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AIMS AND SCOPE

Turkish Journal of Obstetrics and Gynecology (formerly called Türk Jinekoloji ve Obstetrik Derneği Dergisi) is the official peer-reviewed journal of the Turkish Society of Obstetrics and Gynecology and is published quarterly on March, June, September and December.

It is an independent peer-reviewed international journal published in English language since 2014 September. Manuscripts are reviewed in accordance with "double-blind peer review" process for both referees and authors.

The target audience of Turkish Journal of Obstetrics and Gynecology includes gynecologists, obstetricians, urogynecologists, reproductive medicine specialists, gynecological oncologists and primary care physicians interested in gynecology practice. It publishes original work on all aspects of obstetrics and gynecology. The aim of Turkish Journal of Obstetrics and Gynecology is to publish high quality original research articles. In addition to research articles, reviews, editorials, letters to the editor and case presentations are also published.

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Instructions for authors page of the journal is available in the journal content and at www.tjoddergisi.org

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The "Turkish Journal of Obstetrics and Gynecology" is the official publication of the Turkish Society of Obstetricians and Gynecologists. The journal is published quarterly (March, June, September and December) in English and publishes original peer-reviewed articles, reviews, case reports and commentaries in the fields of gynecology, gynecologic oncology, endocrinology and reproductive medicine and obstetrics. The journal gives publication priority to original research articles over case reports. Reviews are considered for publication only if they are prepared by authors who have at least three published manuscripts in international peer-reviewed journals on the topic of the review and these studies should be cited in the review. Otherwise only invited reviews will be considered for peer-review from qualified experts in the area.

The "Turkish Journal of Obstetrics and Gynecology" is a peer-reviewed journal and adheres to the highest ethical and editorial standards. The editors also adhere to the Committee on Publications Ethics (COPE) recommendations (http://publicationethics.org).

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appropriately investigated and resolved. The statement about the authors' contributions should be placed in the cover letter. All persons who contributed to the work, but not sufficiently to be authors, must be acknowledged.

Cover Letter

Cover letter to the editors addressing the following points:

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· Verification that the manuscript is not under consideration elsewhere, and indication from the authors that it will not be submitted elsewhere until a final decision is made by the editors of Turkish Journal of Obstetrics and Gynecology.

· The declaration of transparency from the corresponding author.

· Clinical trial registration, if applicable.

· Institutional review board (IRB) approval or exemption.

· Informed consent.

· Any explanations related to reporting guidelines.

· The statement about the authors' contributions.

Preparation of Manuscripts

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CONSORT statement for randomized controlled trials (Moher D, Schultz KF, Altman D, for the CONSORT Group. The CONSORT statement revised recommendations for improving the quality of reports of parallel group randomized trials. JAMA 2001; 285: 1987-91) (http://www.consort-statement.org/),

PRISMA for preferred reporting items for systematic reviews and meta-analyses (Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 2009; 6(7): e1000097.) (http://www.prisma-statement.org/),

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).

CARE guidelines are designed to increase the accuracy, transparency, and usefulness of case reports. (Gagnier JJ, Kienle G, Altman DG, Moher D, Sox H, Riley D; the CARE Group. The CARE Guidelines: Consensus-based Clinical Case Reporting Guideline Development.) (http://www.care-statement.org/)

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The editors reserve the right to reject manuscripts that do not comply with the above mentioned requirements. The author will be held responsible for false statements or for failure to fulfill the above mentioned requirements.

Authors must provide statement on the absence of conflict of interests between authors and provide authorship contributions and declare if any financial/material support.

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All manuscripts must be submitted as Microsoft Word (.doc or .docx) files. All manuscript pages (including references, tables, and figure legends) must be double-spaced. Use a standard, 12-point typeface such as Times New Roman. Top, bottom, and side margins should be set at 1 inch. Authors must include the following in the manuscript file:

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Title Page

A separate title page should list;

-The manuscript title, which should contain no more than a total of 100 characters (counting letters and spaces) and should not be declarative; do not use abbreviations or commercial names in the title.

- 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.

Several types of articles can be submitted for publication in Turkish Journal of Obstetrics and Gynecology: Original research, case reports, systematic reviews, current commentaries, procedures and instruments, and letters. Stated word counts and page limits were shown in Table 1. Copyright transfer forms, the cover letter, and figures do not contribute to the page limits.

Table 1. Manuscript length at a glance

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

Conclusion

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

Conflict of Interest

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:

Introduction, Case Report, Discussion and References.

References

References are numbered (Arabic numerals) consecutively in the order in which they appear in the text (note that references should not appear in the abstract) and listed double-spaced at the end of the manuscript. The preferred method for identifying citations in the text is using within parentheses. Use the form of the "Uniform Requirements for Manuscripts" (http://www.icmje.org/about-icmje/faqs/icmje-recommendations/). If number of authors exceeds seven, list first 6 authors followed by et al.

Use references found published in peer-reviewed publications that are generally accessible. Unpublished data, personal communications, statistical programs, papers presented at meetings and symposia, abstracts, letters, and manuscripts submitted for publication cannot be listed in the references. Papers accepted by peer-reviewed publications but not yet published ("in press") are not acceptable as references.

Journal titles should conform to the abbreviations used in "Cumulated Index Medicus".

Examples

Journals; Zeyneloglu HB, Onalan G. Remedies for recurrent implantation failure. Semin Reprod Med 2014;32:297-305.

Book chapter; Ayhan A, Yenen MC, Dede M, Dursun P, Gultekin M. How to Manage Pre-Invasive Cervical Diseases? An Overview. In: Ayhan A, Gultekin M, Dursun P, editors. Textbook of Gyneaecological Oncology. Ankara, Turkey: Gunes Publishing; 2010. p. 28-32.

Book; Arici A, Seli E. Non-invasive Management of Gynecologic Disorders. In: Arici A, Seli E (eds). London: Informa Healthcare; 2008.

Tables and Figures

Tables should be included in the main document after the reference list. Color figures or gray-scale images must be at minimum 300 DPI resolutions. Figures should be submitted in "*.tiff", "*.jpg" or "*.pdf" format and should not be embedded in the main document. Tables and figures consecutively in the order they are referred to within the main text. Each table must have a title indicating the purpose or content of the table. Do not use internal horizontal and vertical rules. Place explanatory matter in footnotes, not in the heading. Explain all abbreviations used in each table in footnotes. Each figure must have an accompanying descriptive legend defining abbreviations or symbols found in the figure. If photographs of people are used, the subjects must be unidentifiable and the subjects must have provided written permission to use the photograph. There is no charge for color illustrations.

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Units of measurement should be in Systéme International (SI) units. Abbreviations should be avoided in the title. Use only standard abbreviations. If abbreviations are used in the text, they should be defined in the text when first used.

Revisions

Revisions will be sent to the corresponding author. Revisions must be returned as quickly as possible in order not to delay publication. Deadline for the return of revisions is 30 days. The editorial board retains the right to decline manuscripts from review if authors' response delays beyond 30 days. All reviewers' comments should be addressed a revision note containing the author's responses to the reviewers' comments should be submitted with the revised manuscript. An annotated copy of the main document should be submitted with revisions. The Editors have the right to withdraw or retract the paper from the scientific literature in case of proven allegations of misconduct.

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LETTER FROM THE PRESIDENT

Dear TJOD Family, colleagues and My Country;

On February 6, we woke up to a cold morning that tore our hearts out. After the earthquake with a magnitude of 7.7 in Kahramanmaraş and the second earthquake with a magnitude of 7.4 that occurred in Elbistan approximately 9 hours after this earthquake, we went into a deep sadness, life almost stopped. This earthquake storm, which affected Kahramanmaraş, Malatya, Adıyaman, Diyarbakır, Gaziantep, Hatay, Kilis, Osmaniye, Şanlıurfa and Adana, actually deeply upset all of Turkey and broke our hearts. I would like to state that we are in deep mourning as the country and our community. I wish Allah's mercy to all our citizens who passed away and lost their relatives after this great disaster, and I would like to express my wishes to all our people who were injured and have financial loss.

As the TJOD family, we organized the day after the earthquake and came together with the branch heads of our 11 provinces, where the state of emergency was declared, to draw up a list of the needs in the region, and the necessity of gynecologists and obstetricians, especially in terms of providing health services, was evaluated. The housing problem, which is the most important problem of our colleagues working in the earthquake area and health workers, was tried to be solved with the containers provided by the Turkish Gynecology and Obstetrics Association, these containers were transported to the region by us and delivered to those in need.

The March issue of the Journal of the Turkish Society of Gynecology and Obstetrics was published under these conditions. I would like to personally thank all of our employees who contributed.

Dear colleagues;

We should not forget that science is an ongoing process. Therefore, the faster we heal our wounds, the more visionary we need to act in realizing our scientific studies and scientific activities. In this context, I would like to state that we continue to work devotedly for the 20th National Gynecological and Obstetrics Congress, which will be held in Cyprus on May 17-21, 2023.

I wish to meet you in the June issue of our magazine, happier, more hopeful, but always in the light of science.

Bulent Tiras, Prof. MD
President of TJOD

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EDITORIAL

Dear Collegeaus,

We are very happy and honored to be in front of you, our esteemed gynecologists and obstetricians, with the March issue of the brand new and richly scientific Turkish Journal of Obstetrics and Gynecology, the first issue of 2023. In this issue, there are 8 clinical studies, 2 reviews, a meta-analysis and a letter to editor.

While the March issue is being prepared, we convey our wishes for God's mercy to our citizens who lost their lives and their relatives after the earthquake storm that occurred on the morning of February 6 and is defined as the disaster of the century, and we wish our injured and financially damaged citizensto get well soon. I would also like to state that we, as the whole magazine team, continued our work with our broken hearts and tears in our eyes after this great disaster we experienced while preparing this issue.

We would like to invite all our colleagues to the 20th National Gynecology and Obstetrics Congress, which will be held in Cyprus on 17-21 May 2023, in these days when we heal our wounds after the earthquake that took place in Kahramanmaraş on February 6th.

Believing that we will be stronger together, lets have a more beautiful and scientific future...

Ercan Yilmaz, Prof.MD
Fatih Sendag, Prof.MD
Editors in TJOG

Clinical Investigation / Araştırma

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Turk J Obstet Gynecol 2023;20:1-7

Antenatal azithromycin to prevent preterm birth in pregnant women with vaginal cerclage: A randomized clinical trial

Vajinal serklajlı gebelerde preterm doğumu önlemek için antenatal azitromisin: Randomize bir klinik çalışma

Rania Hassan Mostafa Ahmed, Hassan Awwad Bayoumy, Sherif Ahmed Ashoush, Wessam Kamal Lotfy Gabr

Department of Gynecology and Obstetrics, Faculty of Medicine, Ain Shams University, Cairo, Egypt

Abstract

Objective: To assess whether antenatal azithromycin given to pregnant women with vaginal cerclage can reduce preterm birth or not.

Materials and Methods: We randomized 50 pregnant ladies who underwent cerclage at Ain Shams University Maternity Hospital in group A (receiving 500 mg Azithromycin oral tablets (Zithrokan®, Hikma, Egypt) one tablet orally twice daily for three days in 3 courses at 14th, 24th and 32nd week, plus usual antenatal care) and an identical group B (receiving usual antenatal care). Our primary outcome was gestational age at delivery, and secondary outcomes were birthweight, mode of delivery, and maternal, and perinatal complications. This study was registered on ClinicalTrials.gov with number: NCT04278937.

Results: Pregnancy was more prolonged in the Azithromycin group (delivery at 36.8 weeks vs 34.1 weeks; p=0.017). Also, a higher birthweight was observed in the Azithromycin group (2932.6 gm vs 2401.8 gm; p=0.006). No significant difference was found between the two groups as regards to other outcomes (miscarriage, stillbirth, neonatal intensive care unit admission, antepartum hemorrhage, postpartum pyrexia, need for blood transfusion).

Conclusion: Adding antenatal azithromycin to women undergoing cerclage prolongs pregnancy and reduces the risk of preterm birth, with a slight increase in birthweight.

Keywords: Azithromycin, birth weight, cerclage, cervical, premature birth

Öz

Amaç: Bu çalışmanın amacı vajinal serklaj uygulanan gebelere antenatal azitromisinin erken doğumu azaltıp azaltamayacağını değerlendirmektir.

Gereç ve Yöntemler: Ain Shams Üniversitesi Doğum Hastanesi'nde serklaj yapılan 50 hamile kadını grup A'ya [500 mg Azitromisin oral tablet (Zithrokan®, Hikma, Mısır) günde iki kez üç gün boyunca 14., 24. ve 32. haftalarda rutin doğum öncesi bakım ile birlikte uygulandı] ve serklaj yapılan 50 hamile kadın rutin doğum öncesi bakımın uygulandığı grup B'ye randomize edildi. Birincil sonlanım doğumdaki gebelik yaşı idi ve ikincil sonlanımlar doğum ağırlığı, doğum şekli, maternal ve perinatal komplikasyonlardı. Bu çalışma ClinicalTrials.gov'da NCT04278937 numarasıyla kayıtlıdır.

Bulgular: Azitromisin grubunda gebelik süresi daha uzundu (36,8 haftaya karşılık 34,1 hafta; p=0,017). Ayrıca Azitromisin grubunda daha yüksek doğum ağırlığı gözlendi (2932,6 g'ye karşılık 2401,8 g; p=0,006). Diğer sonlanımlar (düşük, ölü doğum, yenidoğanın yoğun bakıma yatış, antepartum kanama, postpartum pireksi, kan transfüzyonu ihtiyacı) açısından iki grup arasında anlamlı fark bulunmadı.

Sonuç: Serklaj uygulanan kadınlara antenatal azitromisin eklenmesi gebeliği uzatır, doğum ağırlığında hafif bir artış sağlar ve erken doğum riskini azaltır.

Anahtar Kelimeler: Azitromisin, doğum ağırlığı, serklaj, servikal, erken doğum

PRECIS: We concluded that adding azithromycin as antenatal prophylaxis in women undergoing vaginal cerclage prolongs pregnancy, with slight increase in birth weight.

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Introduction

Preterm birth (PTB) is defined as any birth occurring before completed 37 weeks gestation[1]. It represents an estimate of 10.6% of livebirths worldwide; of which, more than 80% were born in South Asia and Sub-Saharan Africa[2]. Complications of PTB are the main cause of mortality in children under 5 years of age, responsible for approximately 1 million deaths in 2015; most of which could be prevented with current, cost-effective interventions[3]. These interventions include cervical cerclage, tocolytic, progestational agents, and infection treatment or prophylaxis[4].

It's now been established that cervical cerclage reduces PTB in women at a high risk of PTB[5]. National Institute for Health and Care Excellence (NICE) guidelines recommend choosing either vaginal progesterone or cervical cerclage as prophylaxis for women at risk of PTB with a transvaginal ultrasound performed between 16 and 24 weeks of pregnancy revealing a cervical length of less than 25 mm[6].

Amniocentesis-proved intra-amniotic infection was observed in 8-52% of patients with cervical insufficiency, and these patients have poor pregnancy outcomes even if cerclage was done[7]. Furthermore, cervical insufficiency is associated with intra-amniotic inflammation, sometimes with no identifiable microorganisms, caused by alarming signals that elicit an intra-amniotic inflammatory response leading to early preterm delivery, neonatal complications, and maternal morbidity[7]. Antimicrobials, mainly macrolides, may be used against vaginal flora colonizers (Ureaplasma species, Chlamydia trachomatis, and Mycoplasma hominis) to prevent amniotic fluid infection and subsequent inflammation cascade, thereby lowering the risk of preterm labor[8]. Also, macrolides have immunomodulatory properties, suppressing intra-amniotic inflammation by downregulating the expression of proinflammatory transcription factors that induce the production of proinflammatory cytokines[7].

This study aimed to explore the value of adding azithromycin as an antibiotic prophylaxis in preventing PTB in pregnant women who underwent vaginal cerclage at Ain Shams University Maternity Hospital (ASUMH).

Materials and Methods

This randomized clinical trial was conducted in ASUMH, Cairo, Egypt over the period of one year (April 2019-April 2020). The study protocol was approved by the ethical committee, the Faculty of Medicine, Ain Shams University (FMASU 1304/2019). The study was conducted in accordance with the Declaration of Helsinki and registered at ClinicalTrials.gov (NCT04278937). Informed consent was obtained from all participants before recruitment in the study. All data were collected confidentially.

We included 20-35-year-old pregnant ladies who had vaginal cerclage (history- or ultrasound-based) performed at ASUMH. We excluded patients with multiple pregnancy, current or past medical disorders, structural fetal anomalies, allergy to azithromycin, and bacterial vaginal infection, as detected by high vaginal swab, before cerclage. McDonald cerclage was the procedure adopted at our institution. Under general anesthesia, with the patient in the Trendelenburg position, a reinforcing purse-string suture was placed around the proximal cervix, using the Mersilene suture. After tightening leaving the cervical canal open 3-5 mm, the knot was tied posteriorly. The patients were usually discharged on the same day, with instructions to avoid excess physical effort.

We randomized eligible women using a computer-generated sequence 1:1 using MedCalc© version 13 either to Azithromycin group (group A) or non-Azithromycin group (group B). Allocation and concealment were performed using sealed opaque envelopes. Every woman was requested to pull out an envelope, and with the letter within she was allocated to either: Group A: Women received azithromycin 500 mg (Zithrokan®, Hikma, Egypt) one tablet orally twice daily for three days in 3 courses at 14th, 24th and 32nd week, in addition to routine antenatal care; group B: women received usual antenatal care without antibiotic prophylaxis after cerclage. Follow-up through antenatal care was done at 4 weeks-interval till 28 weeks of gestation, and then fortnightly till delivery. Women were subjected to: History taking (with special comment on pain, bleeding, or offensive vaginal discharge); examination (with special comment on signs of infection as fever and tachycardia, and investigations as routine labs (complete blood count and urinalysis) and ultrasound. Although the Society of Maternal and Fetal Medicine recommends giving 17-alpha-hydroxy-progesterone caproate 250 mg intramuscularly weekly to women with short cervical length and history of PTB in addition to cerclage; in our study, we adopted the NICE guidelines, which recommend choosing either vaginal progesterone or cervical cerclage as prophylaxis to those cases[6]. In this study, we selected women undergoing cerclage.

