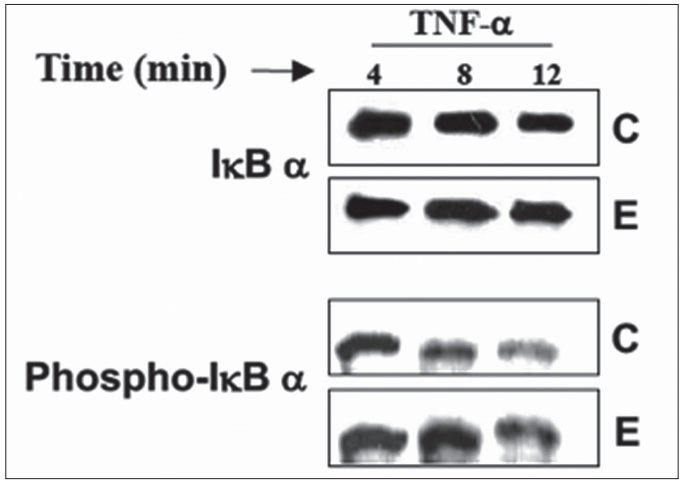

Figure 5. Regulation of inhibitory kappa B alpha (IκBα) in endometrial glandular cells by estradiol and tumor necrosis factor-alpha (TNF-α). Endometrial glandular cells were pre-treated with estradiol (E_2); 10^{-8} M, or vehicle (C; control) for 24 h prior to treatment with TNF-α (1 mg/mL) for 4-12 min. E_2 pre-treatment inhibited IκBα degradation compared with control

Phospho-IκBα: Phosphorylation of inhibitory kappa B-alpha, TNF: Tumor necrosis factor-alpha

Regulation of TNF-α– and IL-1α-induced activation of NF-κB by E_2 as assessed using an NF-κB binding assay

To understand whether the TNF-α– and IL-1α-induced IκBα levels in E_2-treated cells was associated with a decrease in free NF-κB, ESCs were treated with serum-free, phenol red-free media as control, and with E_2 (10^{-8} M) alone, TNF-α (2 ng/mL) alone, IL-1α (2 ng/mL) alone, E_2 combined with TNF-α or IL-1α for 15 min. Free NF-κB levels in control cells and E_2-treated cells were lower than those in TNF-α– and IL-1α-treated cells. On the other hand, E_2 decreased the TNF-α– and IL-1α–induced free NF-κB levels as compared with cells treated with TNF-α alone or IL-lα alone (Figure 7).

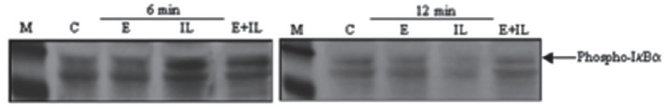

Figure 6. Regulation of inhibitory kappa B (IκBα) in endometrial stromal cells by estradiol and interleukin (IL)-1α. Endometrial stromal cells were treated for 6 and 12 min with estradiol (E_2); 10^{-8} M, IL-1α (IL; E_2 ng/mL), estradiol with IL-1κ (E_2+IL), or vehicle (C; control) and were analyzed for phospho-IκBα. Estradiol treatment suppressed IL-1α-induced IκBα degradation at 6 and 12 min

Phospho-IκBα: Phosphorylation of inhibitory kappa B-alpha, IL: Interleukin

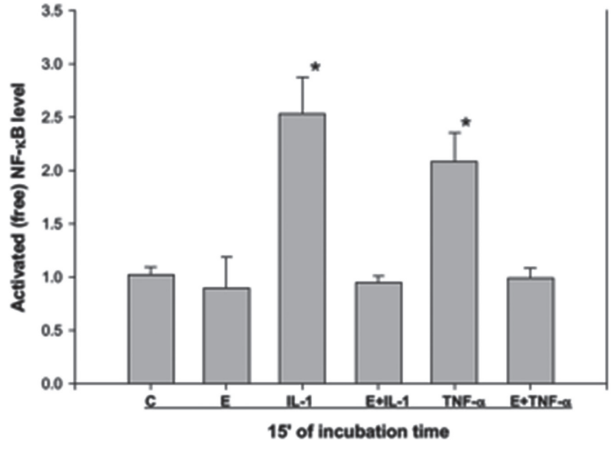

Figure 7. Regulation of active nuclear factor kappa B level in endometrial stromal cells by estradiol. The amount of activated NF-κB in endometrial stromal cells after 15 min of treatment with estradiol (E_2); 10^{-8} M, interleukin (IL)-1α (IL-1; 2 ng/mL) and E_2+IL-1 (10^{-8} M and 2 ng/mL), tumor necrosis factor-alpha (TNF-α) (TNF; 2 ng/mL) and E_2+TNF (10^{-8} M and 2 ng/mL) were compared with control cells. Experiments were repeated on three occasions with similar results and a representative graph from one experiment is presented

NF-κB: Nuclear factor kappa B, IL: Interleukin, TNF-α: Tumor necrosis factor-alpha