Our primary outcome was gestational age at delivery; whereas the secondary outcomes included birthweight, neonatal intensive care unit (NICU) admission, stillbirth, miscarriage, hospital-stay, antepartum hemorrhage, postpartum pyrexia, need for blood transfusion, and maternal intensive care unit admission.

To decrease the risk of bias, the observer collecting the data was blinded as regards whether the patients were the Azithromycin group or non-Azithromycin group.

Sample Size Justification

Sample size was calculated using STATA® program, setting alpha error at 5%, power at 80% and drop-out rate at 10%. Previous results, from the study by Illia et al.[9], showed that only 5.7% of the women in the azithromycin group had low and very low birthweight newborns, as compared to 45.6% of the non-azithromycin group. Hence, the required total sample size was calculated to be 50 women.

Statistical Analysis

The collected data were revised, coded, tabulated, and introduced to a PC using (SPSS 20.0.1 for windows; SPSS Inc, Chicago, IL, 2001). Quantitative variables were expressed as mean and standard deviation, or median and interquartile range according to the distribution of data. Qualitative variables were expressed as frequencies and percentages. Student t-test and the Mann-Whitney test were used to compare a continuous variable between the two groups. The chi-square test and Fishers' exact test was used to examine the relationship between categorical variables. A p-value <0.05 was considered statistically significant. Intention-to-treat analysis was employed.

Results

Eighty-four pregnant women who underwent vaginal cerclage at ASUMH were recruited and assessed for eligibility; of which, 50 women were enrolled and randomized into two groups: Azithromycin group (n=25) and non-azithromycin group (n=25). The process of recruitment and follow-up of the study population are shown in the CONSORT diagram (Figure 1).

A summary of the baseline demographic characteristics, and obstetric history of the two groups being studied is shown in Table 1. The two groups were comparable regarding the baseline characteristics: age, body mass index, parity, and obstetric history.

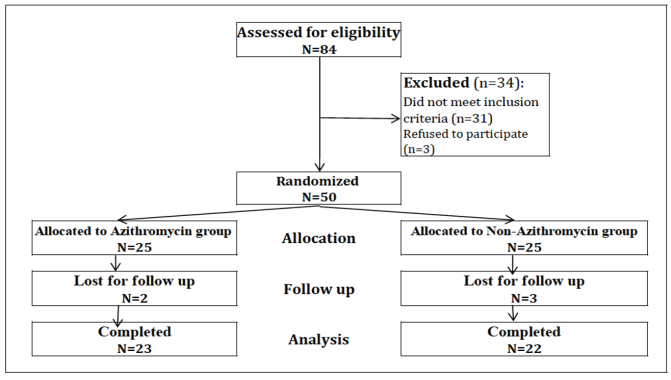

Figure 1. CONSORT flowchart

At enrollment, both groups had a mean gestational age of 13 weeks; however, upon follow-up, pregnancy was more prolonged in the Azithromycin group (delivery at 36.8 weeks vs 34.1 weeks; p=0.017), with significant prolongation of pregnancy in the Azithromycin group (23.7 weeks vs 21.1 weeks; p=0.005) (Table 2).

Regarding birthweight, it was significantly higher in the Azithromycin group (2932.6 gm vs 2401.8 gm; p=0.006). Although there were fewer babies with low birthweight (<2.500 gm) in the Azithromycin group (3 vs. 7 babies in non-azithromycin group), this difference was not statistically significant (p=0.157), as in Table 3.

There was no significant difference between the two groups as regards to mode of delivery. Ten women in the Azithromycin group were delivered by cesarean section, as compared to 12 women in the non-azithromycin group (p=0.569).

Two pregnancies (8%) in non-azithromycin group ended in miscarriage, and two (8%) ended with a stillbirth, whereas there were no miscarriages or stillbirths in the Azithromycin group. Also, there was no significant difference between the two groups as regards to NICU admission and duration of NICU stay; however, we should consider that 4 cases in the non-azithromycin group was not NICU admitted as they were losses (2 miscarriages and 2 stillbirths) (Table 4).

Maternal complications (antepartum hemorrhage, blood transfusion, intensive care unit admission, and postpartum pyrexia) did not differ significantly between the two groups, as shown in Table 5. Postoperative hospital-stay (in days) was significantly shorter among the Azithromycin group (1.2±0.9 vs. 1.9±1.0; p=0.009).

Discussion

This randomized clinical trial evaluated the efficacy of antenatal Azithromycin given prophylactically to reduce PTB in pregnant women who underwent vaginal cerclage in ASUMH. Our results demonstrated that adding azithromycin prolonged pregnancy and reduced the risk of PTB compared to the non-azithromycin group. Additionally, birthweight was significantly higher in the azithromycin group.

Table 1. Demographic characteristics and obstetric history among the study groups

Items		Azithromycin (n=25)	Non-azithromycin (n=25)	p-value
Age (years), mean ± SD		30.0±4.8	29.7±3.9	0.824[a]
BMI (kg/m²)		27.8±1.5	27.4±2.2	0.444[a]
Obstetric history				
Parity, (n, %)	Primiparous	3 (12.0%)	4 (16.0%)	1.000[b]
	Multiparous	22 (88.0%)	21 (84.0%)	
Previous preterm birth		7 (28.0%)	4 (16.0%)	0.306[b]
Previous miscarriage		15 (60.0%)	16 (64.0%)	0.771[b]

[a]: Independent t-test, [b]: Fisher's Exact test, BMI: Body mass index, SD: Standard deviation

Table 2. Gestational age at enrolment and at delivery (weeks) among the study groups

Time	Measures	Azithromycin	Non-azithromycin	p-value
GA at enrolment	**Total**	**25**	**25**	0.626[a]
	Mean ± SD	13.0±0.6	13.0±0.5	
GA at delivery	**Total**	**23**	**22**	**0.017**[a]
	Mean ± SD	36.8±0.9	34.1±4.8	
Prolongation	**Total**	**23**	**22**	**0.005**[a]
	Mean ± SD	23.7±1.0	21.1±4.8	

[a]: Independent t-test, GA: Gestational age, SD: Standard deviation

Table 3. Birthweight (gm) and percentage of low birthweight (<2.500 gms) among the study groups

Measures	Azithromycin (n=23)	Non-azithromycin (n=22)	p-value
Birthweight mean ± SD	2932.6±246.6	2401.8±851.5	**0.006**[a]
	Azithromycin (n=25)	**Non-azithromycin (n=25)**	**p-value**
Low birthweight (n, %)	3 (12.0%)	7 (28.0%)	0.157[b]

[a]: Independent t test, [b]: Chi-square test, SD: Standard deviation

Table 4. Pregnancy fate and neonatal outcomes among the study groups

Fate	Azithromycin (n=25)	Non-azithromycin (n=25)	p-value
Miscarriage	0 (0.0%)	2 (8.0%)	0.490[a]
Stillbirth	0 (0.0%)	2 (8.0%)	0.490[a]
NICU admission	1 (4.0%)	5 (20.0%)	0.189[a]
Duration of NICU stay			
One week	1 (100.0%)	4 (80.0%)	1.000[a]
Two weeks	0 (0.0%)	1 (20.0%)	

[a]: Fisher's Exact test, NICU: Neonatal Intensive Care Unit

Table 5. Maternal complications among the study groups

Complications	Azithromycin (n=25)	Non-azithromycin (n=25)	p-value
Antepartum hemorrhage	0 (0.0%)	4 (16.0%)	0.110[a]
Blood transfusion	0 (0.0%)	1 (4.0%)	1.000[a]
Postpartum pyrexia	1 (4.0%)	2 (8.0%)	--
ICU admission	0 (0.0%)	0 (0.0%)	--

[a]: Fisher's Exact test, ICU: Intensive care unit

Prolonged antibiotic therapy with azithromycin has been previously evaluated in a study by Illia et al.[9]; a prospective study that analyzed pregnancy outcomes in women who had previous perinatal losses due to amnionitis. Pregnant women were treated with prolonged antibiotic therapy (azithromycin 500 mg/day for 3 days; repeated every 10 days and continued till the 34th week of gestation) in a group with cervical cerclage and another group without cerclage. In the group of 35 patients with cerclag, prematurity was decreased from 65.7% to 5.7% (p<0.001)[9].

Also, other studies have used different antibiotic prophylaxis and adjunct therapies with cerclage. A randomized clinical trial evaluated whether indomethacin and antibiotics (cefazolin or clindamycin) given at the time of examination-indicated cerclage resulted in prolonging pregnancy. More pregnancies were prolonged by at least 28 days in patients who received indomethacin and perioperative antibiotics [24 (92.3%) vs. 15 (62.5%), p=0.01]; But there was no notable difference between the two groups in the gestational age at delivery and neonatal outcomes[10].

This contrasted a retrospective observational cohort study by Goyer et al.[11], who evaluated the role of adding azithromycin to the usual treatments (cerclage, tocolysis, rest, etc.) to prolong gestation in patients with intact membranes who were at risk of, or already in preterm labor. They concluded that the median gestational age in the control group was 36 weeks, but 32 weeks in the group receiving azithromycin[11]. However, we must consider that patients in the azithromycin group were more at risk for PTB, having conditions such as: Chorioamnionitis, short cervix, amniotic sludge, bulging membranes, cerclage, or polyhydramnios. However, once adjusted for most confounding factors, prolongation of pregnancy and gestational age at the

event did not differ between the groups, considering that chorioamnionitis could not be excluded or adjusted for. This might explain the earlier age at delivery in the azithromycin group. Also, azithromycin was given as one course, and not as a prophylactic repeated course[11].

Our results showed a higher birthweight among the Azithromycin group (2932.6 gm vs 2401.8 gm; p=0.006). Babies who had low birthweight (<2.500 gm) were non-significantly less frequent in the azithromycin group (3 cases vs. 7 cases; p=0.157). Also, in the study by Illia et al.[9], in the combination of the cerclage and azithromycin group, the number of newborns with low birthweight was non-significantly reduced from 11.4% to 5.7% (p=0.671). However, the percentage of newborns with very low birthweight was significantly reduced from 34.2% to 0 (p<0.001)[9]. An important point to consider when comparing our results with those concluded by Illia et al.[9], is the difference in methodology, and that they compared a group with cerclage to another group without cerclage; therefore, so they evaluated the combined effect of both azithromycin and cerclage.

Azithromycin is an antibiotic of the macrolide group, that inhibits bacterial protein synthesis. It's highly accumulated in cells, especially phagocytes, and thus, it reaches a high concentration at areas of infection and inflammation[12]. Itis a broad-spectrum antibiotic acting against various gram-positive and gram-negative bacteria, and especially obligate intracellular pathogens such as *Chlamydia* species. *Chlamydia* is one of the main causative organisms of cervicitis/urethritis in women, and may cause PTB, preterm rupture of membranes, fetal demise, or endometritis. Azithromycin is highly effective in the treatment of chlamydial cervicitis/urethritis, as well as other non-gonococcal urethritis caused by Ureaplasma urealyticum or *Mycoplasma genitalium*[12]. Another beneficial effect of azithromycin is its immunomodulatory action. Intra-amniotic inflammation has been noted in patients with short cervix and is related to PTB and adverse pregnancy and neonatal outcomes[7]. Late immunomodulatory effects of azithromycin comprise reduction in neutrophil oxidative responses, down-regulation of myeloperoxidase formation, enhanced neutrophil-programmed death, and reducing chemokine [interleukin (IL)-8]- and leukotriene (LT)B4-dependent and chemokine-independent neutrophil chemotactic effects; by inhibition of different transcription factors[12]. Azithromycin also decreases the prostaglandin E2 formation by reducing the expression of prostaglandin synthetic enzymes (COX-1 and COX-2) in leukocytes and monocytes. Azithromycin also decreases tumor necrosis factor-α and granulocyte-macrophage colony-stimulating factor synthesis in monocytes[12].

Other interventions have been investigated previously to reduce PTB; these include using prophylactic antibiotics only without cerclage. Different studies have showed conflicting results. A 2007 meta-analysis to evaluate macrolides (known to be effective against mycoplasma species) on the rate of PTB concluded that erythromycin was associated with a lower risk of premature delivery (odds ratio=0.72; confidence interval 95% 0.56-0.93)[13].

However, another study included 97 women; 51 women were given antibiotics orally (46 received azithromycin and 5 received moxifloxacin), and 46 were not given antibiotics. There was no difference in the median latency from diagnosis to delivery (p=0.47). Neither there was a difference in birthweight (p=0.99). NICU admission was not affected by antibiotic treatment (p=0.08). The average NICU stay did not differ between the treated and untreated groups[14]. These different contradicting results can be explained by different regimens of used antibiotics, different inclusion criteria, and these cases mostly did not have cerclage before starting antibiotic therapy.

In a Cochrane review to assess the benefit of prophylactic antibiotics on maternal and perinatal outcomes during second and third trimester pregnancies, the authors concluded that antibiotic prophylaxis did not decrease the risk of preterm prelabor rupture of membranes or PTB (apart from the subgroup of women with a previous PTB who had bacterial vaginosis); but it decreased the risk of postpartum endometritis, term prelabor rupture of membranes, and gonococcal infection when given routinely to all pregnant women[15].

This was affirmed in another Cochrane review to evaluate the effects of prophylactic antibiotics given to women with PTB and intact membranes, on maternal and neonatal outcomes[16]. That review did not demonstrate any benefit in important neonatal outcomes, although maternal infection may be reduced[16]. However, there were concerns about the short- and longer-term harm for children of mothers treated with antibiotics. The evidence supported not giving antibiotics routinely to women with PTB and intact membranes with no sure signs of infection[16].

More research is needed to evaluate the long-term neurodevelopmental effects of prophylactic antibiotics used during pregnancy. Also, we need more reliable readily available methods to assess subclinical maternal infection, which is still an important factor in the pathogenesis of PTB[16], as intra-amniotic infection is identified in 10% of patients who had PTB[7].

Intra-amniotic microbial invasion could be detected by amniocentesis and cultivation techniques as well as broad-range polymerase chain reaction (PCR) and mass spectrometry[17]. Previous studies have demonstrated microorganisms in the amniotic cavity in 8-52% of pregnant women with cervical insufficiency, and mostly initiate an inflammatory response that predisposes to preterm delivery and neonatal complications[7]. However, "sterile" intra-amniotic inflammation was also evident in patients with preterm labor, where no organism could be detected in the amniotic fluid either by culture or PCR, but analysis of the amniotic fluid showed high concentrations of IL-6. This was observed in the study by Romero et al.[17], where they found that sterile intra-amniotic inflammation was more frequently observed than microbial -associated intra-

amniotic inflammation, but with similar rates of acute placental inflammation and adverse neonatal outcome, and the patients delivered at comparable gestational ages as patients with microbial-associated intra-amniotic inflammation[17].

A study was performed to assess the benefit of antibiotics in treating intra-amniotic infection or intraamniotic inflammation in patients with preterm labor and intact membranes. Treatment of intra-amniotic inflammation or intra-amniotic infection was objectively proven by analysis of amniotic fluid after giving antibiotics in 75% of patients[7]. These findings offer new therapeutic options after personalized evaluation of patients to identify those who can benefit from this intervention[7].

Another potential biomarker of PTB is the amniotic fluid sludge. Evidence suggests that PTB, birth at earlier gestational age, and lower birthweight and NICU admission and neonatal death are increased in patients with amniotic fluid sludge[18]. In addition, another study found that the rates of histological chorioamnionitis with grades II and III were higher when amniotic fluid sludge was present[19]. This simple ultrasound sign might identify patients at risk of PTB who could benefit from adding antibiotics, as proposed in the study by Hatanaka et al.[20]. They concluded that giving antibiotics to high-risk patients with amniotic fluid sludge can effectively reduce the frequency of spontaneous PTB and can increase the birthweight[20].

Study Limitations

Our study has both strength and limitations. To the best of our knowledge, this is the first prospective randomized clinical trial to assess the additional effect of azithromycin in women who already underwent cervical cerclage, with comparable baseline characteristics in both groups. In addition, we reported different neonatal outcomes and maternal complications. However, our study was limited by the small sample size, which might not be large enough to explore whether there is a significant difference in the other outcomes. Also, we did not evaluate the patients' compliance to the drug and its possible side effects. Another important limitation is that we did not assess the presence/absence of intra-amniotic infection/inflammation at the time of delivery, as this was not feasible in the current study. Amniotic fluid culture and analyses of placental chorioamnionitis are not routinely available at our institution; thus definitive diagnosis of intra-amniotic infection/inflammation, or histologic chorioamnionitis couldnot be proved. This would have added more to prove the presumed benefit of using azithromycin.

Conclusion

Using azithromycin as antenatal prophylaxis in women undergoing vaginal cerclage prolongs pregnancy and reduces the risk of PTB, with a slight increase in birthweight. However, there was no clear effect on the incidence of low birthweight, or perinatal morbidity/mortality. Future research is needed to evaluate the long-term neurodevelopmental outcomes of prolonged antibiotic therapy. Also, we must identify a subset of women with subclinical infection who will benefit most out of this therapy.

Ethics

Ethics Committee Approval: The study protocol was approved by the ethical committee, the Faculty of Medicine, Ain Shams University (FMASU 1304/2019).

Informed Consent: Informed consent was obtained from all participants before recruitment in the study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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The expression of stanniocalcin-1, estrogen receptor and progesterone receptor in endometrioid endometrial cancer

Endometrioid endometrial kanserde staniokalsin-1, östrojen reseptörü ve progesteron reseptörü ekspresyonu

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Abstract

Objective: To evaluate the expression of stanniocalcin-1 (STC-1) and to investigate the correlation of STC-1 with expression of estrogen receptor (ER), progesterone receptor (PR) and clinical parameters, histopathological findings and prognostic factors in endometrioid endometrial cancer (EEC).

Materials and Methods: In this retrospective study, STC-1 (cytoplasmic), ER (nuclear), and PR (nuclear) stainings were applied to tissue microarray sections of 89 EEC, 27 endometrial intraepithelial neoplasia (EIN), and 21 normal endometrium (NE). Prognostic factors such as age, tumor size, depth of myometrial invasion, lymphovascular invasion, perineural invasion, and lymph node metastasis were compared with the expression of these markers.

Results: ER showed significantly higher positivity in grade 1 EEC. PR expression was also higher in grade 1 EEC, but these findings were not statistically significant. Strong expression of STC-1 was observed in EIN and EECs compared with NE. STC-1 showed low staining in the NE, and high staining was also noted in the EIN foci adjacent to the NE. STC-1 expression was positively correlated with grade 1 EECs.

Conclusion: STC-1 expression was positively correlated with low histologic grade in EECs. STC-1 can be used for distinguishing low-grade endometrioid tumors and high -grade endometrioid tumors in curretage specimens. Since STC-1 is related to well differentiated tumors, it can also be regarded as a good prognostic factor in EECs.

Keywords: Stanniocalcin-1, estrogen receptor, progesterone receptor, endometrioid endometrial cancer

Öz

Amaç: Bu çalışmanın amacı stanniokalsin-1 (STC-1) ekspresyonunu değerlendirmek ve endometrioid endometrial kanserde (EEK) STC-1 ile östrojen reseptörü (ER), progesteron reseptörü (PR) ekspresyonları ile, klinik parametreler, histopatolojik bulgular ve prognostik faktörlerin korelasyonunu araştırmaktır.

Gereç ve Yöntemler: Bu retrospektif çalışmada 89 EEK, 27 endometrial intraepitelyal neoplazi (EİN) ve 21 normal endometriumun (NE) doku mikroarray kesitlerine STC-1 (sitoplazmik), ER (nükleer), PR (nükleer) boyamaları uygulandı. Yaş, tümör boyutu, myometriyal invazyonun derinliği, lenfovasküler invazyon, perinöral invazyon ve lenf nodu metastazı gibi prognostik faktörler bu belirteçlerin ekspresyonu ile karşılaştırıldı.

PRECIS: Stanniocalcin-1 (STC-1) expression was associated with well differantiated endometriod endometrial cancer. Decreased expression of STC-1 was observed in poor differentiated endometriod endometrial cancer.

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Bulgular: ER, grade 1 EEK'de anlamlı olarak daha yüksek pozitiflik gösterdi. PR ekspresyonu da grade 1 EEK'de daha yüksekti, ancak bu bulgu istatistiksel olarak anlamlı değildi. EİN ve EEK'de NE'ye kıyasla güçlü STC-1 ekspresyonu gözlendi. STC-1, NE'de zayıf boyanma gösterdi ve NE komşuluğundaki EİN odaklarında yüksek boyanma kaydedildi. STC-1 ekspresyonu, grade 1 EEK'ler ile pozitif korelasyon gösterdi.

Sonuç: STC-1 ekspresyonu, EEK'de düşük histolojik derece ile pozitif korelasyon gösterdi. STC-1, küretaj örneklerinde düşük dereceli ve yüksek dereceli endometrioid tümörleri ayırt etmek için kullanılabilir. STC-1, iyi diferansiye tümörlerle ilişkili olduğu için EEK'de iyi bir prognostik faktör olarak kabul edilebilir.

Anahtar Kelimeler: Staniokalsin-1, östrojen reseptörü, progesteron reseptörü, endometrioid endometrial kanser

Introduction

Endometrial cancer (EC) is mostly seen in peri-postmenopausal women. The incidence and mortality rates increase with age[1]. Hormone receptor status has a prognostic role in EC that estrogen receptor (ER) or progesterone receptor (PR) positivity are good prognostic markers[2].

Endometrioid endometrial cancer (EEC) and serous/clear cell endometrial cancers (SEC), show significantly different clinicopathological features[2]. Endometrioid type is mostly associated with overexposure to the estrogen. There are 2 forms of estrogen hormone, alpha and beta. The alpha form was associated with poor survival. Progesterone hormone is used to treat early-stage PR -positive ECs. The progesterone response is low in advanced and recurrent ECs. Progesterone response is an important prognostic indicator in ECs[3]. SECs have a worse prognosis. They are unrelated to estrogen exposure[4].

Increasing the incidence of EC makes it important to forecast the likelihood of recurrence and prognosis after diagnosis to reduce mortality and morbidity. Studies have shown that tumor stage, histological grade, histopathological type, invasion depth, and lymphovascular space involvement status are crucial parameters in predicting recurrence[5,6]. However, it is critical to search for more indicators in predicting prognosis and recurrence.

Stanniocalcin-1 (STC-1), is regarded as a prognostic marker, in predicting the grade and size of tumor and risk of metastasis[7]. STC-1 is a glycoprotein hormone that has a role in cell proliferation, calcium metabolism, programmed cell death, and oxidative stress responses[8].

STC-1 was first detected in humans in 1995[9]. It was determined that ovarian cancers showed higher STC-1 expression than other cancer types[10]. High expression of STC-1 has been detected in hepatocellular carcinoma, thyroid cancer, colon cancer, and lung adenocarcinoma. Conversely, downregulation of STC-1 was shown in cervical cancer[11].

The expression of STC-1 in gynecological cancers has not yet been clarified. In our study, we evaluated the expression of ER, PR and STC-1 expression, which are important in ECs, and clinical, prognostic parameters, histopathological findings.

Materials and Methods

Patients and Tissue Samples

In this retrospective study, tissue samples from 89 EECs, 27 EIN, and 21 normal endometrium (NE) diagnosed between 2011 and 2020 were used. The date of patient age, tumor size, myometrial invasion depth, lymphovascular invasion (LVI), perineural invasion, distant organ metastasis, and lymph node metastasis were obtained from the hospital records.

Since deep myometrial invasion ≥50%[12] and tumor diameter ≥2 cm were previously reported as poor prognostic markers[13], patients were divided into groups using these indicators. Histological grades of EEC, and hematoxylin and eosin (H&E) stained sections of the cases were revised by experienced in gynecologic pathology to verify the diagnosis and differentiate the area most respresentating the tumor.

The histopathological grade of EEC was determined according to the International Federation of Gynaecology and Obstetrics by considering the non-squamous solid areas of the tumor. Grade 1 tumors comprised solid areas 5% or less, grade 2 tumors comprised solid areas between 5 and 50%, and grade 3 tumors comprised more than 50% solid areas[14].

Areas with no necrosis or hemorrhage were considered representative. A manual tissue conditioner was used to manually embed at least two core biopsies into new paraffin blocks from specified tumor regions (Thermo-Labvision, Fermont CA, USA). Each core was 2 mm in diameter. Sections of 5 µm thickness were taken from TMA paraffin blocks. Four slides were cut from all TMA blocks and 1 was used for immunohistochemical staining, and 1 for H&E, to confirm that the correct areas were selected.

The study protocol was been approved by the Ethics Committee of Süleyman Demirel University with the decision numbered 72867572-050.01.04-47478.

Immunohistochemical Staining

ER, PR, and STC-1 markers were immunohistochemically applied to the prepared TMA slides.

STC-1

Anti-STC-1 (rabbit polyclonal antibody, ab229477; Abcam) antibody: Five micrometer tissue sections were deparaffinized for an hour, rinsed with xylene for fifteen minutes, and rehydrated in an alcohol series for twenty minutes after being fixed with 10% formalin at 22 degrees for two days. Sections were blocked for 15 min at 37 °C with 10% goat serum, then incubated with anti-STC-1 primary antibody for 2 h at 37 °C (1: 100), providing endogenous peroxidase inactivation and antigen retrieval. Following this, the sections were incubated for an additional hour at 22 degrees with the kit's included secondary antibody rabbit immunoglobulin G conjugated to horseradish peroxidase. No further rarefaction was necessary.

After 15 min at 37 °C in horseradish enzyme-labeled chain avidin solution, the sections were washed. Later, the proteins were imaged using 3.3'-diaminobenzidine.

Pictures were taken under a microscope with x200 and x400 objectives. When a disagreement occurred between the observers, the final score was decided after a third observer was evaluated. The rate of positively stained cells and the staining intensity -measured as the number of positively stained cells per 100 cells- was used to calculate the IHC score. The rate of staining positive was calculated as follows: The percentage of cytoplasmic staining in the epithelium was evaluated as score 0: 0, score 1: 1-10%, score 2: 11-50%, score 3: 50-75%, score 4: 76% and above. Additionally, staining intensity was manually evaluated in the manner described below: Score 0: Negative/unstained, score 1: Yellow, score 2: Brown, and score 3: Dark brown.

It was calculated in the final quantification by multiplying two scores. The overall score was described as 0 being negative, 1-4 being weak, 5-8 being positive, and 9-12 being strong. The staining intensity and percentage were used to determine the final score. Low STC-1 expression was defined as an IHC value of 5 and below, and high STC-1 expression as a score more than 5 (Figure 1A-D)[15].

ER

ER alpha clone EP1 (rabbit monoclonal antibody, code GA084, Dako Omnis) antibody was ready to use and nuclear staining was considered positive. The evaluation was performed with respect to the method defined by Carcangiu et al.[16]. The evaluation was made according to the percentage of stained cells and the strength of the nuclear stain. The percentage of stained nuclei-positive cells was rated according to the following scale: Score 0: no staining/negative, score 1: 1-25%, score 2: 26-75%, and score 3: More than 76% were considered positive. This is how staining intensity was graded: Score 0: No staining, score 1: Poor; score 2: Strong and score 3: Compelling. The IHC score was calculated by adding both parameters. The IHC score was used to categorize tumors into three groups. Category 1: Corresponded to 2 points, Category 2: 3 or 4 points, and Category 3: 5 or 6 points[16]. Category 1 tumors were considered negative, Category 2 tumors were considered moderately positive, and Category 3 tumors were considered highly positive (Figure 1E-H).

PR

PR, clone PgR 1.294 (mouse monoclonal antibody, code GA090, Dako Omnis) antibody, ready to use, nuclear staining was considered positive. The evaluation was performed with respect to the method defined by Carcangiu et al.[16]. The evaluations was made according to the percentage of stained cells and the strength of the nuclear stain. The percentage of stained nuclei-positive cells was rated according to the following scale: Score 0: No staining/negative, score 1: 1-25%, score 2: 26-75%, and score 3: More than 76% were considered positive. This is how staining intensity was graded: score 0: No staining, score 1: Poor; score 2: Strong and score 3: Compelling. The IHC score was calculated by adding both parameters. The IHC score was used to categorize tumors into three groups.

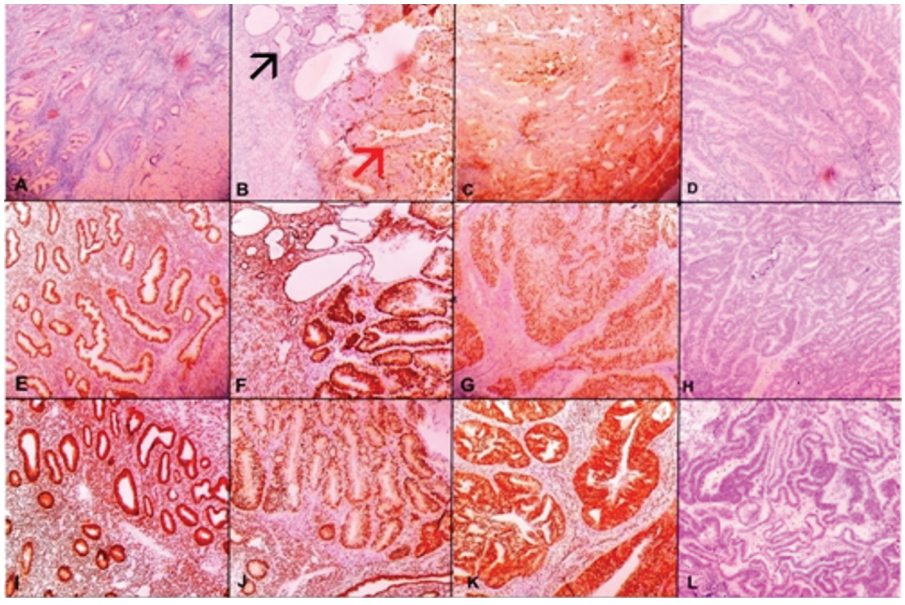

Figure 1. A-D) Staniocalcin-1 (STC-1) cytoplasmic expression [A- normal endometrium (NE) (low staining), B- Low staining was observed in NE (black arrow) foci, while high staining with STC-1 was noted in adjacent endometrial intraepithelial neoplasia (EIN) (red arrow) foci, C- Endometrioid endometrial cancer (EEC) (high staining), D- EEC (low staining), x100], E-H) Estrogen receptor nuclear expression [E- NE (highly positive staining), F- EIN (highly positive staining), G-EEC (highly positive staining), H-EEC (negative staining), x100], I-L) Progesterone receptor nuclear expression [I- NE (highly positive staining), J- EIN (highly positive staining), K- EEC (highly positive staining), L- EEC (negative staining), x100]

Table 1. Clinical and histopathological features of endometrioid endometrial cancer

	Mean ± SD
Age	56.6±9.1
Accompanying myoma/adenoma	n (%) (n=89)
No	53 (59.6)
Myoma	31 (34.8)
Adenoma	3 (3.4)
Myoma + adenoma	2 (2.2)
Tm grade	
Grade 1	47 (52.8)
Grade 2	30 (33.7)
Grade 3	12 (13.5)
Endocervical involvement	
Positive	12 (13.5)
Negative	77 (86.5)
Deep myometrial involvement	
Positive	20 (22.5)
Negative	60 (77.5)
Lymphovascular invasion	
Positive	6 (6.7)
Negative	83 (93.3)
Perineural invasion	
Positive	0 (0)
Negative	89 (100)
Metastatic lymph node	
Positive	3 (3.4)
Negative	86 (96.6)
STC-1 expressions	
Low	53 (59.6)
High	36 (40.4)
Progesterone expressions	
Negative	7 (7.9)
Moderately positive	23 (25.8)
Highly positive	59 (66.3)
Estrogene expressions	
Negative	6 (6.7)
Moderately positive	20 (22.5)
Highly positive	63 (70.8)

Tm: Tumor, STC-1: Stanniocalcin-1, SD: Standard deviation

Category 1: Corresponded to 2 points, Category 2: 3 or 4 points, and Category 3: 5 or 6 points[16]. Category 1 tumors were considered negative, Category 2 tumors were considered moderately positive, and Category 3 tumors were considered highly positive (Figure 1I-L).

As a control, the expression of STC-1, ER, and PR in NEs and cases with EIN and in malignant cases were compared.

Statistical Analysis

The difference between age, gender, STC-1, ER, and PR scores of patients with EEC were compared with the chi-square test. STC-1, ER, PR scores, and tumor grade of patients with EEC were analyzed by the Spearman correlation test. SPSS 21.0 (IBM, Chicago, IL, USA) software was used for statistical analysis, and p-value <0.05 was considered statistically significant.

Results

The mean age was 56.6 in the study population. In 31 (34.8%) cases accompanying leiomyoma was observed. Forty-seven (52.8%) EECs were grade 1, 30 (33.7%) grade 2, 12 (13.5%) grade 3 EECs. Endocervical involvement was observed in 12 (13.5%) patients, and deep myometrial invasion was observed in 20 (22.5%) patients, and 3 (3.4%) patients had metastatic lymph nodes (Table 1). In 89 EEC patients, 19 (21.3%) EINs and 2 (1.05%) endometrial polyps were observed at the time of diagnosis. The invasion to the bladder was detected in 1 (1.1%) patient with grade 3 EEC.

EIN and EECs showed increased ER expression compared to NE (p=0.044). High expression of STC-1 in EIN and EECs compared with NE was noted (p<0.001). Low staining was observed in the NE with STC-1 (Figure 1A), and staining was noted in EIN foci observed adjacent to the NE in Figure 1B, (Table 2). There was no significant difference between EIN and EEC expression of STC-1, ER, and PR (p=0.171, p=0.157 and, p=0.269).

STC-1 expression was significantly high in grade 1 and grade 2 EEC compared to grade 3 EEC (p=0.021) (Table 3). ER was statistically highly positive in grade 1 EEC (p=0.017) (Table 4). Grade 1 EECs have much less LVI than grade 3 tumors (p=0.005). As expected, grade 1 EECs exhibited considerably less myometrial invasion than grade 3 EEC (p=0.006) (Table 4). Patients with grade 2 EECs were statistically significantly older than the patients with grade 1 EEC (p=0.042). Tumor size was significantly higher in patients with grade 3 EEC than in grade 1 EC (p=0.034) (Table 4).

There was a negative association between tumor grade (r=-0.390; p<0.001) and myometrial invasion, but a positive correlation between ER and PR (r=0.559; p<0.001) (r=-0.281; p=0.008).

Discussion

In this study, STC-1, ER, and PR stains were evaluated immunohistochemically in the samples taken from patients

Table 2. Comparision of immunohistochemical findings in NE, EIN, EEC

		NE (n=21)	EIN (n=27)	EEC (n=89)	p*
ER expressions	Negative	0 (0)	1 (3.7)	6 (6.7)	0.044
	Moderately positive	9 (42.9)	2 (7.4)	20 (22.5)	
	Highly positive	12 (57.1)	24 (88.9)	63 (70.8)	
PR expressions	Negative	0 (0)	0 (0)	7 (7.9)	0.566
	Moderately positive	6 (28.6)	7 (25.9)	23 (25.8)	
	Highly positive	15 (71.4)	20 (74.1)	59 (66.3)	
STC-1 expressions	Low	21 (100)	20 (74.1)	53 (59.6)	<0.001
	High	0 (0)	7 (25.9)	36 (40.4)	

*Fisher's Exact test, NE: Normal endometrium, EIN: Endometrial intraepithelial neoplasia, EEC: Endometrioid Endometrial cancer, ER: Estrogen receptor, PR: Progesterone receptor, STC-1: Stanniocalcin-1

with EEC; clinical parameters, histopathological type, and prognostic factors were compared.