Discussion

Steroid hormones classically bind to cognate nuclear receptors to regulate target gene expression[35]. Estrogen takes part in cell and tissue regulation at many stages of human life. In addition to the reproductive tract of women, other systems such as the skeletal and nervous systems are important targets for estrogen action[36,37]. Estrogen mainly affects cells through the genomic pathway[38]. Estrogen actions may also result from non-genomic activity, possibly related to the cell type, receptor type, and the presence of intracellular co-factors that may interact with typical or atypical ERs. Non-genomic effects occur within minutes and appear to include cell membrane-dependent signaling mechanisms such as the nitric oxide cascade, stimulation of p38-mitogen-activated protein kinase, or phosphorylation of protein kinase B, among others[39-42]. In contrast, long-term effects of estrogen, namely genomic effects, arise over hours or longer and are directed in part by DNA estrogen response elements[43]. Some biologic processes can also play a role in both genomic and nongenomic pathways. A previous study showed that the lipopolysaccharide-stimulated activation of NF-κB was reduced by cell-impermeable E_2–bovine serum albumin in mouse bone marrow-derived macrophage cultures in both genomic and nongenomic pathways[44]. Eutopic and ectopic endometrium undergoes cycle-dependent changes predominantly controlled by estrogen and progesterone in their implantation site[45-47]. The present study is focused on the anti-inflammatory effects of estrogen, assessing IκBα phosphorylation and NF-κB activation in endometrial and endometriotic cells. *In vitro* and *in vivo* studies indicate that NF-κB–mediated gene transcription stimulates inflammation, invasion, angiogenesis, and cell proliferation, and reduces apoptosis of endometriotic cells. Excessive activation of NF-κB has been confirmed in endometriotic implants and peritoneal macrophages of patients with endometriosis[48,49]. In inflammatory tissue, an increase in TNF-α is often the first step in the cascade, followed by increases in the expression of various chemokines and the recruitment of leukocytes[27,50-53]. Previous studies have shown that, when bound to their receptors, TNF-α and IL-1 increase IκBα phosphorylation, degradation, and eventually NF-κB activation, which results in increased inflammatory cells and expression of several inflammatory cytokines and chemokines[27,54,55]. Our findings suggest that E_2 may reduce phospho-IκBα and therefore decrease its degradation in endometrial cells. In this way, estrogen may block NF-κB transport into the nucleus and attenuate the inflammatory response. To our knowledge, this is the first study to report IκBα regulation by estrogen in endometrial stromal and glandular cells. It is possible that this increase arises from effects on the transcriptional or translational machinery, because a previous study has shown that E_2 has a down-regulatory effect on IκBα at the mRNA level in phorbol ester-induced HeLa cells[56]. Alternatively, a previous study performed using MCF-7 cells suggested that this increase was related to the increase of p105 protein level[57]. On the other hand, another research group showed that estrogen treatment decreased liver IκB mRNA and protein expression and also increased ethanol-induced liver NF-κB levels and TNF-α expression[58]. These disparate findings are likely to be related to the cell-specific effects of estrogen and merit further analysis. Several cytokines participate in NF-κB activation. In addition to TNF-α, IL-1α also regulates IκBα levels in the cytosol. The similar effects on IκBα levels by E_2 co-treatment with TNF-α and with IL-1α, compared with treatments with TNF-α or IL-1α alone, indicate that the effect of E_2 is not specific for the TNF-α signaling cascade. IL-1α initiates an alternate cascade for IκBα-related NF-κB activation to that of TNF-α. Furthermore, because both signaling pathways merge on IKK activation, the effect of estrogen may be on IKK activation or on subsequent steps. Bulun et al.[59] studied NF-κBα and IκBα expression in human fetal membranes and decidua at preterm and term gestation. The authors observed a marked increase in the nuclear localization of p65 and in the IκBα immunoreactivity in tissues obtained at term compared with tissues delivered preterm, suggesting a role for p65 in the regulation of parturition-related gene transcription in the decidua[59]. Our *in vivo* results show an increase in IκBα levels from early proliferative to the late proliferative phase, and suggest direct or indirect estrogenic regulation of IκBα in human endometrial cells. On the other hand, persistently low levels of IκBα immunoreactivity in ectopic endometrial cells are likely to be related to the increased local inflammation observed in endometriosis and may contribute to the increased inflammatory cytokine levels in the peritoneal cavity of women with endometriosis[60,61]. Endometriosis is an estrogen-dependent disease and implants of endometriosis have sufficient enzymes for the local production of estrogen[54,59,62-64]. The low levels of IκBα in ectopic endometrial cells suggest that the signaling effects of estrogen on IκBα may function similarly to those observed in eutopic endometrium. It seems that there is a lack of the inhibitory effect of E_2 on cytokine-induced IκBα phosphorylation in ectopic endometrium. Supporting this hypothesis, a recent study has shown that E_2 increases phospho-IκB levels, and more interestingly, induces higher IL-8 levels in endometriotic cells when compared with eutopic endometrium[65]. Similarly, Akoum et al.[66] showed that E_2 and IL-1β had synergistic effects on the expression of RANTES, revealing that E_2 enhanced the mRNA stability of RANTES, and IL-1β increased its transcription. A recent study reported the expressions of IκBα, IκBβ, and p50 in human endometrial cells throughout the menstrual cycle[67]. Expression of these inhibitory proteins decreased significantly during the mid-secretory phase of the cycle. The study detected maximal immunoreactivity for IκBα during the late proliferative phase, consistent with our findings. Another study showed an increase in IκBα mRNA levels in the pre-menstrual endometrium, suggesting activation of NF-κB during this phase or alternate regulation of IκBα expression[68]. Our results support the findings of this study because activation of NF-κB requires IκBα phosphorylation

and degradation, low levels of IκBα protein would stimulate high level of IκBα mRNA during the pre-menstrual phase to replenish degraded IκB protein. One reason for the inhibitory effect of estrogen on chemokine expression may be related to decreased IκBα degradation. As a consequence, estrogen may decrease the amount of free-NF-κB in the cytosol, and therefore decrease the level of activation. Recently, we showed that the presence of ligand ERs suppressed free-NF-κB subunits (both p65 and p50) binding to NF-κB response element,[26] suggesting a second mechanism for estrogen-dependent inhibition of NF-κB-mediated gene activation. ERs in ESCs inhibited DNA binding of p50 and p65 subunits of NF-κB. Also, NF-κB activation significantly reduced estrogen responsiveness of ER-alpha–transfected ESCs, but p50 did not impair ER-alpha DNA binding, suggesting possible indirect mechanisms for this type of interaction[26].

Study Limitations

There were some limitations in the present study. This study presented a limitation with regard to experimental circumstances. These results also need to be assessed under *in vivo* conditions.

Conclusion

Our results support the hypothesis that E_2 inhibits NFκB activation through the down-regulation of IκBα phosphorylation and consequent reduction of free NF-κB in the cytosol. These results demonstrate that the regulation of IκBα by E_2 may regulate the inflammatory response in eutopic and ectopic endometrial cells. Our *in vivo* and *in vitro* findings suggest that this effect of estrogen on IκBα may not be optimal in ectopic endometrium, which may be an important factor in the pathogenesis of endometriosis.

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Ethics

Ethics Committee Approval: The study was approved by the Human Investigation Committee of Yale University Local Ethics Committee (approval number: HIC#22334).

Informed Consent: Consent form was filled out by all participants.

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Authorship Contributions

Surgical and Medical Practices: A.A., Concept: A.A., Design: A.A., Ü.A.K., Data Collection or Processing: Ü.A.K., Analysis or Interpretation: S.A., Literature Search: S.A., Writing: Ü.A.K., S.A., A.A.

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Is the measurement of the size of uterine lesions with positron emission tomography consistent in pre- and postmenopausal periods in endometrioid-type endometrial cancer?

Endometrioid tip endometriyal kanserde, pre- ve postmenopozal dönemlerde pozitron emisyon tomografisi ile uterin lezyon boyutunun ölçümleri tutarlı mıdır?

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Abstract

Objective: We aimed to investigate the correlation of the size and volume of uterine tumors obtained using positron emission tomography/computed tomography (PET/CT) and pathology specimens in patients with endometrioid-type endometrial cancer (EEC) in the premenopausal period, and to compare the results with those of postmenopausal women. In the premenopausal period, the endometrium uses more glucose than in the postmenopausal period. Therefore, the measurement of uterine tumor size using PET/CT in the premenopausal period may normally be different.

Materials and Methods: In this retrospective study, we reviewed the records of patients who were diagnosed as having EEC and underwent hysterectomy. Only patients who underwent preoperative PET/CT imaging were included in the study. The thickness and volume of the uterine lesion, and its maximum standardized uptake value as obtained using PET/CT and hysterectomy pathology specimens were recorded.