Morphological and molecular changes occur in endometrium with the different hormonal status, and physiology has been the target of extensive research to make the pathophysiology more understandable[17]. In our study, the expression of STC-1, ER, and PR evaluation in NE. EINand EEC was compared with the TMA. STC-1 expression in EIN and EECs was higher than NE. Secretory phase endometrium showed increased STC-1 expression in patients undergoing assisted reproductive techniques[18]. Patients with unexplained infertility showed decreased endometrial STC-1 expression according to a paper. Low STC-1 expression in the secretory endometrium period in a woman with endometriosis, and in endometrial pathologies were reported. It is yet unclear how STC-1 expresses itself in diseased and normal settings[17]. In our study, NE was taken as the control group, and additional disease information for the cases was not available. The low expression of STC-1 may also be due to additional diseases present in the cases or the presence of endometrial samples belonging to the similar phases.

EECs are typically observed in the peri-postmenopausal period[14]. Similarly, the mean age of all EECs in our study was 56.6±9.1. Patiens who have grade 1 tumors were younger than the patients that have grade 2 tumors.

EIN is predicted as the precursor of EEC; ER, PR positivity is common in EIN and EECs[14]. In our study, EIN in the different focuses accompanying EECs at the time of diagnosis was 21.3%. EIN and EECs showed significantly higher ER expression compared to the NE. However, PR expression was similar between the groups.

Prognostic factors for EC were reported as stage, tumor grade, histopathological type, myometrial invasion, age, and extrauterine spread in EC are among the criteria determined to predict prognosis[19]. In our study, we compared immunohistochemical markers with the mentioned parameters. Many ECs have been shown to express ER and PR[20]. Possible mechanisms for developing endometrial carcinogenesis include loss of ER and PR expression, and LN involvement are significantly poor prognostic indicators in patients with EEC, and their relationship with prognosis and survival has been qualitatively demonstrated[21,22]. Loss of ER and PR was correlated with more aggressive clinicopathological features[22]. In our study, ER was statistically highly positive in grade 1. Although PR was higher in grade 1 EECs, it was not statistically significant. Metastatic lymph nodes were observed in 3 of 89 patients, as expected in EECs, and since the group distribution was not balanced, comparison with immunohistochemical marker expression was not satisfactory.

The expression of STC-1 can vary between different tissues and in a given tissue section[23]. It is therefore possible that STC-1 functions differently among human tumors[24]. STC-1 is thought to be a promising biomarkers with various biological mechanisms in tumor progression due to increased mRNA levels in peripheral blood in cancer patients[25]. The expression of STC-1 and STC-2, evaluated in patients with laringeal squamous cell carcinoma, can be used for predicting recurrence and metastasis[24]. Another study showed that STC-1 and STC2 increase vascular endothelial growth factor (VEGF) prolonging the lifespan of multiple cancer patients in targeted therapy against angiogenesis mediators such as VEGF[26].

In the TMA study of STC-1 in EEC patients, LVI, deep myometrial invasion, and large tumor size were all associated with loss of STC-1 expression. Higher epithelial expression was observed in grade 1 EECs than in grade 3 EECs. Nuclear staining was not observed. No relationship was found between disease-specific survival and expression, and the effect of STC on prognosis could not be proven. The loss of expression in ECs was shown to be associated with increased recurrence[8]. Similarly in our study, STC-1 expression was significantly low in grade 3 tumors. Grade 1 EECs have much less LVI than grade 3 tumors. And no more relationship was found between other prognostic parameters and STC-1 expression.

In our study, cytoplasmic staining was observed in epithelial areas in tumor cells, and nuclear expression was not observed

Table 3. Comparison of STC-1 with clinical features of patients, histopathological features and ER, PR expression in EEC

	Low expression n (%) (n=53)	High expression n (%) (n=36)	p-value
Age (mean ± SD)	56.6±8.8	56.4±9.7	0.634[a]
Tm dimesions (mean ± SD)	4.9±3.6	4.3±3.5	0.279[a]
Accompanying myoma/adenoma			
No	31 (58.5)	22 (61.1)	0.396[b]
Myoma	20 (37.7)	11 (30.6)	
Adenoma	2 (3.8)	1 (2.8)	
Myoma + adenoma	0 (0)	2 (5.6)	
Tm grade			
Grade 1	28 (52.8)	19 (52.8)	0.021[b]
Grade 2	14 (26.4)	16 (44.4)	
Grade 3	11 (20.8)	1 (2.8)	
Endocervical involvement			
Positive	46 (86.8)	31 (86.1)	0.582[b]
Negative	7 (13.2)	5 (13.9)	
Deep myometrial involvement			
Positive	14 (26.4)	6 (16.7)	0.280[c]
Negative	39 (73.6)	30 (83.3)	
Lymphovascular invasion			
Positive	5 (9.4)	1 (2.8)	0.395[b]
Negative	48 (90.6)	35 (97.2)	
Perineural invasion			
Positive	0 (0)	0 (0)	-
Negative	53 (100)	36 (100)	
Metastatic lymph node			
Positive	1 (1.9)	2 (5.6)	0.563[b]
Negative	52 (98.1)	34 (94.4)	
PR expression			
Negative	6 (11.3)	1 (2.8)	0.211[b]
Moderately positive	11 (20.8)	12 (33.3)	
Highly positive	36 (67.9)	23 (63.9)	
ER expression			
Negative	5 (9.4)	1 (2.8)	0.194[b]
Moderately positive	9 (17)	11 (30.6)	
Highly positive	39 (73.6)	24 (66.7)	

[a] Mann-Whitney U test, [b] Fisher's Exact test, [c] chi-square analysis, Tm: Tumor, ER: Estrogen receptor, PR: Progesterone receptor, STC-1: Stanniocalcin-1, EEC: Endometrioid endometrial cancer, SD: Standard deviation

Table 4. Comparison of tumor grade with clinical histopathological and immunohistochemical expressions findings

	Grade 1 (n=40) n (%)	Grade 2 (n=37) n (%)	Grade 3 (n=12) n (%)	p-value
Age (mean ± SD)	54.4±9.9	58.7±6.3	59.6±2.9	0.042[a]
Tm dimesions (mean ± SD)	4.0±3.6	5.2±3.5	6.2±3.5	0.034[a]
Accompanying myoma/adenoma				
No	26 (55.3)	17 (56.7)	10 (83.3)	0.223[b]
Myoma	18 (38.3)	12 (40)	1 (8.3)	
Adenoma	1 (2.1)	1 (3.3)	1 (8.3)	
Myoma + adenoma	2 (4.3)	0 (0)	0 (0)	
Endocervical involvement				
Positive	7 (14.9)	4 (13.3)	1 (8.3)	0.824[b]
Negative	40 (85.1)	26 (86.7)	11 (91.7)	
Myometrial involvement				
Positive	5 (10.6)	9 (30)	6 (50)	0.006[b]
Negative	42 (89.4)	21 (70)	6 (50)	
Lymphovascular invasion				
Positive	0 (0)	3 (10)	3 (75)	0.005[b]
Negative	47 (100)	27 (90)	9 (75)	
Perineural invasion				
Positive	0 (0)	0 (0)	0 (0)	-
Negative	47 (100)	30 (100)	12 (100)	
Metastatic lymph node				
Positive	0 (0)	3 (10)	0 (0)	0.082[b]
Negative	47 (100)	27 (90)	12 (100)	
Progesterone expressions				
Negative	2 (4.3)	2 (6.7)	3 (25)	0.063[b]
Moderately positive	9 (19.1)	10 (33.3)	4 (33.3)	
Highly positive	36 (61)	18 (60)	5 (8.5)	
Estrogene expressions				
Negative	3 (6.4)	0 (0)	3 (25)	0.017[b]
Moderately positive	7 (14.9)	11 (36.7)	2 (16.7)	
Highly positive	37 (78.7)	19 (63.3)	7 (58.3)	
STC-1 expressions				
Low	28 (59.6)	14 (26.4)	11 (91.7)	0.027[c]
High	19 (40.4)	16 (53.3)	1 (8.3)	

[a] Kruskal-Wallis test, [b] Fisher's Exact test, [c] chi-square analysis, Tm: Tumor, ER: Estrogen receptor, PR: Progesterone receptor, STC-1: Stanniocalcin-1, SD: Standard deviation

for STC-1. No specific staining was observed in stromal cells, and the staining in the epithelium with STC-1 was consistent with other studies[17,27], showing that the main target of STC-1 is the epithelium.

Studies evaluating the expression of ovarian serous carcinoma and STC have shown that expression correlates with tumor grade[28]. In another study, it has been shown that STC plays a role in the aggressive course and metastasis by inducing cellular proliferation in tumors and reducing apoptosis[25]. In our study, STC-1 expression was significantly low in grade 3 EEC patients. The loss of expression in STC-1 was reported to be a poor prognostic factor in cervical cancer[11]. In another study, it was observed that high expression and tumor size were inversely correlated in hepatocellular carcinoma of the liver[29].

It has been shown that hyf-1alpha can participate in the proliferation of ccRCCs by inducing the accumulation of STC-1, down-regulating calcium. Distant organ metastasis, tumor diameter, and STC-1 expression were positively correlated with ccRCC[30].

The explanation on the effect of STC-1 on tumor proliferation may that tumor cells may use STC-1 for their growth.

Study Limitations

TMA is a common method used to predict prognosis in many cancers. In our study, tumor samples were taken from 2 different foci from each tumor and statistical analysis were performed by taking the average of the expressions. However, due to tumor heterogeneity, it may not represent the entire tumor.

Conclusion

STC-1 expression was positively correlated with low histologic grade in EECs. STC-1 can be used for discriminating low-grade EECs and high-grade EECs in curretage specimens. Since STC-1 is related to well-differantiated tumors, it can also be regarded as a good prognostic factor in EECs.

Ethics

Ethics Committee Approval: The study protocol was been approved by the Ethics Committee of Süleyman Demirel University with the decision numbered 72867572-050.01.04-47478.

Informed Consent: Retrospective study.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: G.E., K.K.B., İ.M.Ç., E.E., Concept: R.O.Y, Z.A.K., G.E., Design: G.E., Ş.M.B., İ.M.Ç., K.K.B., Data Collection or Processing: G.E., E.E., K.K.B., İ.M.Ç, R.O.Y., Analysis or Interpretation: G.E., K.K.B., İ.M.Ç, R.O.Y., Literature Search: G.E., Z.A.K., Ş.M.D., Writing: G.E., K.K.B., İ.M.Ç., E.E., Z.A.K., R.O.Y.

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The effects of dydrogesterone treatment on first-trimester aneuploidy screening markers and nuchal translucency in women with threatened miscarriage

Düşük tehdidi olan gebelerde oral didrogesteron tedavisinin ilk trimester anöploidi tarama belirteçleri ve ense kalınlığı üzerindeki etkileri

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Abstract

Objective: To evaluate the effects of dydrogesterone treatment on first-trimester aneuploidy screening markers and nuchal translucency (NT) in women with threatened miscarriage.

Materials and Methods: This study is an prospective case-control study. One hundred seven pregnant women who applied for the first-trimester screening test at 11-14th weeks of gestation were included in the study. The study group consisted of 53 pregnant women using oral dydrogesterone due to the threat of miscarriage for at least 2 weeks and without vaginal bleeding for the last 72 h at the time of enrollment. The control group was composed of 54 healty pregnant women. Fetal Crown-rump length (CRL), NT, pregnancy-associated plasma protein-A (PAP-A) level, and free beta-human chorionic gonadotropin (free B-hCG) levels of the patients were measured.

Results: One hundred seven patients included in the study, 54 (50.46%) were in the control group, and 53 (49.54%) were in the study group using dydrogesterone. Age, body mass index, gravida, parity and abortion numbers, gestational weeks, and CRL values of the two groups were congruent. In the comparison-free B-hCG, PAPP-A and NT values of both groups, no statistically significant difference was found between the two groups in terms of first-trimester test results and NT ($p<0.05$).

Conclusion: The use of dydrogesterone in first-trimester pregnancies does not affect first-trimester screening tests and nuchal translucency.

Keywords: Dydrogesterone, nuchal translucency, threatened miscarriage, prenatal screening tests

Öz

Amaç: Gebelerde düşük tehdidi nedeniyle kullanılan oral didrogesteron tedavisinin ilk trimester anöploidi tarama testi parametreleri ve ense saydamlığı (NT) üzerine etkisini değerlendirmek.

Gereç ve Yöntemler: Bu çalışma prospektif olgu kontrol çalışmasıdır. Çalışmaya 11-14. gebelik haftalarında birinci trimester tarama testi için başvuran toplam 107 gebe dahil edildi. Çalışma grubunu düşük tehdidi nedeniyle en az 2 hafta süreyle oral didrogesteron kullanan ve tedaviden sonra son 72 saatte vajinal kanaması olmayan 53 gebe, kontrol grubunu ise 54 sağlıklı gebe oluşturdu. Katılımcıların demografik ve obstetrik özellikleri kayıt edildi. Fetal tepe popo uzunluğu (CRL), NT, gebelikle ilişkili plazma protein-A (PAPP-A) ve serbest beta insan koryonik gonadotropin (free B-hCG) düzeyleri ölçüldü.

Bulgular: Çalışmaya dahil edilen toplam 107 hastanın 54'ü (%50,46) kontrol grubunda, 53'ü (%49,54) didrogesteron kullanan çalışma grubundaydı. İki grubun yaş, vücut kitle indeksi, gravida, parite ve abort sayıları, gebelik haftaları, CRL değerleri birbiri ile eşlenikti. Her iki grubun free B-hCG, PAPP-A ve NT değerleri ile yapılan karşılaştırmada iki grup arasında ikili test sonuçları ve NT açısından istatistiksel anlamlı fark tespit edilmedi ($p<0,05$).

PRECIS: Although progesterone itself is known as a pregnancy hormone, the safety of exogenous use during pregnancy and its effect on first trimester screening tests have been the subject of many studies.In our study, the effect of first trimester dydrogesterone use on the screening test was examined and it was shown that the speculative hypothesis that it impaired the test was not correct.

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Sonuç: İlk trimester gebelerde didrogesteron kulanılması ilk trimester tarama testi parametrelerini ve ense saydamlığını etkilememektedir.
Anahtar Kelimeler: Didrogesteron, ense saydamlığı, düşük tehdidi, doğum öncesi tarama testi

Introduction

Threatened miscarriage is the most common complication of pregnancy, manifested by vaginal bleeding, while the cervix are closed before 20 weeks of gestation, occurring in approximately one-fifth of pregnant women[1-3]. In addition to its negative social and economic effects, it has an important effect on physical and psychological well-being. Research showed that the level of distress associated with miscarriage can be equivalent to the stillbirth of a full-term baby and causes post-traumatic stress disorder[4]. This situation forces physclinicians to take more stringent prevention measures. Empirically, it is attempted to be treated and prevented using progesterone supplementation, anticoagulation, and/or immunomodulatory therapies[5]. The most common practice is to prescribe progesterone[6,7]. It is now understood that progesterone is necessary for the initiation and maintenance of pregnancy at all stages[8,9]. It has been reported that progesterone deficiency causes miscarriage[10].

From the late 1900s to the present, first-trimester screening tests remain important for fetal chromosomal anomaly evaluation[11,12]. Nuchal translucency (NT) is a hypoechoic region located between the skin and soft tissues behind the cervical spine. This hypoechoic area is presumed to represent mesenchymal edema and is frequently associated with distended jugular lymphatics. In some studies, it has been stated that progesterone may cause abnormal blood flow patterns and therefore an increase in NT, but it does not change the result of the screening test[13,14]. It has been demonstrated that progesterone could cause both rapid dose-dependent relaxation of the placental vascular smooth muscle and the proliferation of cultured human vascular smooth muscle cells of the umbilical vein.

Incorrect evaluation of NT, which is a sensitive marker in Down syndrome screening and is accepted as a component of first-trimester screening tests, may lead to false-positive results. Therefore, the parameters affecting NT measurements should be thoroughly investigated.

Although there are many publications on dydrogesterone, which is structurally and pharmacologically similar to microgenized progesterone, in the prophylaxis of threatened miscarriage and its use in early pregnancy[15], as far as we know, there is no study on its effect on first-trimester screening test components. In our study, we evaluated the effect of dydrogesterone use in the first-trimester on NT, pregnancy-associated plasma protein-A (PAPP-A), and free beta-human chorionic gonadotropin (B-hCG) values, which are the components of the first-trimester aneuploidy screening test[16].

Materials and Methods

Our study is a prospective observational case-control study conducted at University Health Sciences Turkey, Gaziosmanpaşa Training and Research Hospital on first-trimester pregnant women who presented to our pregnant outpatient clinic between November 2022 and January 2023 for first-trimester screening tests. Participants were included in the study by invitation. Written informed consent was obtained from all pants who agreed to participate. The inclusion criteria were age 18-39 years, between 11-13.6 weeks of gestation, and confirmation of a crown-rump length (CRL) of 45 mm to 84 mm in ultrasonographic evaluation. Pregnant women with multiple pregnancies, high blood pressure, gestational diabetes, metabolic disease with vascular involvement, renal failure, and chronic drug use during pregnancy were excluded from the study.

In the gynecological evaluation, pregnancies with closed cervix and vaginal bleeding were diagnosed as a threatened miscarriage. All participating pregnant women were questioned about whether they experienced the threat of miscarriage and vaginal bleeding and therefore used oral dydrogesterone. Participants who used oral dydrogesterone for at least 2 weeks and no-bleeding for the last 72 h were included in the case group. Pregnant women who used dydrogesterone for less than two weeks or whose bleeding still continued after treatment were excluded from the study. The control group was composed of 54 healthy pregnant women with the same criteria as the study group but who did not take exogenous dydrogesterone and without bleeding.

The reason for taking the 2-week period as a criterion is in almost all the studies that can be compared in the literature, oral microgenized progesterone was used and the duration of use was taken as 2 weeks. To make an accurate comparison with other studies in the literature, it was sought to use at least two weeks to ensure that the duration was constant. The reason for seeking a condition of 72 h without bleeding after treatment was to eliminate the possible effect of bleeding on the test. Pregnant women who used any progesterone other than dydrogesterone were excluded from the study.