Results: Tumor size (p=0.051) and volume (p=0.404) were not found to be correlated with the imaging method used in premenopausal women and pathologic specimens. However, there was a correlation in postmenopausal women (p<0.001 for tumor size and p<0.001 for tumor volume). PET/CT has higher sensitivity, specificity, and positive predictive value in the postmenopausal period in the detection of >20 mm uterine tumors.

Conclusion: PET/CT has a limited role in the measurement of the size of uterine lesions in all patients, especially in the premenopausal period; therefore, we recommend that frozen-section examinations be used intraoperatively to decide on lymph node dissection.

Keywords: Positron emission tomography/computed tomography, endometrial cancer, premenopausal and reproductive periods

Öz

Amaç: Endometrioid tip endometriyum kanseri (EEK) olan hastalarda pozitron emisyon tomografisi/bilgisayarlı tomografi (PET/BT) ve patoloji örnekleri ile elde edilen uterin tümörlerin boyut ve hacimlerinin korelasyonunu ve premenopozal dönem ile postmenopozal kadınların sonuçlarını karşılaştırmayı amaçladık. Premenopoz dönemlerde, endometriyum, postmenopozal dönemlere kıyasla daha fazla glukoz kullanır. Bu nedenle, premenopozal dönemde uterin tümör büyüklüğünün PET/BT ile ölçümü normalden farklı olabilir.

Gereç ve Yöntemler: Bu retrospektif çalışmada EEK tanısı alan ve histerektomi yapılan hastaların kayıtları gözden geçirildi. Sadece preoperatif PET/BT görüntüleme yapılan hastalar çalışma kapsamına alındı. Uterin lezyonun kalınlığı, hacmi ve maksimum standartlaştırılmış alım değerleri PET/BT ve histerektomi patoloji örnekleri tarafından elde edilen veriler kaydedildi.

Bulgular: Premenopozal çağdaki kadınlarda tümör boyutu (p=0,051) ve hacmi (p=0,404) görüntüleme yöntemi ve patolojik örnekler arasında korelasyon bulunamadı. Bununla birlikte, postmenopozal kadınlarda bir korelasyon vardı (p<0,001 tümör boyutu için ve p<0,001 tümör hacmi için). PET/BT, >20 mm uterin tümörü tanımada postmenopozal dönemde daha yüksek sensitivite, spesifisite ve pozitif prediktif değere sahiptir.

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Sonuç: Tüm hastalarda özellikle premenopozal periyotda, PET/BT'nin uterin lezyonun boyutunun ölçülmesinde sınırlı bir role sahip olduğunu göstermektedir; bu nedenle lenf nodu diseksiyonuna karar vermek için ameliyat sırasında uterusun frozen kesit incelemesine gönderilmesini öneriyoruz.
Anahtar Kelimeler: Pozitron emisyon tomografisi/bilgisayarlı tomografi, endometriyal kanser, premenopozal ve üreme dönemleri

PRECIS: Positron emission tomography/computed tomography does not accurately assess the size of uterine lesions due to physiologic events in the endometrium and uterus in reproductive ages.

Introduction

Endometrial cancer is the most common gynecologic malignancy in developed countries[1-3]. Prognosis is affected by the age of the patient, histologic type and grade of the tumor, cervical invasion, depth of myometrial invasion, lymph node involvement, and distant organ metastasis[1,2]. Fluorine-18 (^{18}F) fluorodeoxyglucose (FDG) positron emission tomography/computed tomography (PET/CT) is an imaging modality used to obtain anatomic and metabolic data on cancer cells in numerous malignancies[2,3]. It is helpful to evaluate tumor perfusion and metabolism screening using the following radioisotopes: carbon-11, ^{18}F, nitrogen-13, oxygen-15 and rubidium-82[4]. Of these, ^{18}F-FDG passes through the cell membrane in the same way as glucose and is effectively trapped when it is phosphorylated and cannot be metabolized by the following enzyme: phosphofructokinase-1. Thus, ^{18}F-FDG remaining within the cell reflects glucose uptake into the cell[4]. The standardized uptake value (SUV) is accepted as an indicator of tumor aggressiveness and a marker for metabolic alterations in cancer tissues[2-4]. The maximum SUV (SUV_{max}) has been associated with the tumor proliferation rate, tumor grade, and expression of glucose transporters[2-4]. About 25% of patients with endometrioid-type endometrial cancer (EEC) are in the premenopausal periods[5]. In women of the premenopausal period, physiologic FDG accumulation in the uterus should be considered when focal FDG accumulation is observed in the pelvis[6]. In the endometrium, normal uptake of ^{18}F-FDG PET/CT in patients who are premenopausal varies cyclically and increases in the ovulatory and menstrual phases[7]. In the premenopausal period, the endometrium consumes constant energy for proliferation and the secretory phases[6]. In the present study, we aimed to investigate the correlation of the size and volume of uterine tumors obtained using PET/CT and pathology specimens in patients with EEC in the premenopausal period and to compare the results with those of postmenopausal women.

Materials and Methods

In this retrospective study, we reviewed the records of patients who were diagnosed as having EEC and underwent hysterectomy at the Tepecik Training and Research Hospital, Clinic of Gynecologic Oncology between January 2012 and August 2016. Only patients who underwent preoperative ^{18}F-FDG PET/CT imaging were included in the study. A flowchart of the study is shown in Figure 1. Diagnosis was confirmed histopathologically in all patients. The thickness and volume of the uterine lesion and its SUV_{max} value as obtained using ^{18}F-FDG PET/CT and hysterectomy pathology specimens were recorded. Data including age, menopausal status, and comorbidities were recorded. Tumor staging was performed based on the International Federation of Gynecology and Obstetrics (FIGO) 2009 staging criteria[8]. The study was approved by the local ethics committee (Katip Çelebi University, approval number: 45, Date: 27/02/2014). Written informed consent was obtained from each patient. The study was conducted in accordance with the principles of the Declaration of Helsinki. All surgical specimens were evaluated by specialized gynecologic pathologists. The inclusion criteria were as follows: 1) all types of histology, 2) no intraoperative evidence of extrauterine spread, 3) performance of pelvic and para-aortic lymphadenectomy, and 4) histopathologically proven cervical stromal involvement. Uterine sections were selected from anterior and posterior aspects of the cervix, lower uterine segment, and uterine corpus. A minimum of 6 sections including a section of the deepest tumoral invasion was obtained for all specimens. Whole-body ^{18}F-FDG PET/CT images were performed using a PET/CT scanner (Philips Gemini TF; Philips Healthcare, Andover, MA, USA), which consisted of a dedicated lutetium orthosilicate full-ring PET scanner and 16-slice CT. Both PET and low-dose CT scanning covered the skull to the proximal thigh. The protocol included 6 h of fasting before image acquisition, and all patients were asked to void before undergoing scanning. On the day of the examination, the serum glucose levels measured before ^{18}F-FDG injections were found to be less than 140 mg/dL. Subsequently, ^{18}F-FDG (6.5-13.4 µCi) was given intravenously 60 to 120 min before the CT scan, and the patients were instructed to rest in a semi-dark, temperate room between the injection and scanning. At 60 min after the administration of ^{18}F-FDG, low-dose CT (50 mAs, 120 kV) covering the area from scull to the proximal thighs was performed to attenuate the correction and precise anatomic localization. An emission scan was then conducted in the three-