In the group using dydrogesterone, it was required to have received treatment at a standard dose for at least 2 weeks. The dose recommended by the pharmaceutical company for dydrogesterone treatment was accepted as the standard dose. To standardize the study, daily use of 10 mg orally every 8 h up to 30 mg was accepted as the standard; doses higher than this were considered high doses and excluded from the study. Only oral preparations were included in the study.

The pregnancy histories, medical histories, smoking habits, age, and height information of the patients were questioned, and the information was recorded. Then, measurements of all participants were made using the same ultrasonography (USG) scanner by the same single physician. Gestational age was calculated using the fetal CRL. All ultrasound examinations

were performed with a 4.5-16.5 MHz transabdominal probe or with a 5-9 MHz transvaginal trans-ducer (Mindray DC8 Expert, Wauwatosa). In cases where it was difficult to visualize the fetus [such as with high body mass index (BMI)], the was examined 2-dimensionally (5-9 MHz). Scans were performed transvaginally using a transvaginal probe. Patients with a CRL less than 45 mm and more than 84 mm, the presence of a non-viable fetus, multiple pregnancies, the presence of major serious fetal anomalies such as anencephaly, the presence of spina bifida, and cardiac anomalies were excluded from the study. NT measurements were performed thrice for each patient and the highest value was recorded[15]. Measurements were made in the sagittal plane, whereas the fetus was in a neutral position, with clear separation of the amnion membrane, and after magnification on the USG screen to cover the fetal head and upper thorax. Patients NT values greater than 2.5 mm were excluded (which is roughly equivalent to the 95th percentile[15]. Immediately after USG evaluations, blood samples for PAPP-A and free B-hCG were taken from the patients.

Ethical approval was obtained from the Ethics Committee of University Health Sciences Turkey, Gaziosmanpaşa Training and Research Hospital before starting the study (date: 23/11/2022, no: 145) and was carried out in accordance with the Declaration of Helsinki.

Statistical Analysis

In the power analysis performed before starting the study, it was found appropriate to detect a difference of at least 0.25 (medium level) effect size between the groups, with 80% power and 5% type error, with a total of 106 people, and a minimum of 53 people in each group. The calculation was made using the G*Power 3.1.9.7 program. It was aimed to reach the numbers specified as the study endpoint. The normality of the distribution of continuous variables was evaluated using the Shapiro-Wilk test. Student's t-test was used to compare normally distributed variables, and the Mann-Whitney U test was used for non-normally distributed variables. Spearman Rho correlation coefficients were calculated to examine the linear relationship between continuous variables. Fisher's Exact test was used in the analysis of categorical data. The analysis of the data was performed using the IBM SPSS 21 program.

Results

Of the 107 patients included in the study, 54 (50.46) were in the control group and 53 (49.54%) were in the dydrogesterone group. The demographic characteristics of the participants in the groups are presented in Table 1. There was no statistically significant difference between the two groups in terms of

Table 1. Demographic parameters of groups

	No medication			Medication			
	Mean ± SD	Median (IQR)	Min-max	Mean ± SD	Median (IQR)	Min-max	p
Age (years)	27.93±5.69	27 (23.75-32.25)	18-39	28.21±6.06	26 (24-33.5)	18-40	0.893
BMI (kg/m^2)	25.53±5.89	25.25 (20.91-28.4)	16.73-47.67	25.19±5.03	25.28 (22.48-27.55)	18.34-47.67	0.810
Gravidity	2.43±1.31	2 (1-3)	1-8	2.32±1.31	2 (1-3)	1-8	0.575
Parity	1.07±0.99	1 (0-2)	0-4	1.04±1.04	1 (0-2)	0-4	0.738
Abortion	0.28±0.66	0 (0-0)	0-3	0.21±0.53	0 (0-0)	0-3	0.632
Curettage	0.02±0.14	0 (0-0)	0-1	0.02±0.14	0 (0-0)	0-1	0.989
Ectopic pregnancy	0.06±0.23	0 (0-0)	0-1	0.06±0.23	0 (0-0)	0-1	0.981
Vaginal birth	0.54±0.77	0 (0-1)	0-3	0.42±0.63	0 (0-1)	0-2	0.494
Cesarean	0.52±0.79	0 (0-1)	0-3	0.58±0.89	0 (0-1)	0-3	0.822

p: Mann-Whitney U test, BMI: Body mass index, SD: Standard deviation, IQR: Interquartile range, Min-max: Minimum-maximum

Table 2. NT, PAPP-A, free B-hCG MoM levels of groups

	No medication			Medication			
	Mean ± SD	Median (IQR)	Min-max	Mean ± SD	Median (IQR)	Min-max	p
Free B-hCG MoM	0.9±0.5	0.81 (0.54-1.11)	0.25-3.02	0.83±0.39	0.76 (0.55-1.1)	0.28-2.17	0.654
PAPP-A MoM	1.04±0.66	0.88 (0.62-1.34)	0.3-4.15	0.99±0.78	0.74 (0.61-1.08)	0.35-4.15	0.299
NT MoM	0.91±0.22	0.92 (0.79-1.02)	0.19-1.45	0.95±0.17	0.93 (0.83-1.04)	0.58-1.45	0.241*

p: Mann-Whitney U test, *Student's t-test, NT: Nuchal translucency, MoM: Multiple of the median, PAPP-A: Pregnancy-associated plasma protein-A, SD: Standard deviation, IQR: Interquartile range, Min-max: Minimum-maximum

Table 3. Change of NT, PAPP-A, free B-hCG MoM levels of the groups according to CRL

	No medication			Medication			p
	Mean ± SD	Median (IQR)	Min-max	Mean ± SD	Median (IQR)	Min-max	
Free B-hCG MoM							
45-54 (n=23/17)	0.86±0.4	0.76 (0.51-1.25)	0.25-1.73	0.79±0.41	0.56 (0.49-1.27)	0.29-1.43	0.665
55-64 (n=17/16)	0.74±0.27	0.7 (0.52-0.96)	0.28-1.18	0.77±0.3	0.71 (0.56-0.94)	0.28-1.51	0.723*
65-74 (n=7/7)	1.07±0.5	0.94 (0.74-1.06)	0.72-2.17	0.94±0.57	0.72 (0.62-1.02)	0.43-2.17	0.259
75-84 (n=7/13)	1.28±0.94	1.31 (0.35-1.73)	0.32-3.02	0.89±0.37	0.8 (0.65-1.28)	0.32-1.44	0.438
PAPP-A MoM							
45-54 (n=23/17)	1.2±0.86	1.03 (0.62-1.43)	0.36-4.15	1.4±1.22	0.84 (0.62-1.54)	0.36-4.15	0.935
55-64 (n=17/16)	0.83±0.45	0.65 (0.45-1.18)	0.3-1.65	0.66±0.21	0.73 (0.44-0.74)	0.35-1.04	0.790
65-74 (n=7/7)	0.99±0.19	0.99 (0.82-1.06)	0.76-1.33	0.89±0.37	0.92 (0.46-1.28)	0.39-1.33	0.545*
75-84 (n=7/13)	1.11±0.56	1 (0.6-1.48)	0.57-2.06	0.93±0.34	1.07 (0.61-1.2)	0.43-1.48	0.387*
NT MoM							
45-54 (n=23/17)	1.01±0.15	1.02 (0.91-1.05)	0.76-1.3	1.04±0.14	1.03 (0.92-1.13)	0.89-1.3	0.607
55-64 (n=17/16)	0.83±0.24	0.83 (0.76-0.92)	0.19-1.45	0.93±0.22	0.86 (0.78-1.1)	0.65-1.45	0.326
65-74 (n=7/7)	0.88±0.3	0.92 (0.58-1.02)	0.47-1.38	0.88±0.17	0.92 (0.77-1.02)	0.58-1.05	0.983*
75-84 (n=7/13)	0.77±0.14	0.73 (0.66-0.83)	0.6-1.03	0.89±0.11	0.84 (0.82-1.01)	0.66-1.06	0.943*

p: Mann-Whitney U test, *Student's t-test, NT: Nuchal translucency, MoM: Multiple of the median, CRL: Crown-rump length, PAPP-A: Pregnancy-associated plasma protein-A, SD: Standard deviation, IQR: Interquartile range, Min-max: Minimum-maximum

demographic characteristics, and the groups were conjugate with each other.

The NT, PAPP-A, and free B-hCG multiple of the median (MoM) levels of the groups are shown in Table 2. There were no significant differences in NT MoM levels, maternal serum PAPP-A, and free B-hCG MoM levels between the study and control groups.

Both groups were divided into four subgroups to determine the relationship between NT and dydrogesterone use for specific CRL measurements. CRL in the first, second, third, and fourth groups were 45-54 mm, 55-64 mm, 65-74 mm, and 75-84 mm, respectively. The NT, PAPP-A, and free B-hCG MoM levels of the groups are shown in Table 3. There were no significant differences in NT levels, maternal serum PAPP-A, and free B-hCG levels between the study and control groups for all subgroups of CRL measurements.

When examining whether fetal NT measurements were related to maternal and fetal parameters, no significant relationship was found between these parameters and NT MoM values. A negative significant relationship was found between NT MoM and CRL mm only in drug users and non-users ($p<0.05$). This means that as CRL measurements increase, regardless of drug use, the NT MoM value decreases (Table 4).

Table 4. The relationship between fetal nuchal translucency measurements and maternal and fetal parameters

NT MoM		No medication	Medication
Age (years)	r	-0.102	0.061
	p	0.464	0.663
BMI (kg/m^2)	r	-0.086	0.081
	p	0.538	0.562
Gravidity	r	-0.102	0.136
	p	0.463	0.332
Parity	r	-0.192	0.056
	p	0.165	0.693
Abortion	r	0.176	-0.042
	p	0.204	0.767
Vaginal birth	r	-0.016	0.029
	p	0.906	0.837
Cesarean	r	-0.183	0.091
	p	0.184	0.515
CRL (mm)	r	-0.519	-0.418
	p	<0.001	0.002
Free B-hCG MoM	r	-0.077	-0.206
	p	0.578	0.139
PAPP-A MoM	r	0.081	0.048
	p	0.560	0.732

p: Spearman Rho correlation, MoM: Multiple of the median, BMI: Body mass index, PAPP-A: Pregnancy-associated plasma protein-A, NT: Nuchal translucency

Discussion

In our study, we investigated the effects of dydrogesterone treatment on first-trimester aneuploidy screening markers and NT in women with threatened miscarriage. We found similar NT levels in patients who used dydrogesterone because of the

threat of miscarriage and in the control group that did not. We also found similar levels of PAPP-A and free B-hCG between the groups. The use of dydrogesterone in first-trimester pregnancies does not affect first-trimester screening tests and nuchal translucency.

The use of first-trimester screening tests is very common because it is non-invasive. It does not require special training other than NT measurement, it can be performed as soon as the patient is seen in outpatient clinics, and it is still one of our powerful weapons as a screening test. It is of great importance that it is performed meticulously and its accuracy has increased. Therefore, the factors that may affect the parameters should be investigated thoroughly. In the study that was the starting point of our study, Giorlandino et al.[13] were the first to examine the relationship between first-trimester progesterone therapy and the fetus in a total of 3.716 pregnant women and they found that the use of exogenous progesterone increased NT. Moreover, they showed that this increase was independent of maternal age, BMI, smoking, and gestational age. They stated that this increase did not change the results of first-trimester screening tests. However, although they evaluated many progesterone formulations in their studies, dydrogesterone was excluded. Additionally, in the correspondence with Bellver et al.[17] on the subject after these studies, it was stated that the NT increase was only in the 11th week, and it did not occur in the following weeks. Again, in the same correspondence, it was emphasized that different formulations might change the fetal circulation differently and thus affect NT. Based on this criticism, we included pregnant women who used a single preparation for the same period in our study.

In the study by Namlı Kalem et al.[18] in 2018, 121 pregnant women using intravaginal progesterone for assisted reproduction treatment (ART) and 124 healthy pregnant women who did not use progesterone were compared and it was found that NT increased in the progesterone group and this increase was statistically significant. However, in this study, the women who became pregnant spontaneously and who did not use drugs were chosen as the control group and compared with the case group, which became pregnant with assisted reproduction. Whether assisted reproduction pregnancy itself affects NT is unknown. To exclude this factor from our study, we included pregnant women who became pregnant spontaneously and excluded the ART group from the study. In a study by Keçecioğlu et al.[14] that was designed retrospectively in 2016, groups that did and did not receive micronized progesterone were compared and it was shown that oral progesterone treatment increased only NT and free B-hCG values without causing abnormal results in the test result. In their ROC analysis, the area under the curve for NT was found as 0.634, which was distinctive, and a correlation was found between treatment time and NT[14].

In a study conducted in 2021, Karadağ et al.[19] divided case and control groups by dividing women who did and did not use progesterone into subgroups according to their CR, not according to their gestational week. As a result, they found no differences in MoM levels of NT, PAPP-A, and free B-hCG in all CRL groups[19].

In the study of Karaca et al.[20] in 2022, which we found as the most recent in the literature, the participants were divided into three groups according to bleeding and progesterone use, and they found that free B-hCG increased in the group that used progesterone regardless of whether the women had bled, compared with the group that did not use progesterone.

Study Limitations

The main limitation of our study is The small number of patients and not being compared with other progesterone types. New works with other types of progesterone can be detailed. There are no detailed studies on the duration of progesterone use in the literature. New studies can be performed by comparing the duration of use. The other limitation is the dose regimen of dydrogesterone; we gaved all patients orally 30 mg/day dydrogesterone, but the patient weight was different. Although we excluded in the study the patients >30 kg/m^2 BMI, different weights of patients could obtain different dose regimens.

Conclusion

The strength of our study is that the speculative hypothesis that the use of progesterone affects fetal circulation and increases nuchal translucency, thus increasing the risk in first-trimester screening tests, is not true.However, all of these studies were performed either with micronized progesterone or with other progesterone preparations that excluded dydrogesterone. There is no other study in the literature comparing NT, PAPP-A, and free B-hCG levels with the use of dydrogesterone. Oral use is very convenient and preferred compared with vaginal use in women with bleeding. Additionally, because it did not change any parameters, it had no negative effect on the reliability of the first-trimester screening test and the objectivity of the NT value, which provides an advantage in terms of use in first-trimester pregnancies. If our study is supported by studies involving larger numbers of participants, we think that dydrogesterone will come to the forefront during pregnancy because it does not affect first-trimester tests.

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Ethics

Ethics Committee Approval: Ethical approval was obtained from the Ethics Committee of University Health Sciences Turkey, Gaziosmanpaşa Training and Research Hospital before starting the study (date: 23/11/2022, no: 145) and was carried out in accordance with the Declaration of Helsinki.

Informed Consent: Written informed consent was obtained from all pants who agreed to participate.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: E.Y., Concept: E.Y., Design: E.Y., Data Collection or Processing: E.Y., Analysis or Interpretation: E.Y., B.T., Literature Search: E.Y., B.T., Writing: E.Y., B.T.

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Increase in transforming growth factor-β didnot affect trombospondin1 in preeclampsia placentas

Transforming growth faktör-β'daki artış, preeklampsili plasentalarda trombospondin1'i etkilemedi

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Abstract

Objective: The abnormalities of the placental growth process are a theory causing pre-eclampsia. Antiangiogenic factors contributed to it, such as thrombospondin-1 (TSp-1) that could stimulate transforming growth factor-beta (TGF-β), or vice versa. Some research showed that an increase in TGF-β did not always figurized its signaling. Therefore, we conducted a study to examine the TGF-β signaling proteins through its receptors and TSp-1 expression in preeclampsia placentas.

Materials and Methods: This observational study used 33 normal and 33 pre-eclampsia placental storaged samples, for examination of TGF-β and TGF-βR 1 and 2, SMAD2 using ELISA, and SMAD2 and TSp-1 mRNA using the reverse transcription polymerase chain reaction method. Data were analyzed using SPSS version 20.0, normality test by Kolmogorov-Smirnov, and significancy was analyzed using nonparametric Mann-Whitney test, or t-test for parametric, with confidence interval 95%. Spearman correlation was used for non-parametric data, besides the Pearson correlation for parametric data.

Results: Results showed that there were significant differences between preeclampsia and normal placenta in TGF-β, its receptors, SMAD2, and TSp-1 mRNA. Normal-TGF-β=1.19 (0.713-2.051) pg/mg; preeclampsia-TGFB=2.69 (0.906-10.252) pg/mg; p=0.001; normal-TGFBR1=1.025 (0.622-1.402) ng/mg; preeclampsia-TGFBR1=1.223 (0.372-2.553) ng/mg; p=0.004; Normal-TGF-βR2=0.959 (0.644-1.634) pg/mg; preeclampsia-TGFBR2=1.490 (0.775-3.645) pg/mg; p=0.0001; normal-SMAD2=2.087 (1.279-4.300) ng/mg; preeclampsia-SMAD2=3.508 (1.842-22.489) ng/mg; p=0.0001. The SMAD2 mRNA relative expression (Livax) in the normal placenta was=0.71 (0.03-7.25); pre-eclampsia placenta (PE)=0.49 (0.01-40.71); p=0.075, the normal TSp-1 mRNA expression=1.08 (0.09-5.31); PE=0.21 (0.002-24.06); p=0.002. The correlation test showed a strong correlation between TGF-β with TGFBR1 and 2 in the normal placenta, conversely, there was no correlation in the preeclampsia placenta. There was also no correlation between SMAD2 and TSp-1 mRNA in both normal and pre-eclampsia.

Conclusion: TGF-β signaling in the preeclampsia placenta was changed due to the increased of the protein signaling it self without correlation between TGF-β to its receptors and TSp-1 relative expression.

Keywords: Pre-eclampsia placenta, TGF-β, TGF-βRs, SMAD2, and thrombospondin-1

Öz

Amaç: Plasental büyüme sürecindeki anormalliklerin preeklampsiye neden olması bir teoridir. Transforming growth faktör beta'yı (TGF-β) uyarabilen trombospondin-1 (TSp-1) gibi antianjiyojenik faktörler buna katkıda bulunmuştur veya bunun tersi de geçerlidir. Bazı araştırmalar, TGF-β'daki bir artışın her zaman onun sinyalini yansıtmadığını göstermektedir. Bu nedenle, preeklampsili plasentalarda reseptörleri ve TSp-1 ekspresyonu yoluyla TGF-β sinyal proteinlerini incelemek için bu çalışma planlanmıştır.

PRECIS: The levels of TGF-β, TGFBR1 and 2, SMAD2 were increased in the preeclampsia placenta, but conversely the thrombospondin1 mRNA relative expression, it considered that there was no correlation between TGF-β and its receptors.

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Gereç ve Yöntemler: Bu gözlemsel çalışmada, 33 normal ve 33 preeklampsili plasental depolanmış numunede, TGF-β, TGF-βR 1 ve 2 ve SMAD2 düzeyleri ELISA metodu kullanılarak ve SMAD2 ve TSp-1 mRNA transkripsiyonları ters transkripsiyon polimeraz zincir reaksiyonu yöntemi kullanılarak ölçüldü. Veriler SPSS versiyon 20.0 ile analiz edildi. Kolmogorov-Smirnov normallik testi kullanıldı. Anlamlılık, %95 güven aralığı ile, normal dağılmayan verilerde parametrik olmayan Mann-Whitney testi kullanılarak ve normal dağılan verilerde parametrik t-testi kullanılarak analiz edildi. Normal dağılmayan veriler için Spearman korelasyonu, normal dağılan veriler için Pearson korelasyonu kullanıldı.

Bulgular: Preeklampsi ve normal plasenta grupları arasında TGF-β, reseptörleri, SMAD2 ve TSp-1 mRNA ölçümleri açısından farklılıklar saptandı. Normal-TGF-β=1,19 (0,713-2,051) pg/mg; preeklampsi-TGFB=2,69 (0,906-10,252) pg/mg; p=0,001; normal-TGFBR1=1,025 (0,622-1,402) ng/mg; preeklampsi-TGFBR1=1,223 (0,372-2,553) ng/mg; p=0,004; normal-TGF-βR2=0,959 (0,644-1,634) pg/mg; preeklampsi-TGFBR2=1,490 (0,775-3,645) pg/mg; p=0,0001; normal-SMAD2=2,087 (1,279-4,300) ng/mg; preeklampsi-SMAD2=3,508 (1,842-22,489) ng/mg; p=0,0001. Normal plasentadaki SMAD2 mRNA göreceli ifadesi (Livax)=0,71 (0,03-7,25); preeklampsili plasentada=0,49 (0,01-40,71); p=0,075; normal TSp-1 mRNA ifadesi=1,08 (0,09-5,31); preeklampsili plasentada=0,21 (0,002-24,06); p=0,002. Korelasyon testi normal plasentada TGF-β ile TGFBR1 ve 2 arasında güçlü bir korelasyon gösterdi, aksine preeklampsili plasentada korelasyon yoktu. Hem normal plasentalarda hem de preeklampsili plasentalarda SMAD2 ve TSp-1 mRNA ekspresyonu arasında bir korelasyon yoktu.

Sonuç: Preeklampsili plasentadaki TGF-β sinyali, TGF-β, TGF-β'nın reseptörleri ve göreceli TSp-1 ekspresyonu arasında korelasyon olmaksızın, kendi kendine sinyal veren proteinin artması nedeniyle değişmiştir.

Anahtar Kelimeler: Preeklampsili plasenta, TGF-β, TGF-βR'ler, SMAD2 ve trombospondin-1

Introduction

In Indonesia, maternal mortality rate (MMR) is one indicator of success to achieve Sustainable Development Goals in 2030[1]. The MMR in Indonesia was still relatively high, equal to 133 per 100,000 live birth[2]. Among them, 27.1% with hypertension in pregnancy[1]. Maternal hypertension in pregnancy can be caused by the presence of preeclampsia, and characterized by hypertension and proteinuria at over 20 weeks of gestation[3]. Every year there was approximately 10 million pregnant women in the world with preeclampsia and 76,000 cases were death[4]. One theory developed about the cause of preeclampsia was the presence of an ischemic placenta, placental failured to supply oxygen and nutrients that result in a pathophysiological disorder. The failure of the placenta was due to the failure of the placental development process, both in the invasion of trophoblastic cells in the maternal decidua during implantation and spiral artery remodeling. Both of these processes involved many proteins that play a role in angiogenesis, proangiogenesis, and antiangiogenesis[5,6].

One protein that plays a role in angiogenesis in the placenta is TGF-β, a multifunctional signal that activates the intracellular signal transduction pathway by binding to its serine/threonin transmembrane receptor, TGF-β receptor 1 (TGFBR1) and TGF-β receptor 2 (TGFBR2)[7,8]. Some previous studies, TGF-β has been shown to be antiangiogenic and its expression is increased in preeclampsia[9-11]. The association between TGF-β with both of TGFBR1 and TGFBR2 triggers the activation of the SMAD proteins, one of them is the SMAD2, and signaling continues into to the nucleus and plays a role on regulation of gene expression[12,13]. Various tissues demonstrated the role of SMAD2, as an antiantiogenic peptide, it regulates the expression of the thrombospondin-1 (TSp-1) and sFLT-1 genes, an antiangiogenesis factor[14]. Therefore, it is considered that an increase in SMAD2 mRNA expression because of TGF-β signaling via TGF-β receptor 2 failured the placental vascular development in preeclampsia. This study was conducted to observe wether any different expression and correlation of TGF-β, TGFBR1 and 2, SMAD2, and Tsp-1 expression between normal and preeclampsia placentas, also to examine the possibility of TGF-β signal transduction disturbed through SMAD2 relative expression.

Materials and Methods

This observational study was a cross-sectional design, conducted in Molecular Biology Laboratory for Oxidative Stress, Department of Biochemistry and Molecular Biology, Faculty of Medicine, Universitas Indonesia. The samples used were biological material stored as many as 33 normal cases and 33 cases of pre-eclampsia. This research has been approved by the Ethic Committe of the Faculty of Medicine, University of Indonesia, number 0878/UN2.F1/ETIK/2018.

The examination of protein concentration: The first placenta was made to be homogenate. A total of 100 mg of placental tissue was balanced (Sartorius) and placed into a 1.5 mL microtube and then added with 500 µL PBS to be homogenized. After the tissue was completely blended, 500 µL PBS was added again and the homogenization process was resumed. The homogenate was then centrifuged for 10 min at 5.000 g, the supernatant formed was taken for the total protein content measured by spectrofluorophotometer (Varioscan) and compared to the standard curve formula of bovine serum albumin.

Examination of TGF-β and TGF-β protein receptor 1 and 2 levels was performed using the ELISA method. A total of 100 µL of standard solutions of all dilutions and samples were put into the microplate wells, sealed and incubated for 90 min at 37 °C. After that, the sample and standard solutions were removed from the wells, and filled with 100 µL Biotinylated Detection Ab Working Solution that was already diluted 100 times, and then incubated for 60 min at 37 °C. After, the solution was discharged, the well was washed using a 350 mL Wash Buffer (diluted 25 times), and repeated 3 times. In each standard and samples, 100 mL HRP Conjutae Working Solution (diluted 100

times) were put, and incubated for 30 min at temperature 37 °C. Then, after the solution was removed, the wells washed again with 350 µL wash buffer and repeated 5 times. A total of 90 µL Substrate Reagent was added to each well and incubated for 15-30 min to develop color, and the last, reaction was discontinued by adding 50 µL Stop Solution. The target protein concentration was examined by measuring the absorbance at a 450 nm wavelength and the result is calculated using the equation formula of the standard curve to obtain the concentration.

Measurement of SMAD2 and TSp-1 mRNA relative expression by reverse transcription polymerase chain reaction method (RT-PCR) method was performed using Sensifast™ SYBR No-ROX One Step Kit RT-PCR (Meridian Bioscience). Before performing the relative expression, total RNA isolation was measured using a total RNA minikit (Geneaid). The primers used are forward primer and reverse primer: SMAD2 Forward primer: 5'-ACC-GAA-ATG-CCA-CGG-TAG-AA-3', Reverse primer: 5'-TGG-GGC-TCT-GCA-CAA-AGA-T-3'; TSp-1 Forward primer: 5'-AGC-ATG-GTC-CTG-GAA-CTC-AG-3', Reverse primer: 5'-CAG-CTC-ATT-GGC-CAA-CTC-TT-3'; 18s rRNA Forward primer: 5'-AAA-CGG-CTA-CCA-CAT-CCA-AG-3', Reverse primer: 5'-CCT-CCA-ATG-GAT-CCT-CGT-TA-3'.

To obtain the relative expressions of mRNAs, optimization of the primary attachment temperature was prepared. The protocol of mRNA relative expression was in the following steps: Into each well the mix solution was added, it consisted of 10 µL Sensifast™ SYBR No-ROX One Step mix, 0.8 µL forward primer, 0.8 µL reverse primer, 0.4 µL Ribosafe RNase Inhibitor, 0.2 µL Reverse Transcriptase, 4 µL mRNA template, and DEPC-H_2O maximum 16 µL. Each sample was run in triplo and using NTC as a negative control. The samples were then incubated on a qRT-PCR machine (PCR max Ecosains) with the appropriate protocol kit temperature.

The result of one-step qRT-PCR is then analyzed further to determine the relative expression of the gene using the following formula:

$$\text{Relative Expresssion} = 2^{-\Delta\Delta Cq}$$

Statistical Analysis

Statistical analysis was performed using the statistical package for social science (SPSS) software for window version 2.0. All data were tested for their normality using Kolmogorov-Smirnov test. Furthermore, homogeneity tes, the normal and homogeneous data will be presented in mean and standard deviation using the unpaired t-test. Besides, the data that were not normally distributed, the Mann-Whitney test was used. Assessment of the correlation between two variables, the Pearson correlation

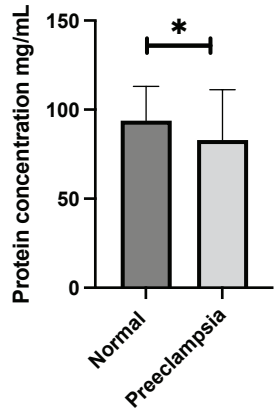

Figure 1. Placental protein concentration

It showed that the protein concentration in the normal placenta was significantly higher that the preeclampsia placenta, p=0.01

Table 1. Data distribution and significances

Placental Markers	Median (min-max)	p#	p$
TGFB-N (ng/mg)	1.19 (0.713-2.051)	0.0001	0.0001*
TGFB-PE (ng/mg)	2.69 (0.906-10.252)	0.0001	
TGFBR1-N (ng/mg)	1.025 (0.622-1.402)	0.0001	0.002*
TGFBR1-PE (ng/mg)	1.223 (0.372-2.553)	0.0001	
TGFBR2-N	0.959 (0.644-1.634)	0.0001	0.0001*
TGFBR2-PE	1.490 (0.775-3.645)	0.0001	
SMAD2-N (ng/mg)	2.087 (1.279-4.300)	0.0001	0.0001*
SMAD2-PE (ng/mg)	3.508 (1.842-22.489)	0.0001	
SMAD2 mRNA-N (ratio)	0.71 (0.03-7.25)	0.0001	0.075
SMAD2mRNA-PE (ratio)	0.49 (0.01-40.71)	0.0001	
TSp-1mRNA-N (ratio)	1.08 (0.09-5.31)	0.0001	0.0001*
TSp-1mRNA-PE (ratio)	0.21 (0.002-24.06)	0.0001	

#: Shapiro-Wilk test; $: Mann-Whitney test; *: Significantly different; N: Normal placenta; PE: Pre-eclampsia placenta, min: Minimum, max: Maximum, TSp-1: Thrombospondin-1

was used for normally distributed data and the Spearman test was used for the data thet were not normally distributed. The significant limit used was p<0.05.

Results

Protein Level of TGF-β

Before calculating the TGF-β concentration, the total protein of the placentas was measured. There was a significant different between normal and preeclampsia placenta protein contents (Figure 1). Measurement of TGF-β using the ELISA sandwich method showed that using the Mann-Whitney test it was proffed a significantly different between the relative expression of normal and preeclampsia markers. TGF-β levels in the preeclampsia placenta was 2.69 (0.906-10.252) ng/mg, it was increased significantly compared with normal placenta, 1.19 (0.713-2.051) ng/mg, p=0.0001, (Table 1, and Figure 2).

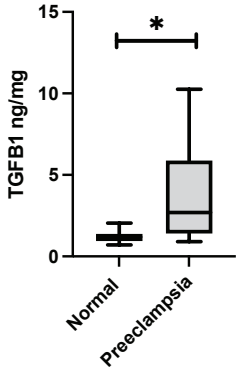

Figure 2. TGF-β protein levels in the placenta of normal pregnancy and pre-eclampsia. There was a significant difference between normal protein TGF-β levels and pre-eclampsia (Mann-Whitney, p=0.0001)

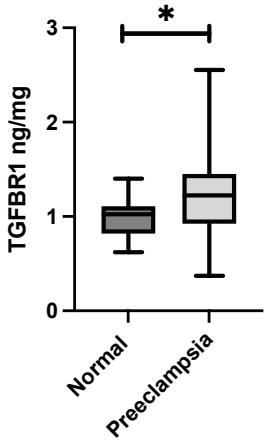

Figure 3. TGFBR1 protein levels in the placenta of normal pregnancy and pre-eclampsia. There was a significant difference between normal protein TGF-β levels and preeclampsia, (Mann-Whitney test, p=0.002)

Protein Level of TGF-β Receptor 1

Measurement of TGF-BR1 showed that its concentration was increased significantly in preeclampsia 1.2239 (0.372-2.553 ng/mg) compared to normal placenta 1.025 (0.622-1.402 ng/mg), p=0.002, (Figure 3 and Table 1).

Protein Level of TGF-β Receptor 2

TGF-β receptors 2 (TGFBR2) protein level were examined using the sandwich ELISA method (Figure 4). It showed that levels of TGF-β receptor 2 in the preeclamptic placenta 1.490 (0.775-3.645) ng/mg, it was higher significantly than normal 0.959 (0.644-1.6340) ng/mg. The Mann-Whitney test results showed a significant difference between the TGFBR2 relative expression of normal and preeclampsia with p=0.0001 (p<0.05), (Table 1).

Relative Expression of SMAD2 mRNA

The SMAD2 mRNA relative expression of preeclampsia was not significantly different compared to normal placentas, (Mann-Whitney test, p=0.075), Figure 5.

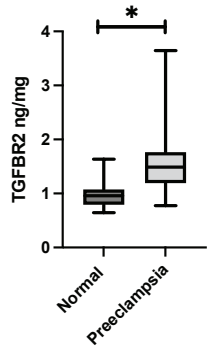

Figure 4. Protein levels of TGF-β receptor 2 in placental tissue normal pregnancy as control and pre-eclampsia. There was a significant difference between TGF-β protein levels. Normal receptor 2 and pre-eclampsia (Mann-Whitney test, p=0.0001)

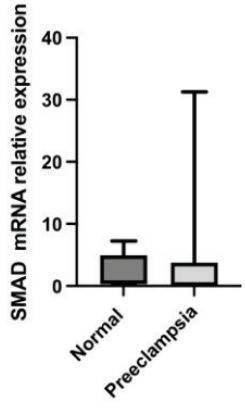

Figure 5. SMAD2 mRNA Relative Expression on normal placenta and pre-eclampsia. There was no significant difference between the relative expression of normal placental SMAD2 mRNA and preeclampsia (p=0.075)

Protein Level of SMAD2

SMAD 2 protein concentration showed that its concentration in the preeclampsia placenta 3.508 (1.842-22.489) ng/mg was significantly higher than normal 2.087 (1.279-4.300) ng/mg, (Mann-Whitney, p=0.0001), (Table 1, and Figure 6).

Thrombospondin mRNA Relative Expression

The relative expression of TSp-1 mRNA was decreased significantly in the preeclampsia placenta compared to normal, (Mann-Whitney, p=0.0001), (Table 1, and Figure 7).

Comparison of Protein Markers Correlation Between Preeclampsia and Normal Placentas

The correlation between TGF-β and TGF-BR1 in the normal placenta was strong, (Spearman correlation, R=0.789, p=0.0001), but in the preeclampsia placenta, there was no correlation between TGF-β and TGF-BR1 in the normal placenta, (Spearman correlation, R=0.053, p=0.76), (Table 2). A similar results shown in the correlation between TGF-β and TGF-BR2. In the preeclampsia placenta, there was no correlation between TGF-β and TGF-BR2 (Spearman correlation R=0.028, p=0.878), but in the normal placenta it showed a strong correlation (Spearman correlation R=0.623, p=0.0001), (Table 2). Correlation between TGF-BR1 and TGF-BR2 showed strong correlation both in normal (Spearman correlation, R=0.799) and preeclampsia placenta, (Table 2).

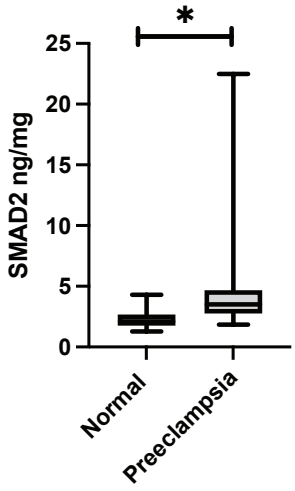

Figure 6. SMAD2 placental protein. There was a significant difference between normal and preeclampsia placenta, (Mann-Whitney, p=0.0001)

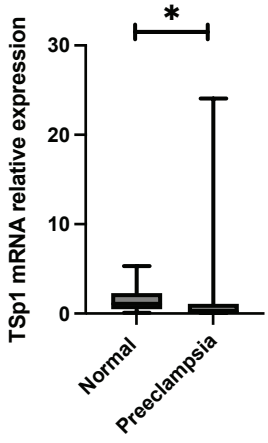

Figure 7. Thrombospondin-1 mRNA relative expression. There was a significant different between TSp-1 mRNA relative expression between preeclampsia and the normal placenta

Mann-Whitney test, p=0.0001.