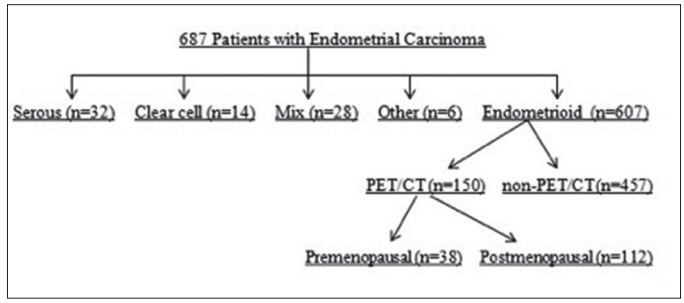

Figure 1. Flowchart of the study

PET/CT: Positron emission tomography/computed tomography

dimensional mode. All images were reconstructed and stored as axial, coronal, and sagittal slices. The total scanning time was about 20 min per patient. The SUV_{max} was estimated for each hypermetabolic lesion.

Statistical Analysis

This study was calculated to have 94% power and 71% effect size using the G power analysis program (Faul, Erdfelder, Lang and Buchner, 2007; version 3.0). Statistical analysis was performed using the Med-Calc for Windows version 16.0 statistical software (MedCalc Software, Mariakerke, Belgium). Descriptive data are expressed in mean ± standard deviation and percentages. Student's t-test was used to compare the mean values between two independent groups, and the chi-square (χ^2) test was used to compare nominal values between the two groups. Correlation analysis was performed using bivariate correlation analysis. The sensitivity, specificity, negative and positive predictive values of the ^{18}F-FDG PET/CT were also calculated. Receiver operating characteristic (ROC) curve analysis was used to determine the optimal cut-off value of predictive tumor size >2 cm in uterin lesion with EEC. A p value of <0.05 was considered statistically significant.

Results

Of all patients with EEC who underwent ^{18}F-FDG PET/CT, 38 women were premenopausal, and 112 were postmenopausal. The demographic and clinical characteristics of the patients are shown in Table 1.

The largest tumor size and total volume of both premenopausal and postmenopausal patients in the ^{18}F-FDG PET/CT reports were compared with the pathology reports. The correlation analysis results are shown in Figures 2 and 3. The tumor size and volume were not found to be correlated with the imaging method used in premenopausal women and pathologic specimens for tumor size and tumor volume (p=0.051, correlation coefficient: 0.319; p=0.404, correlation coefficient: 0.139, respectively). However, there was a correlation in postmenopausal women for tumor size and tumor volume (p<0.001, correlation coefficient: 0.772; p<0.001 and correlation coefficient: 0.695, respectively). Sensitivity and specificity tests were performed in the premenopausal women and postmenopausal women by dividing the tumor size into the two groups (≤20 mm; >20 mm) in both ^{18}F-FDG PET/CT reports and pathology specimens. In the former group, the sensitivity of ^{18}F-FDG PET/CT to detect >20 mm tumors was 19/21 (90.4%), specificity was 6/17 (35.2%), the negative predictive value was 6/8 (75.0%), and

Table 1. Demographic characteristics and clinical characteristics of the patients

	Premenopausal (n=38)	Postmenopausal (n=112)	p
Age, mean ± SD	44.1±4.8	62.6±7.5	<0.001
Gravida, mean ± SD	2.4±1.9	3.3±2.2	0.082
Parity, mean ± SD	1.9±1.6	2.8±1.9	0.046
BMI, mean ± SD	30.1±5.1	33.1±5.7	0.093
CA125, mean ± SD	136.7±264.4	219.9±570.0	0.394
Hemotocrit, mean ± SD	38.0±4.3	38.8±3.6	0.234
Hypertension, n (%)	6 (15.7)	45 (40.1)	<0.001
Diabetes, n (%)	4 (10.5)	31 (27.6)	0.005
Histologic grade, n (%)			0.774
I	15 (39.4)	38 (33.9)	
II	18 (47.3)	62 (55.3)	
III	5 (13.1)	12 (10.7)	
LVSI, n (%) positive	13 (34.2)	44 (39.2)	0.633
SUV_{max}, mean ± SD	13.4±5.9	15.7±6.5	0.062

BMI: Body mass index, CA125: Cancer antigen 125, LVSI: Lymphovascular space invasion, SUV_{max}: The maximum standardized uptake value, SD: Standard deviation

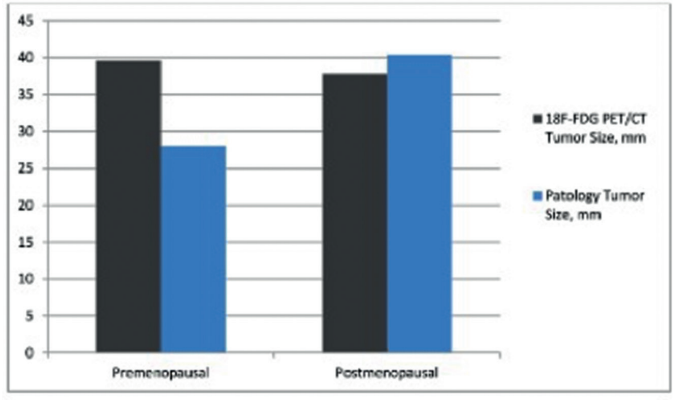

Figure 2. Correlation analysis* between ^{18}F-FDG PET/CT and pathology report of tumor size

*^{18}F-FDG PET/CT: 18-Florin-Fluorodeoxyglucose-positron emission tomography/computed tomography, *: Spearman's correlation analysis, p=0.051 for premenopausal, p<0.001 for postmenopausal*

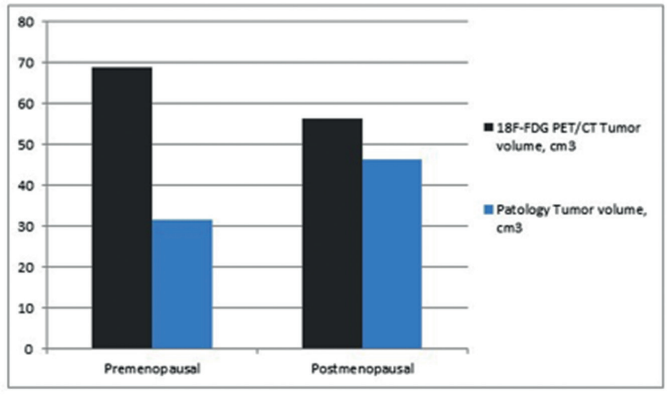

Figure 3. Correlation analysis* between ^{18}F-FDG PET/CT and pathology report of tumor volume