Discussion

Preeclampsia is a disorder in pregnancy characterized by the presence of hypertension (blood pressure ≥140/90 mmHg) and proteinuria (urinary protein level ≥300 mg/24 h) at gestational age above 20 weeks[15,16]. Preeclampsia has a negative impact on the mother may lead to eclampsia and lead to HELLP syndrome, a set of syndromes characterized by microangiopathy, elevated liver enzymes (liver dysfunction) and low platelets[16,17]. Fetus of preeclampsia, may suffered to retardation during fetal growth, premature birth, low birth weight, and neonatal death[18,19].

There were several developing theories about the pathophysiology of preeclampsia, but in developmental research on pre-eclampsia was focused on the placenta. Placental ischemia in preeclampsia is placental failure in supplying oxygen and nutrients that results in pathophysiological disorders and affects fetal development and maternal health. The failure of the placenta is due to an interruption of the placental development process. In the process of placental development, during the invasion, trophoblasts induce spiral artery remodeling to form a low -resistance vascular system and it is essential for fetal development. This developmental stage is characterized by a physiological change in oxygen pressure at the base of the intervillous region[20].

During the first few weeks of pregnancy, the trophoblast is in a relatively low-oxygen environment. Maternal blood flow to the placenta tends to be low for trophoblast invasion, although this low oxygen pressure state is important for normal embryo and placental development. As soon as the spiral artery remodeling is completed, maternal blood flow to the fetus increases and

Table 2. Correlation between placental TGFB signaling proteins

Groups	Normal		Pre-eclampsia	
	R	p	R	p
TGFB and TGFBR1	0.789	**0.0001***	0.053	0.76
TGFB and TGFBR2	0.623	**0.0001***	0.028	0.878
TGFBR1 and TGFBR2	0.799	**0.0001***	0.783	**0.0001***
TGFBR1 and SMAD2	0.704	**0.0001***	0.675	**0.0001***
TGFBR2 and SMAD2	0.650	**0.0001***	0.539	**0.001***
SMAD2 and TSp-1 mRNA	0.013	0.943	0.151	0.401

*: Spearman correlation, TSp-1: Thrombospondin-1

hypoxia may be discontinued[20]. However, this condition does not occur in the placenta with impaired in trophoblast invasion, such as preeclampsia. Lack of optimum trophoblast invasion, cause the spiral artery remodeling process does not occur properly, leading to a decrease in maternal blood flow to the fetus, and hypoxia in pregnancy continues, leading to preeclampsia[3].

The trophoblastic invasion process involved tissues, multiple cytokines, growth factors, and angiogenesis factors that are mutually coordinated. These molecules interact with each other to provide biological effects, by triggering the transcription of certain proteins. Therefore, failure in coordination and interaction between cytokines and growth factors may impact the disruption of trophoblast invasion that triggers placental abruption. A cytokine that play a role in the development of the placenta is TGF-β. In this study, we found that there was an increase in TGF-β levels of placental pre-eclampsia. This agreed with previous studies suggesting that TGF-β protein levels are higher in placenta preeclampsia because TGF-β has an anti-angiogenesis effect through smad2 but proangiogenesis through smad3[21,22]. Additionally, Extra Villous Trophoblastic cells treated with TGF-β1, TGF-β2, and TGF-β3 showed decreased invasion by decreasing protease activity[22,23].

Increased levels of TGF-β in the placenta pre-eclampsia are also triggered by the presence of placental hypoxia. In a hypoxic state, the cells produce HIF-1 proteins that regulate the transcription of certain genes in response to their environment. This increase in HIF-1 also triggers an increased expression of TGF-β mRNA in trophoblasts[24]. Increased levels of TGF-β by HIF-1 may be intended to trigger angiogenesis to reduce hypoxi; it is considered that TGF-β via another receptor signaling molecule may also be proangiogenic. However, when the state of hypoxia did not goes down, the TGF-β levels increased excessively and eliminated its proangiogenic effec; also, the increased in SMAD2 led to suppress the trophoblast ability to make network[25].

Similar results were found in the examination of levels of TGF-β receptor 2 protein, which were higher in the preeclampsia than in the normal placenta. The interaction of the TGF-β ligand with its receptor triggers both of these receptor types for association, which are then followed by the phosphorylation of type 1 receptors by the type 2 receptors in the domain of the serine-threonin kinase. This activated type 1 receptor followed by passing the signal into an intracellular mediator called SMADs[6]. In line with elevated levels of TGF-β and TGF-β receptor 2 proteins, the relative expression of SMAD2 mRNA in preeclamptic placentas also significantly increased compared to normal placentas. This increase in SMAD2 mRNA expression was not very high compared with elevated levels of TGF-β and TGF-β receptor 2 proteins, it was considered that may be other factors affecting the regulation of SMAD2 mRNA expression.

In this study, the correlation analysis showed a weak positive correlation relationship between TGF-β protein and the relative expression of SMAD2 mRNA in the normal placenta. Conversely, a correlation analysis of both proteins in the preeclamptic placenta groups did not show any correlation. In the other side, a correlation test also performed on TGF-β receptor 2 protein levels with SMAD2 mRNA expression, it also showed no correlation between both parameters, either on normal or pre-eclampsia placentas.

In various studies on the TGF-β signaling pathway in preeclampsia, it mentioned that this protein will bind to both receptors, which can then give a pleiotropic effect: Proangiogenesis or antiangiogenesis[26]. It was also mentioned that TGF-β signaling via TGF-β receptor 1 (ALK5) and TGF-β Receptor 2 phosphorylates the antiangiogenesis SMAD2 protein[27,28]. This suggests that the TGF-β signaling pathway was more influential on the activation of latent SMAD2 protein, compared with its mRNA expression. We consider that any other molecule affects SMAD2 mRNA expression, based on the result that there was no correlation between TGF-β protein levels and their receptor with SMAD2 mRNA expression, presented in this study. Although, both groups of these parameters were increased in the preeclampsia placenta compared with normal.

Study Limitation

There was a limitation in this study due to using storaged placentas that not yet be parafined therefore the immunohystochemistry preparation couldnot be performed to observe TSp-1, and fibrotic area due to the high level of the TGF-β.

Conclusion

TGF-β protein level, TGF-β receptors and SMAD2 were increased in the preeclampsia placenta. Additionally, there was a mild positive correlation between TGF-β protein and relative expression of SMAD2 mRNA in the normal placenta, but was not found in the preeclamptic placenta. There may be other factors contributing to the regulation of SMAD2 mRNA expression.

Ethics

Ethics Committee Approval: This research has been approved by the Ethic Committe of the Faculty of Medicine, University of Indonesia, number 0878/UN2.F1/ETIK/2018.

Informed Consent: Not necessary.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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

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Fetuin B may be a potential marker for predicting maternal and neonatal outcomes in intrahepatic cholestasis: Prospective case-control study

Fetuin B, intrahepatik kolestazda maternal ve neonatal sonlanımı öngörmede potansiyel bir belirteç olabilir: Prospektif olgu kontrol çalışması

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Abstract

Objective: To investigate the levels of serum fetuin B in healthy pregnant women and women with intrahepatic cholestasis of pregnancy (IHCP) and their association with pregnancy outcomes.

Materials and Methods: This was a prospective case-control study, we included sixty singleton pregnant women with IHCP and sixty healthy-matched pregnant women in their third trimester. The serum fetuin B levels of these patients were analyzed. All the patients were followed up prospectively until delivery and data related to maternal, perinatal, and neonatal outcomes were obtained.

Results: Total bile acid levels and liver function tests were significantly higher in the IHCP group than in the control group ($p<0.0001$ and <0.0001, respectively). The serum fetuin B concentrations were higher in the IHCP group than in the control group, without any significant group difference ($p=0.105$). Preterm delivery, iatrogenic preterm delivery, and birth weight ≤2.500 gm are only significantly associated with serum fetuin B levels respectively ($p<0.05$). The diagnostic performance of serum bile acids [area under the curve (AUC)=0.998] was significantly better than that of fetuin B (AUC=0.586) (DeLong's test $p\leq0.001$).

Conclusion: We neither noted a significant difference between the IHCP and control groups concerning the serum fetuin B levels nor could we correlate its levels with adverse maternal and perinatal outcomes except with birth weight, thereby serum fetuin B was not an effective marker for use in shedding light on the pathophysiology of IHCP.

Keywords: Total bile acids, intrahepatic cholestasis of pregnancy, farnesoid X receptor ursodeoxycholic acid, maternal-fetal outcomes

Öz

Amaç: Bu çalışmanın amacı sağlıklı gebelerde ve intrahepatik gebelik kolestazı (IHCP) olan kadınlarda serum fetuin B düzeylerini ve bunların gebelik sonuçları ile ilişkisini araştırmaktır.

Gereç ve Yöntemler: Bu prospektif olgu kontrol çalışmasına, IHCP'li 60 tekil gebeliği olan kadını ve 60 sağlıklı eşleştirilmiş gebeyi üçüncü trimesterde dahil ettik. Bu hastaların serum fetuin B seviyeleri analiz edildi. Tüm hastalar prospektif olarak doğuma kadar takip edildi ve maternal, perinatal ve neonatal sonlanımlara ilişkin veriler elde edildi.

Bulgular: Toplam safra asidi seviyeleri ve karaciğer enzim seviyeleri, IHCP grubunda kontrol grubuna göre anlamlı derecede yüksekti (sırasıyla $p<0,0001$ ve $p<0,0001$). Serum fetuin B konsantrasyonları istatistiksel olarak anlamlı olmasa da IHCP grubunda kontrol grubuna göre daha yüksekti ($p=0,105$). Preterm

PRECIS: We investigated the levels of serum fetuin B in healthy pregnant women and pregnant women with IHCP and its association with pregnancy outcomes in IHCP.

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doğum, iyatrojenik preterm doğum ve ≤2,500 g doğum ağırlığı serum fetuin B düzeyleri ile anlamlı şekilde ilişkili bulunmuştur (p<0,05). Serum safra asitlerinin tanısal performansı [eğrinin altındaki alan (AUC)=0,998], fetuin-B'ninkinden (AUC=0,586) anlamlı olarak daha iyiydi (DeLong testi p≤0,001).

Sonuç: Serum fetuin B düzeyleri açısından IHCP ve kontrol grupları arasında anlamlı bir fark gözlemlemedik ve serum fetuin B düzeylerini doğum ağırlığı dışında olumsuz maternal ve perinatal sonlanımlarla ilişkilendiremedik, bu nedenle serum fetuin B, IHCP'nin patofizyolojisine ışık tutmak için etkili bir belirteç değildir.

Anahtar Kelimeler: Toplam safra asitleri, intrahepatik gebelik kolestazı, farnesoid X reseptörü, ursodeoksikolik asit, maternal-fetal sonlanımlar

Introduction

One of the most prevalent pregnancy-induced liver diseases is intrahepatic cholestasis of pregnancy (IHCP), the incidence of this disorder ranges between 1.5% to 4%[1]. The typical features of this disease are pruritus localized to the palms and soles mostly in the second half of the gestation, with raised bile acid levels (≥10 mmol/L), and/or abnormal transaminase levels. IHCP has a relatively nonthreatening course in women with symptoms, usually, liver enzymes and serum bile acids decrease within a few weeks after birth but it is associated with serious fetal complications[2]. IHCP has a varied impact on both mother and fetus. This can manifest upon the fetus leading to preterm birth, meconium-stained amniotic fluid (MSAF), asphyxia or respiratory distress syndrome (RDS), neonatal intensive care unit (NICU) admission, and in extreme cases non-reassuring fetal status and fetal death[2-6]. It appears to have a genetic linkage where in susceptible women develop altered biliary transport and excretion of bile acids due to elevated levels of reproductive hormones[7].

The metabolism of cholesterol in the liver leads to the production of bile acids. The various actions in the gastrointestinal tract like the metabolism of cholesterol, absorption of fat and fat-soluble vitamins, and regulation of the gut microbiome, are regulated by bile acids. Increased serum bile acid in IHCP leads to abnormal metabolic processes. Various pregnancy-related morbidities like preeclampsia, gestational diabetes mellitus, dyslipidemia, and impaired glucose tolerance are quite prevalent among women with IHCP due to a common pathophysiology[8,9]. The proper regulation of bile acid synthesis plays an enormous role in the prevention of the danger effects of bile acids. Hepatocytes and enterocytes express the Farnesoid X receptor (FXR), a member of the nuclear receptor superfamily this is central to the control of multiple metabolic pathways. FXR on its activation by bile acids regulates the synthesis, conjugation, and transport of bile acids and coordinates various aspects of lipid and glucose metabolism[10]. Fetuin B expression is enhanced by the treatment with an FXR agonist. This expression of fetuin B occurs in human primary hepatocytes and in the human hepatoma HepG2 cell line[11]. Fetuin B belongs to the cystatin superfamily of cysteine protease inhibitors documented to have diverse functions. Women with IHCP express higher levels of fetuin B vis-a-vis healthy pregnant women[12]. Thereby we can infer that FXR agonists in human hepatocytes increase fetuin B gene expression[12,13].

Considering the critically important role of FXR, a regulator of the fetuin B gene expression will take part in the regulation of bile acid homeostasis. The concept of serum fetuin B and its use in IHCP is a very recent idea. Hardly any studies are available in the current literature. Based on this information, we set our primary objective to investigate the serum levels of fetuin B in pregnant women who are in good health and pregnant women diagnosed with IHCP. We kept our secondary objectives to determine the association between serum fetuin B, total bile acids (TBAs), and damaging pregnancy outcomes in IHCP and to compare the diagnostic performance of serum fetuin B with serum TBAs in IHCP cases. Based on our data, we speculate if a relationship between serum fetuin B, serum TBAs, and adverse pregnancy outcomes could be established it would prevent many unfavorable maternal and perinatal outcomes by using serum fetuin B as a predictive marker for IHCP similar to serum TBAs.

Materials and Methods

This was a prospective comparative study, conducted in the Department of Obstetrics and Gynecology of a tertiary care hospital and medical college from January 2019 to December 2021. This study was conducted after the approval of the Institutional Ethics Board and after obtaining informed written consent from the participants. We carried out our research according to the Helsinki Declaration 1975. For this study, we included singleton pregnant patients between ≥28 weeks and 40 weeks of pregnancy presented with serious unexplained persistent pruritus in the absence of any primary skin lesions and/or abnormal liver function test (LFT), alanine aminotransferase (ALT >40 IU/L)/aspartate aminotransaminase (AST >40 IU/L) with serum TBA levels ≥10 micromol/liter, who could fit into the category of obstetric cholestasis as a case group (IHCP) and matched pregnant women in good health were recruited as the control group (non-IHCP).

We excluded pregnant women with any alternative causes like acute fatty liver of pregnancy, chronic liver diseases (symptomatic cholelithiasis, cholecystitis, primary biliary cirrhosis, primary sclerosing cholangitis, hepatitis B, hepatitis C), human immunodeficiency virus infection, skin diseases with itching and rashes, addiction to smoking or alcohol, gestational hypertension or preeclampsia or HELLP syndrome, diabetes mellitus, thyroid disease and women who were in labor during recruitment. These exclusions were based on a detailed history, and clinical examination followed by hepatobiliary ultrasound.

Sample Size

Based on a previous study,[14] the un-adjusted odds ratio for the 3 adverse outcomes that is preterm delivery, stillbirth,

and neonatal intensive care admission, lies between 1.81-4.6, by taking an average odds ratio of 3, since we have no data, assuming exposed control by chance of 50%, the power of the study to a minimum of 80%, alpha error of 5%, we got a total sample size of 120, which was equally divided as 60 each in two groups. In one group 60 consecutive pregnant women diagnosed with IHCP were taken as cases and similarly, age and gestational age-matched 60 pregnant women in good health were included as controls.

From the case controls, demographic characteristics, obstetrics details, gestational age at the time of diagnosis of IHCP, and gestational age at biochemical testing were recorded. At 11-13 weeks, fetal crown-rump length was measured for estimation of the gestation age. Before any start of treatment, blood samples were drawn upon admission to the hospital from women with IHCP. Blood samples were obtained from the age and gestational age-matched control group and were tested for LFT, serum bile acids, and serum fetuin B. All the patients in the IHCP group were given symptomatic treatment along with a tablet of ursodeoxycholic acid (15 mg/kg body weight). Till delivery fetal surveillance was performed on a weekly basis with non-stress tests and fetal Doppler. At 37 weeks of gestation, an elective termination was planned for the patients in the IHCP group and the relevant data were collected. Whereas the patients of the control group were followed up prospectively till delivery and data related to maternal, perinatal, and neonatal outcomes were obtained. To determine the incidence of the disease, the total number of deliveries was also noted during the study period.

The serum biochemical test included were LFTs, total serum bile acids, and serum fetuin B tests, which were determined using routine laboratory methods. A commercial enzyme-based colorimetric assay was used for measuring the serum bile acid levels and a standard manufacturer-recommended protocol was followed. Serum preparation for Fetuin B measurement was done by centrifugation of the samples at 1000 g for 20 min followed by freezing at -80 °C for subsequent analysis. The levels of serum Fetuin B were assessed using a Human FETUB (Fetuin B) sandwich ELISA kit, Wuhan Fine Biotech Co. Ltd (catalogue No. EH3055, Detection range: 93.75-6.000 pg/mL, sensitivity: <56.25 pg/mL). However, the test samples were run in 1:50 dilution and the results were obtained by multiplying with 50 (dilution factor).

The parameters studied were the history of IHCP in a previous pregnancy, gestational age at sampling, serum biochemical test (LFT), TBAs and fetuin B level at diagnosis, gestational age at delivery, mode of termination of pregnancy, spontaneous preterm delivery/iatrogenic preterm delivery, birth weight, pathological cardiotocograph (CTG) immediately before delivery, MSAF at the time of delivery, NICU admission, stillbirth, a composite adverse maternal outcome, and neonatal outcome. The definition of composite adverse maternal outcome is the presence of either of the following events like postpartum hemorrhage, prolonged hospital stay, transfusion of blood and blood product, and maternal death. The definition of composite adverse neonatal outcome is the presence of either of the following events like NICU admission, Apgar 1 and 5 min ≤7, meconium aspiration, hypoglycemia, hyperbilirubinemia, RDS, transient tachypnea of the newborn, use of mechanical ventilation, use of oxygen by nasal cannula, newborn pneumonia, and stillbirth.