*^{18}F-FDG PET/CT: 18 Florin-Fluorodeoxyglucose positron emission tomography/computed tomography, *: Spearman's correlation analysis, p=0.404 for premenopausal, p<0.001 for postmenopausal*

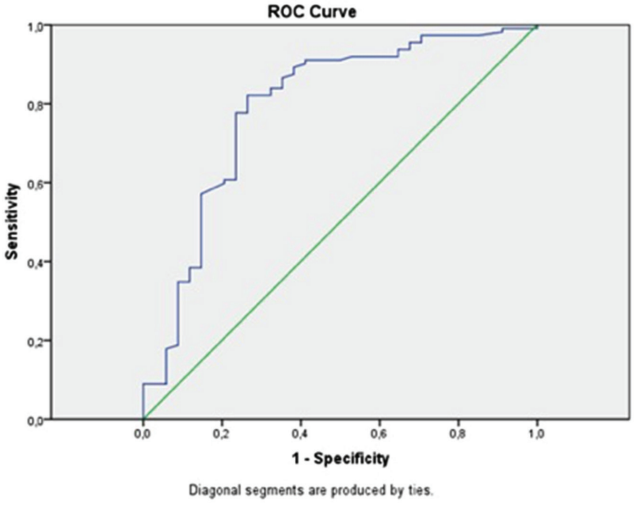

Figure 4. ROC curve associated with the maximum standardized uptake value to identify patients with tumor size >20 mm. The area under the curve was 0.791 (p<0.001)
SUV_{max}: The maximum standardized uptake value

the positive predictive value was 19/30 (63.3%). The pooled diagnostic indices to detect tumors >20 mm in postmenopausal women were as follows: sensitivity 88/94 (93.6%), specificity 11/18 (61.1%), negative predictive value 11/17 (64.7%), and positive predictive value 88/95 (92.6%). The optimal SUV_{max} value was investigated using ROC analysis to distinguish patients with tumor size >2 cm. The ROC analysis is shown in Figure 4 (p<0.001, area under the curve=0.791). SUV_{max} values of 10.5 and above were found as 82.1% sensitivity and 73.5% specificity for tumors with tumor size >2 cm. In addition, the ^{18}F-FDG PET/CT showed 4/5 (80.0%) sensitivity, 28/33 (84.8%) specificity, 28/29 (96.6%) negative predictive value, and 4/9 (44.4%) positive predictive value to detect lymph node involvement in premenopausal women. In the postmenopausal period, however, the pooled diagnostic indices for lymph node involvement were as follows: sensitivity 12/15 (80.0%), specificity 91/97 (93.8%), negative predictive value 94/97 (96.9%), and positive predictive value 9/15 (60.0%).

Discussion

In this retrospective study, we evaluated the accuracy of ^{18}F-FDG PET/CT in the assessment of the size and volume of uterine lesions associated with EEC in premenopausal and postmenopausal patients with EEC. Based on our study results, we found that tumor volume and size were correlated in postmenopausal women, but not in premenopausal women. Previous studies showed that PET/CT had 81.8% sensitivity and 89.8% specificity in the detection of primary uterine tumors in patients with EEC[9]. In our study, the sensitivity of ^{18}F-FDG PET/CT for detecting tumors >20 mm in premenopausal women was 90.4%, specificity was 35.2%, and the negative and positive predictive values were 75.0% and 63.3%, respectively. In the postmenopausal period, the sensitivity of ^{18}F-FDG PET/CT to detect >20 mm tumors was 93.6%, specificity was 61.1%, and the negative and positive predictive values were 64.7% and 92.6%, respectively. The proposed cut-offs for SUV_{max} for these parameters to identify deep myometrial invasion in the literature is a relatively wide range, 9-18[8,10]. There was a significant association reported between the SUV_{max} of the primary tumors and maximum tumor size (p=0.001), but not between the SUV_{max} and menopause state (p=0.522)[11]. In our cohort, SUV_{max} >10.5 had 82.1% sensitivity and 73.5% specificity for tumors >2 cm. The ^{18}F-FDG PET/CT imaging modality uses the intracellular glucose metabolism of tumor cells[4]. In the premenopausal period, the endometrium uses different amounts of glucose for menstruation, proliferation, ovulation, and secretion processes; however, the endometrium in the postmenopausal period uses less glucose[6]. In a study of endometrial ^{18}F-FDG uptake in gynecologic malignancies in premenopausal women by Lerman et al.[7] the mean SUV values were 5.0±3.2 in the menstrual phase, 2.6±1.1 in the proliferation phase, 3.7±0.9 in the ovulation phase, and 2.5±1.1 in the secretory phase (p<0.001). In the aforementioned study, the mean SUV value of the patients with abnormal cycles was 3.4±1.4 in patients with oligomenorrhea and 1.9±1.2 in patients with amenorrhea (p=0.02). PET may be influenced by tissue type[9]. The efficacy of PET/CT may be affected by the size of the tumor, and thus PET/CT may be limited for the detection of small tumors[9]. Furthermore, oral contraceptive use has been shown to affect the ^{18}F-FDG uptake in the endometrium[12]. In our study, we hypothesized that ^{18}F-FDG uptake in premenopausal women and the calculated tumor size and volume would be less correlated with the pathology specimens compared with postmenopausal women. In our study population, the calculated tumor size (p=0.051) and volume (p=0.404) on ^{18}F-FDG PET/CT imaging in premenopausal women were not correlated with the pathology specimens. However, in the postmenopausal period, tumor size (p<0.001) and volume (p<0.001) on the ^{18}F-FDG PET/CT scan were found to correlate with the pathology specimens. In addition, the sensitivity and specificity of the PET/CT to detect lymph node metastasis in premenopausal women was 80.0% and 84.8%, respectively, compared with 80.0% and 93.8% in postmenopausal women, respectively. The sensitivity was 40.9% in micrometastatic lymph nodes with (metastasis >2 mm) and 52.9% in those with (metastasis >5 mm)[9]. The sensitivity and specificity of PET/CT for detecting nodal metastases were 51.1-78.6% and 98.4-99.8%, respectively[13,14]. Although the sensitivity and specificity values for detecting lymph node involvement are similar in pre- and postmenopausal women, estimating the size of primary uterine lesions showed limited correlation in premenopausal women. In our cohort, there was no statistically significant difference between the SUV_{max} values of pre- and postmenopausal period uterine lesions. Therefore, we consider that PET/CT has a limited role in deciding for lymph node dissection in premenopausal women, and frozen-section examinations should be performed during surgery. In

our cohort, we found that there were statistically significantly more patients with diabetes among the postmenopausal patients (p=0.005). However, previous studies reported that PET/CT could be applied to diabetics[15]. In all patients (diabetics and non-diabetics), the serum glucose levels measured before ^{18}F-FDG injections were found to be less than 140 mg/dL.

Study Limitations

Nonetheless, there are some limitations to this study. First, the study has a retrospective design. Second, the sample size is relatively small. Third, the endometrial phases of premenopausal women are still missing aspects of the study. Despite these limitations, the similarities of the demographic characteristics in the study population and analysis reports of the expert pathologists and radiologists increased the validity of our results and diminished the weaknesses. However, further large-scale, prospective studies are required to shed light on the role of ^{18}F-FDG PET/CT in EEC.

Conclusion

In conclusion, our study results suggest that ^{18}F-FDG PET/CT has a limited role in the measurement of the size of the uterine lesion in all patients, especially in the premenopausal period; therefore, we recommend that frozen-section examinations should be performed intraoperatively to decide on lymph node dissection.

Ethics

Ethics Committee Approval: The study was approved by the Katip Çelebi University Local Ethics Committee (approval number: 45, Date: 27/02/2014).
Informed Consent: Consent form was filled out by all participants.
Peer-review: External and internal peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: İ.A.Ö., M.S., Concept: V.G., K.G., Design: M.K., K.G., Data Collection or Processing: M.K., Ö.Ç.G., Analysis or Interpretation: V.G., Ö.Ç.G., Literature Search: V.G., İ.A.Ö., Writing: V.G., M.K.
Conflict of Interest: No conflict of interest was declared by the authors.
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The importance of uterosacral ligament anatomy in overactive bladder: A preliminary study

Aşırı aktif mesanede uterosakral ligament anatomisinin önemi: Ön çalışma

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Abstract

Objective: To evaluate whether uterosacral ligament (USL) thickness measured using magnetic resonance imaging (MRI) was associated with overactive bladder (OAB) in otherwise healthy women.

Materials and Methods: The study comprised 27 women with OAB and 27 healthy women (control group) who were followed up at the Obstetrics and Gynecology Department of a tertiary referral center. All subjects were evaluated using pelvic MRI to determine the transverse USL thickness. These measurements were compared between the two groups. p values less than 0.05 were considered statistically significant.

Results: The mean age of women in the OAB and control groups were 43.88±9.36 years and 39.92±5.36 years, respectively. The mean body mass index in the OAB group was 29.77±4.82 kg/m^2 and 27.49±3.44 kg/m^2 in the control group. In the comparison of Pelvic Organ Prolapse Quantification system stages between the groups, no statistically significant relationship was determined. In the OAB group, the mean right USL thickness was 2.04±0.34 mm, and the mean left USL was 2.04±0.52 mm. In the control group, the mean right USL thickness was 2.17±0.47 mm, and the mean left USL was 2.09±0.51 mm. There were no statistically significant differences in terms of USL thickness between the OAB and control groups (p>0.05).

Conclusion: No previous studies have been identified in the literature that have investigated the relationship between USL thicknesses and urinary incontinence. In the present study, no significant relationship could be demonstrated between right and left USL thicknesses of the OAB and control groups. This was a preliminary study, and further research with larger sample sizes is required to reach a final conclusion.

Keywords: Integral theory, magnetic resonance imaging, overactive bladder, uterosacral ligaments

Öz

Amaç: Aşırı aktif mesane (AAM) tanısı alan kadınlarda manyetik rezonans görüntüleme (MRG) ile ölçülen uterosakral ligament (USL) kalınlıklarının, sağlıklı kadınlarda aşırı aktif mesane (OAB) ile ilişkili olup olmadığını değerlendirmek.

Gereç ve Yöntemler: Çalışmaya, Ocak 2013-Aralık 2015 tarihleri arasında jinekoloji polikliniğinde takip edilen, yaşları 21-55 arasında değişen, AAM tanılı 27 ve sağlıklı 27 kadın (kontrol grubu) dahil edildi. Tüm olgular, transvers USL kalınlığını belirlemek için pelvik MR ile değerlendirildi. Bu ölçümler iki grup arasında karşılaştırıldı. P değerlerinin 0,05'ten küçük olması istatistiksel olarak anlamlı kabul edildi.

Bulgular: OAB ve kontrol grubundaki kadınların yaş ortalaması sırasıyla 43,88±9,36 yıl ve 39,92±5,36 yıl idi. Kontrol grubunda OAB grubunda ortalama vücut kitle indeksi 29,77±4,82 kg/m^2 ve 27,49±3,44 kg/m^2 bulundu. Pelvik Organ Prolapsus Kantifikasyon sistem evrelerinin gruplar arasında karşılaştırılmasında istatistiksel olarak anlamlı bir ilişki saptanmadı. OAB grubunda ortalama sağ USL kalınlığı 2,04±0,34 mm, sol USL değeri 2,04±0,52 mm idi. Kontrol grubunda sağ USL kalınlığı 2,17±0,47 mm idi ve sol USL değeri 2,09±0,51 mm idi. OAB ve kontrol grupları arasında USL kalınlığı açısından istatistiksel olarak anlamlı farklılık yoktu (p>0,05).

Sonuç: Literatürde USL kalınlıklarının üriner inkontinans ile ilişkisini inceleyen bir çalışma yoktur. Araştırmamızda, AAM ile sağ ve sol USL kalınlıkları arasında istatistiksel olarak anlamlı bir ilişki bulunmadı (p>0,05). Bu çalışmada OAB ve kontrol gruplarının sağ ve sol USL kalınlıkları arasında anlamlı bir ilişki gösterilemedi. Bu bir ön çalışma niteliğinde olup, kesin bir sonuca varılabilmesi için daha çok olgu içeren araştırmalara ihtiyaç vardır.

Anahtar Kelimeler: İntegral teori, manyetik rezonans görüntüleme, aşırı aktif mesane, uterosakral ligamentler

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PRECIS: No difference was identified in terms of right and left USL thicknesses of the OAB and control groups. This was a preliminary study, and further research with larger sample sizes is required to reach a conclusion.

Introduction

Overactive bladder (OAB) is a significant health problem that can negatively affect quality of life[1]. "OAB" is a term that describes a syndrome of urinary urgency with or without incontinence, which is often accompanied by nocturia and urinary frequency[2,3]. The terms "urgency incontinence" and "OAB with incontinence" are often used interchangeably. Significant risk factors for urinary incontinence are primarily known as age, obesity, births, menopause, hysterectomy, and cigarette smoking[4]. It is thought to result from detrusor overactivity, leading to uninhibited detrusor muscle contractions during bladder filling[3]. In the etiology, neurologic disorders (e.g., spinal cord injury), bladder abnormalities, increased or altered bladder microbiome may be other reasons, or this may be idiopathic[5]. The integral theory describes the pathophysiology of urinary incontinence. The integral theory indicates that pelvic organ prolapses and abnormal pelvic symptoms such as urge, frequency, nocturia, and pelvic pain are usually caused by connective tissue laxity in the vagina or its supporting ligaments[6]. In the theory, the pelvic floor muscle forces the vaginal membrane to stretch against the suspensory ligaments to stimulate the micturition stretch receptors. Laxity in the membrane or suspensory ligaments may activate stretch receptors, which are perceived by the cortex as urgency, frequency, and nocturia untimely. The cortex then perceives this stimulation as urgency, frequency, and nocturia[7]. Imaging methods are playing an increasingly important part in the diagnosis of pelvic floor disorders. In many studies, pelvic floor disorders have been evaluated with magnetic resonance imaging (MRI)[8]. Measurements of the uterosacral ligaments (USL), which are strong ligaments of the uterus, have previously been made with ultrasound in cadaver studies and in patienst with endometriosis[9-11]. The aim of the current study was to investigate the role of USL anatomy in stretching the vaginal membrane in patients with OAB.

Materials and Methods

Approval for this controlled clinical study was granted by the Adana Numune Training and Research Hospital Ethics Committee (approval number: 122/03.11.2015) and written informed consent was obtained from all participants. The study included a total of 27 patients who had been diagnosed as having OAB in our clinic between January 2013 and December 2015. Patients were excluded from the study if they were determined to have concomitant stress urinary incontinence or a malignant pathology. The control group comprised 27 healthy women. The diagnosis of OAB was made from the anamnesis and physical examination. On the first presentation, the patients were questioned in respect of the times that urine leakage occurred, the amount of urine leakage, the reason for the leakage, and what increased or decreased the leakage. Questions were also asked regarding the presence of additional diseases that could cause urine leakage. A physical and pelvic examination was performed to all patients. The stress test was applied. The patients kept a urine diary and this was examined. Patients with OAB who met the study criteria were included in the evaluation. For all the patients included in the study, a record was made of age, height, weight, body mass index (BMI), parity, type of births, and the Pelvic Organ Prolapse Quantification system (POP-Q) was used, which was first published in 1996, in an article by Bump et al.[12] The hymen acts as the set point of indication throughout the POP-Q staging. There are six described points for quantity in the POP-Q system. Anterior: Aa, Ba, C, posterior: Ap, Bp, D. Three others milestones: genital hiatus, the vaginal length (TVL), and perineal body. Each is measured in centimeters above or proximal to the hymen (negative number) or centimeters below or distal to the hymen (positive number) with the plane of the hymen being defined as zero (0). Stage 0: no prolapse; Stage I: the most distal portion of prolapse is >1 cm above level of hymen; Stage II: the most distal part of prolapse is <1 cm proximal to or distal to the plane of hymen; Stage III: the most distal portion of the prolapse protrudes more than 1 cm below the hymen but no farther than 2 cm less than the total TVL (for example, not all of the vagina has prolapsed); Stage IV: complete vaginal eversion is needed, full urine test and urine culture. Lower abdominal MRI was taken using a 1.5 Tesla MRI system (Siemens Magnetom Avanto, Philadelphia, USA). The same protocol was applied to all patients and the control group. Axial T1 and T2 sequences and coronal and sagittal T2 sequences were used. The parameters for the sequences used in the study were as follows: T1-weighted axial repetition time (TR): 370 ms, echo time (TE): 7.11 ms, slice thickness: 3 mm, field of view (FOV): 34x34 mm; T2-weighted axial TR: 3185 ms, TE: 105 ms, slice thickness: 3 mm, FOV: 33x33 mm; T2-weighted turbo spin echo (TSE) sagittal TR: 5117 ms, TE: 120 ms, slice thickness: 3 mm, FOV: 30x30 mm; T2-weighted TSE coronal TR: 5484 ms, TE: 120 ms, slice thickness: 3 mm, FOV: 32x32 mm. The images were evaluated on a separate workstation by a radiology specialist with 10 years' experience who was blinded to the study. All images were investigated in respect of pelvic pathology, then the USLs on both sides were identified and an evaluation was made concerning their thickness and nodularity. The USL thickness was measured using MRI at the closest points to the cervix and the sacrum and at the mid point between those two points, and the results of the OAB group were compared with those of a control group. Any patients with nodularity that was found to be significant for endometriosis were excluded from the study. A measurement was made of the transverse thickness of the area

observed as hypointense on T1 and T2-weighted sequences from the closest points to the cervix and the sacrum and from the midpoint of those two points (Figure 1). The mean of the three measurements was then calculated for the right and left USL.

Statistical Analysis

The statistical analysis of the study data was made using Statistical Package for Social Sciences (SPSS) v. Twenty-one software (SPSS , Chicago, IL, USA). Comparisons were made between the OAB patient group and the control group in respect of the above-mentioned clinical parameters and the USL thickness measured on MRI. Categorical variables were stated as number and percentage (%) and numerical variables as mean (minimum-maximum) ± standard deviation (SD). Conformity of the data to normal distribution was assessed using the the Kolmogorov-Smirnov test. In the comparisons of categorical data between the groups, the chi-square test or Fisher's exact test was used. In the comparison of numerical data, Student's t-test was used when there was conformity to normal distribution and the Mann-Whitney U test where there was non-normal distribution. A value of $p<0.05$ was accepted as statistically significant.

Results

The study included a total of 33 patients with OAB and 30 control subjects who met the study criteria within the specified period. A total of 6 patients with OAB and 3 control group subjects were excluded from the final evaluation because the MRIs were not clear. The mean age was 43.88±9.36 years in the OAB group and 39.92±5.36 years in the control group. The mean BMI value was 29.77±4.82 kg/m^2 in the OAB group and 27.49±3.44 kg/m^2 in the control group. The mean parity was 3.37±1.59 in the OAB group and 2.7±1.68 in the control group (Table 1). In the OAB group, previous births were performed with vaginal delivery in 59.3%, caesarean section in 25.9%, and with vaginal delivery and cesarean in 14.8%. In the control group, previous births were performed with vaginal delivery in 55.6%, with cesarean section in 37%, and with vaginal delivey and cesarean in 7.4% ($p>0.05$) (Table 2). No statistically significant difference was determined between the groups in respect of episiotomy ($p=0.08$). In the comparison of the POP-Q stages between the groups, no statistically significant relationship was determined ($p>0.05$) (Table 3). The mean (± SD) thickness of the right USL and left USL of both the OAB and control groups was 2.10±0.4 mm and 2.06±0.51 mm, respectively. In the OAB group, the mean thickness of the right USL was 2.04±0.34 mm and the mean thickness of the left USL was 2.04±0.52 mm. In the control group, the mean thickness the right USL was 2.17±0.47 mm and the mean thickness of the left USL was

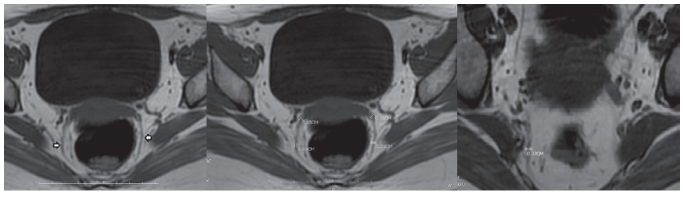

Figure 1. Measurement points of the uterosacral ligament on magnetic resonance imaging

Table 1. Demographic characteristics of overactive bladder and control groups

	OAB group (mean ± SD)	Control group (mean ± SD)	p value
Age (years)	43.88±9.36	39.92±5.36	0.063
BMI (kg/m²)	29.77±4.82	27.49±3.44	0.051
Parity	3.37±1.59	2.7±1.68	0.141

Data are given as mean ± SD
SD: Standard deviation, OAB: Overactive bladder, BMI: Body mass index

Table 2. Obstetric history of overactive bladder and control groups

	OAB group (n=27)	Control group (n=27)	p value
Vaginal delivery	16 (59.3%)	15 (55.6%)	0.537
Cesarean delivery	7 (25.9%)	10 (37%)	
Vaginal and cesarean delivery	4 (14.8%)	2 (7.4%)	

Data are presented as n (%)
OAB: Overactive bladder

Table 3. Pelvic Organ Prolapse Quantification system staging of overactive bladder and control groups

POP-Q	OAB group (n=27)	Control group (n=27)	p value
Stage 0	12 (44.4%)	12 (44.4%)	0.375
Stage 1	8 (29.6%)	8 (29.6%)	
Stage 2	5 (18.5%)	7 (25.9%)	
Stage 3	2 (7.4%)	0 (0%)	

Data are presented as number (%)
POP-Q: Pelvic Organ Prolapse Quantification system, OAB: Overactive bladder

Table 4. Uterosacral ligament thickness of overactive bladder and control groups

	OAB group (mean ± SD)	Control group (mean ± SD)	p value
Right USL thickness (mm)	2.04±0.34	2.17±0.47	0.71
Left USL thickness (mm)	2.04±0.52	2.09±0.51	0.206

Data are given as mean ± SD
USL: Uterosacral ligament, OAB: Overactive bladder, SD: Standard deviation

2.09±0.51 mm. No statistically significant difference was found between the groups in respect of the thickness of the right USL (p=0.71) and the thickness of the left USL (p=0.206) (Table 4).

Discussion

In current study, we aimed to evaluate thickness of the USL using MRI to find anatomic disorders of USL that may cause laxness of the vaginal membrane. We hypothesized that the thickness of the USL might correlate with OAB. The role of imaging methods in the evaluation of pelvic floor dysfunctions has been questioned in many studies. MRI in particular has become increasingly useful in the diagnosis of pelvic organ prolapse and pelvic floor disorders[12,13]. In previous studies, a correlation has been determined between pelvic floor measurements made with MRI and POP clinical staging[14]. However, as study data are limited and cannot be compared, there is no standardized method as yet for MRI measurements[15]. In a study by Tan et al.[8] pelvic MRI was performed in young healthy females and cadavers, clearly showing the pelvic and urogenital diaphragm and the previously defined uterus-supporting tissues, and the periuretal and parauretal ligaments were able to be seen anatomically. Stoker et al.[16] examined the whole pelvic floor aiming to find a solution to both urinary and anal dysfunction. The integral theory of female urinary incontinence states that stress and urge symptoms both derive from the same anatomical defect, a lax vagina[17]. According to the integral theory, urge, frequency, and nocturia are neurogenic symptoms and can happen with minimal prolapse[6]. The integral theory suggests sustentation of the mid-urethra (the anterior vaginal wall along the arcus tendineous, and the vaginal cuff along the uterosacral "neoligament") will prevent a lax vaginal membrane, which will cure OAB and/or urge incontinence symptoms. This is based on the presence of hypothesized stretch receptors at the proximal urethra and bladder neck[6]. On the other hand, hysterectomy has been implicated as a risk factor for the development of urinary incontinence[18]. Urinary incontinence after hysterectomy can be the result of a lasting injury to the pelvic plexus at the time of uterosacral/cardinal ligament complex transection, bladder flap formation, and possibly disruption of the anatomic support to the bladder neck and urethra[19]. The USL is an important ligament that supports the pelvic floor. No systematic mapping has been performed to define the length and thickness of the USL[10] and there are very few studies in the literature with any information related to USL thickness. In a cadaver dissection study by Vu et al.[9], the USL thickness was reported to be a mean 5-20 mm in the cervical region, 5 mm in the central region, and 5 mm in the sacral region. In addition to cadaver studies, USL thickness has been evaluated in endometriosis. In a study by Bazot et al.[20] MRI findings showed USL thickness as >9 mm in patients with endometriosis. In our study, we were also able to identify the POP-Q stages between the control and OAB groups. However, a statistically significant relationship was not determined between the two groups. It is possible that laxness of the USL may be correlated with both thickness of the ligament and the distribution and function of the collagen types within the tissue[21-23]. In the whole group of the current study, including the OAB patients and the control group, the mean thickness of the left USL was 2.06±0.51 mm, and the right was 2.10±0.40 mm. None of the current study patients had endometriosis or any pelvic pain. In the OAB group, the mean thickness of the right USL was determined as 2.04±0.34 mm, and the left as 2.04±0.52 mm. In the control group, the mean thickness of the right USL was 2.17±0.46 mm, and the left was 2.09±0.51 mm (Table 4). According to the integral theory, vagina and bladder base defects are displayed as "OAB" symptoms. A previous study by Petros and Ulmsten[7] reported that repair of ligament stretch and tension restored anatomy and function. The USLs are major insertion points for the directional vectors that stretch the vaginal membrane to block premature activation of the micturition reflex. All different appearances of a prematurely activated micturition reflex such as urge incontinence depend on the link between loose ligaments and diminished striated muscle force. The Testicular Feminisation syndrome creates a strong suspension structure to restore muscle contractility and to prevent urge incontinence, as well as urge symptoms[6,23-25].

Study Limitations

This study provides useful preliminary data of the relationship between USL thickness and incontinences. However, the present study has some limitations. First, the sample size was small. Due to the low number of patients, this report should be regarded as a preliminary study. A larger number of patients may be more likely to elucidate the relationship of USL thickness with OAB and the development of urinary problems. Second, a urodynamic test was not used to determine causes of incontinence.

Conclusion

In conclusion, no statistically significant difference was found between the two groups examined in respect of both left and right USL thickness. There is a need for further studies including a larger number of patients.

Ethics

Ethics Committee Approval: The study was approved by the Adana Numune Training and Research Hospital Local Ethics Committee (approval number: 122/03.11.2015).

Informed Consent: Consent form was filled out by all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: C.A., Concept: C.A., Design: C.A., A.S., Data Collection or Processing: C.A., S.A., S.S., G.S., G.U., Analysis or Interpretation: C.A., A.S., D.Y, Literature Search: C.A., S.A., O.Y., Critical Revision: A.S., E.S.S.Y., Writing: C.A., S.A.

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