Statistical Analysis

Excel spreadsheet Program was used for data recording and coding. The data analysis was performed using the statistical package of social sciences (SPSS) version 23 (IBM Corp.). Descriptive statistics were calculated in the forms of mean ± standard deviation (SD) for continuous variables and frequencies and percentages for categorical variables. A graphical representation of the data was chosen for better visualization. Normally distributed variables were compared between groups. The analysis was done using an independent samples t-test and was expressed as mean ± SD. Non-normally distributed data were analyzed with the Wilcoxon test and the chi-square test for categorical data. Fisher's exact test was used if the expected frequency in the contingency tables was found to be <5 for >25% of the cells. All the normally distributed data were analyzed with Pearson's correlation and non-normally distributed data were analyzed with Spearman's correlation. The correlation between continuous and categorical variables was assessed using Point-Biserial Correlation. To determine the predictive cut-off values of TBAs levels and fetuin B levels, receiver operating characteristic (ROC) analysis was used. P values of less than 0.05 were considered statistically significant.

Results

During the study period, the total number of deliveries conducted was 2501, out of which 60 cases of IHCP were diagnosed in women with singleton pregnancies, which fits into the definition of IHCP and as per the inclusion and exclusion criteria. So, the incidence was found to be 2.3%. The baseline parameters and the laboratory values of the study groups are mentioned (Table 1). The demographic parameters were similar however, the gestational week at the time of delivery, history of IHCP, LFT, and the TBAs were significant between the two groups ($p<0.05$). The mean serum fetuin B level was higher in the case group than in the control group, without any significant difference between them ($p=0.105$).

A comparison of obstetric, perinatal, and neonatal outcomes between the two groups is shown in (Table 2). In this study, we found a significant difference in the labor parameters. Among the perinatal outcomes, MSAF, pathological radiography, and admission to the NICU were found to be significant between the two groups.

We also studied the association between the maternal serum bile acids and fetuin B levels with obstetric, perinatal, and neonatal outcomes in the case group. In this study, iatrogenic

Table 1. The demographic and laboratory parameters of the case and control groups

Parameters	Group		p-value
	IHCP Cases (n=60)	Non IHCP Controls (n=60)	
Age (years)	27.63±4.37	26.73±4.82	0.164[1]
BMI (kg/m^2)	24.30±4.13	24.71±3.88	0.385[1]
Gravida			0.577[3]
Primigravida	34 (56.7%)	37 (61.7%)	
Multigravida	26 (43.3%)	23 (38.3%)	
POG at blood sampling (weeks)	35.42±2.35	35.08±2.37	0.510[1]
Past H/O IHCP (yes)***	8 (13.3%)	1 (1.7%)	0.032[4]
POG at delivery (weeks)***	37.18±1.52	38.07±1.51	0.001[1]
Total bile acids (µmol/L)***	26.58±17.54	4.13±2.79	<0.001[1]
ALT (U/L)***	139.52±85.10	26.18±23.32	<0.001[1]
AST (U/L)***	113.88±75.66	22.92±13.86	<0.001[1]
Total bilirubin (mg/dL)***	0.72±0.38	0.48±0.19	<0.001[1]
GGT (U/L)***	32.23±18.37	12.38±16.20	<0.001[1]
S. fetuin B (pg/mL)	77893.85±74855.74	55608.84±38492.48	0.105[1]

***Significant at p<0.05, [1]: Wilcoxon-Mann-Whitney U test, [2]: t-test, [3]: chi-squared test, [4]: Fisher's exact test, BMI: Body mass index, POG: Period of gestation, IHCP: Intrahepatic cholestasis of pregnancy, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, GGT: Gamma glutamyl transferase

preterm delivery, NICU admission, MSAF, pathological CTG, and adverse composite neonatal outcomes were significantly associated with serum TBAs levels respectively (p<0.05). Whereas, preterm delivery, iatrogenic preterm delivery, and birth weight ≤2.500 gm are significantly associated with serum fetuin B levels respectively (p<0.05).

A positive Spearman's correlation was detected between TBA levels and gestational age at blood sampling, AST, ALT, and serum total bilirubin levels, respectively. The correlation analysis could not find a correlation between serum fetuin B and gestational age at sampling, serum AST level, ALT level, total bilirubin level, and TBAs levels (Table 3).

On analyzing the Point-Biserial Correlation between serum TBAs, serum fetuin B level with perinatal outcomes, a positive correlation was obtained between serum TBAs and NICU admission, MSAF, pathological CTG, adverse composite maternal outcomes, and adverse composite fetal outcomes with a medium effect size. We did not find any correlation between Serum fetuin B and NICU admission, meconium-stained liquor, pathological CTG, adverse composite maternal outcomes, and adverse composite neonatal outcomes in the case group. Whereas, by Spearman's correlation analysis, we could note a negative correlation of medium effect size between serum TBAs and birth weight and between serum fetuin B levels and birth weight.

The ROC analysis was performed to compare the diagnostic performance of serum fetuin B with serum TBAs in predicting case (IHCP) versus control (Table 4). At a cut-off of serum bile acids (µmol/L) ≥10 microliters predicted a case group with a sensitivity of 100.0%, a specificity of 95%, a positive predictive value (PPV) of 95.2%, and a negative predictive value (NPV) of 100.0%. The area under the ROC curve (AUROC) for serum bile acids (µmol/L) predicting case versus control was 0.998 [95% confidence interval (CI): 0.996-1], thus demonstrating excellent diagnostic performance. This value was statistically significant (p≤0.001).

At a cut-off of serum fetuin B, (pg/mL) ≥45376.8 predicted the case with a sensitivity of 68%, a specificity of 53% a PPV of 59.4%, and a NPV of 62.7%. The AUROC for serum fetuin-B (pg/mL) predicting case versus control was 0.586 (95% CI: 0.483-0.689), thus demonstrating poor diagnostic performance. This was not statistically significant (p=0.105). Therefore, the diagnostic performance of serum bile acids (µmol/L) (AUC=0.998) was significantly better than that of serum fetuin-B (pg/mL) (AUC=0.586) (DeLong's test p≤0.001) (Figure 1).

Discussion

In this study, the incidence of IHCP was 2.3%, which is similar to the incidence mentioned in a few research articles[1,15]. Serum TBAs, serum fetuin B, serum bilirubin, and liver enzymes were raised in the IHCP group when compared with pregnant women with good health. This has also seen in other studies[12,16-18]. We could found a good interrelation between serum TBAs and gestational age at sampling, serum bilirubin, liver enzymes, birthweight of baby, perinatal outcomes like NICU admission,

Table 2. Comparisons of obstetric, perinatal and neonatal outcomes outcome between both the groups

Parameters	Group		p-value
	Case (n=60)	Control (n=60)	
Induced labour (yes)***	37 (61.7%)	25 (41.0%)	**0.023**[3]
Spontaneous labour (yes)***	22 (36.7%)	9 (15.0%)	**0.007**[3]
LSCS (yes)***	53 (88.3%)	42 (70.0%)	**0.013**[3]
VD (yes)***	7 (11.7%)	18 (30.0%)	**0.013**[3]
Elective CS (yes)	18 (30.0%)	21 (35.0%)	0.699[3]
Emergency CS (yes)***	35 (58.3%)	21 (35.0%)	**0.010**[3]
Preterm delivery (yes)	13 (21.7%)	6 (10.0%)	0.080[3]
Iatrogenic preterm delivery (yes)***	10 (16.7%)	3 (5.0%)	**0.040**[3]
Spontaneous preterm delivery (yes)	3 (5.0%)	3 (5.0%)	1.000[4]
Term delivery (yes)	47 (78.3%)	54 (90.0%)	0.080[3]
Birth weight (g)	2735.32±593.93	2846.73±494.20	0.266[2]
Birth weight ≤2500 g (yes)	19 (31.7%)	18 (30.0%)	0.843[3]
Birth weight ≤10th percentile (yes)	18 (30.0%)	21 (35.0%)	0.559[3]
NICU admission (yes)***	13 (21.6%)	3 (5.0%)	**0.007**[3]
MSL (yes)***	23 (38.3%)	13 (21.7%)	**0.048**[3]
Stillbirth (yes)	0 (0.0%)	0 (0.0%)	1.000[3]
Pathological CTG (yes)***	25 (41.7%)	7 (11.7%)	**<0.001**[3]
Composite adverse maternal outcome (yes)	8 (13.3%)	6 (10.0%)	0.570[3]
Composite adverse neonatal outcome (yes)	14 (23.3%)	6 (10.0%)	0.051[3]

***Significant at p<0.05, [1]: Wilcoxon-Mann-Whitney U test, [2]: t-test, [3]: chi-squared test, [4]: Fisher's exact test, LSCS: Lower segment Caesarean section, VD: Vaginal delivery, CS: Caesarean section, PTD: Preterm delivery, NICU: Neonatal intensive care unit, MSL: Meconium-stained liquor, CTG: Cardiotocograph

Table 3. Correlation analysis of TBAs and Serum Fetuin B in IHCP/Case group (n=60)

	Age (years)	BMI (kg/m^2)	GA at sampling (weeks)	AST (IU/L)	ALT (IU/L)	GGT (IU/L)	TBAs (µmol/L)	Sr. total bilirubin (mg/dL)
IHCP group (n=60)								
S. TBAs (µmol/L) r	0.08	-0.25	0.51	0.56	0.58	0.077	_	0.51
p-value	0.05	0.54	0.00003[a]	<0.00001[a]	<0.00001[a]	0.595	-	<0.00003[a]
IHCP group (n=60)								
S.Fetuin B (pg/mL), r	0.039	-0.022	0.131	0.037	0.164	-0.082	0.135	0.039
p-value	0.797	0.867	0.153	0.773	0.210	0.503	0.305	0.767

[a]: At the 0.05 level (2-tailed) a significant correlation was established and p values in bold refer to a statistically significant result, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, GGT: Gamma-glutamyl transferase, TBAs: Total bile acids, GA: Gestational age, IHCP: Intrahepatic cholestasis of pregnancy

Table 4. Comparison of the diagnostic performance of serum fetuin B and serum total bile acids in predicting group: case vs control (120)

Predictor	AUROC	95% CI	P	Sn	Sp	PPV	NPV	DA
S. fetuin-B (pg/mL)	0.586	0.483-0.689	0.105	68%	53%	59%	63%	61%
S. bile acids (µmol/L)	0.998	0.996-1	<0.001	100%	95%	95%	100%	98%

AUROC: Area under ROC curve, CI: Confidence interval, P: P-value, Sn: Sensitivity, Sp: Specificity, PPV: Positive predictive value, NPV: Negative predictive value, DA: Diagnostic accuracy

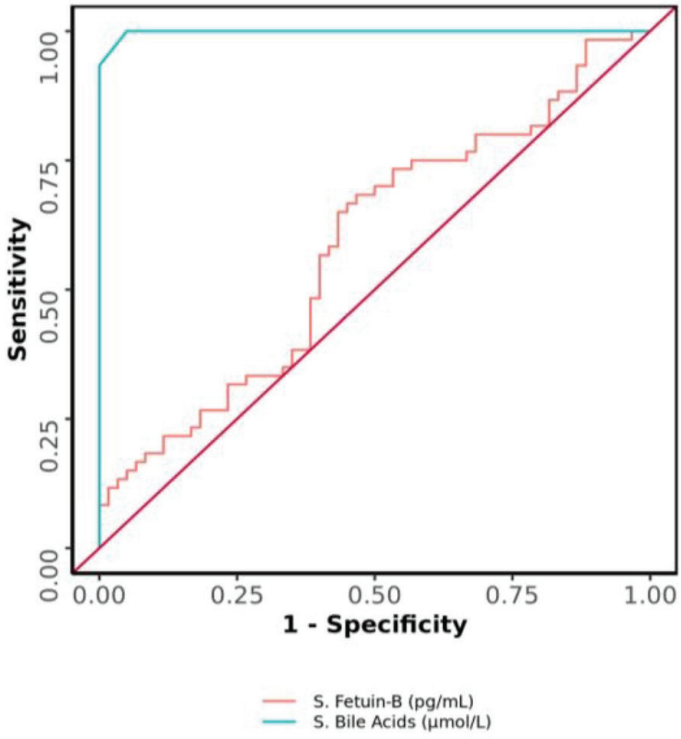

Figure 1. ROC curve showing comparison of diagnostic performance of serum fetuin B and serum bile acids (µmol/L) in predicting cases verses controls (n=120)

ROC: Receiver operating characteristic

MSAF, pathological CTG, composite adverse maternal outcomes, and composite adverse neonatal outcomes. Whereas, we could not establish any interrelation between serum fetuin B and any similar factor except with the birthweight of the baby. In this study, the diagnostic performance of serum bile acids was significantly better than that of serum fetuin B.

Although IHCP is relatively mild for the mother, the threat of having fetal complications is very high in pregnancies affected by IHCP. Pregnancy outcomes observed in this study were, women with IHCP were seen to have a high probability for the induction of labor, iatrogenic preterm delivery, increased cesarean delivery rate, and increased emergency cesarean delivery rate, but no stillbirth. The incidence of stillbirth occurs if pregnancy is continued beyond 37 weeks of gestation and with serum bile acid levels ≥100 µmol/L[19,20]. The absence of stillbirth in our study could be due to the reason that most of our patients had late preterm deliveries with serum TBAs not reaching very severe levels and the deliveries were conducted at a tertiary center. For preventing stillbirth in patients with IHCP interventions like treatment with ursodeoxycholic acid, frequent fetal monitoring, and early deliveries at 37 weeks of gestation were conducted. This could have resulted in observations like higher rates of iatrogenic preterm labor, induction of labor, and emergency cesarean section for pathological CTG. Increased spontaneous labor in women with IHCP may be explained by an increased effect of bile acids on uterine contractility through activation of the oxytocin receptor pathway, as suggested by a study conducted on rodents[21,22]. This study depicts an association between IHCP and MSAF, admission to the NICU, and an enhanced rate of pathological CTG. MSAF may be elucidated by an outcome of enhanced colonic peristalsis owing to higher maternal bile acids[4]. Several earlier studies have concluded that MSAF was observed in severe IHCP cases and this finding is consistent with our study[12-23]. Bile acids exert a vasoconstrictive effect on the placental chorionic veins, which can explain the pathological CTG and intrauterine meconium passage[4,24]. Another explanation though unproven for pathological CTG sudden intrauterine fetal death, and arrhythmia, could be the harmful effect of high bile acid levels on cardiocytes[24-26]. There was no antepartum hemorrhage, postpartum hemorrhage, or blood and blood product transfusion in this study.

When comparing IHCP with the control group, this study concludes that the IHCP group recorded composite adverse neonatal outcomes, which were 23.3% in the IHCP group versus 10.0% in the control, without any significant difference. These results may be due to a higher rate of admission to NICU, an earlier birth rate, and an increased rate of RDS in neonates in the cholestasis group. A comparable outcome was seen in a case-control study that showed a high rate of RDS in newborns delivered by IHCP women at two and half times higher (28.6%

vs. 14%) regardless of bile acid levels. The increased neonatal morbidity among newborns delivered by IHCP has been explained by many hypotheses, one probability was, bile acids have inhibitory surfactant activity even authors speculate on bile acid pneumonia due to its direct effect on neonatal lungs[2,27].

In this study, it was found that the mean serum bile acid levels between women IHCP compared to pregnant women in good health were significant. Subsequently, we noticed that the ideal cut-off for serum TBAs levels to differentiate between pregnant women with IHCP from the other group was ≥10 µmol/L.

A triple-arm Indian study conducted in women with IHCP, healthy pregnant women, and non-pregnant women vividly showed raised serum bile acid levels in women with IHCP only. In the same study, taking optimal cut-off levels of serum bile acid to 8.6 µmol/L for differentiating women with IHCP from the other two groups showed a sensitivity of 87.6% and specificity of 93.3%. Upon increasing the cut-off levels of serum bile acid to 10 µmol/L changed the sensitivity and specificity were 83.8% and 95.5% respectively. The authors inferred that the bile acid levels with a cut-off of 8.6 µmol/L or 10.0 µmol/L are very effective in the diagnosis of IHCP in the Indian scenario[28].

Whereas, we couldn't find a significant difference in mean serum fetuin B (pg/mL) between Indian women with IHCP and pregnant women in good health. Further, we identified that the optimal cut-off for serum fetuin B levels to recognize women with IHCP of pregnant women from the pregnant women in good health was ≥45376.8 pg/mL, with a sensitivity of 68.0% and specificity of 53.0%. The area under the ROC analysis (AUROC) for S. fetuin B (pg/mL) predicting IHCP cases was 0.586 (95% CI: 0.483-0.689), thus eliciting a dismal diagnostic performance and it was not statistically significant (p=0.105).

The study conducted by Koroglu et al.,[12] showed dissimilar observations. In their study, the serum fetuin B levels in the IHCP group were higher (p<0001). The area under the ROC for serum fetuin B for the diagnosis of IHCP was 0.758 (95% CI: 0.649-0.847). The ideal cut-off for fetuin B serum concentration was 5540.2 pg/mL and serum values greater than this level had 80% sensitivity and 65% specificity for the diagnosis of IHCP, which was different from our study[12]. Lately, it has been reported that serum fetuin B is increased in patients with coronary heart disease, non-alcoholic fatty liver, obesity, diabetes, and metabolic syndrome[8-10,29]. Compared to the study conducted by Koroglu et al.,[12] we have found a higher cut-off level of serum fetuin B for diagnosing cases of IHCP, this may be due to the higher prevalence of coronary heart disease, metabolic syndrome, and diabetes in Asian population. Another possible cause may be the method of measurement used. Serum fetuin B was measured using a Human FETUB (fetuin B) sandwich ELISA kit similar to the study conducted by Koroglu et al.[12] In our study, the test samples were run in 1:50 dilution so the results were obtained by multiplying with 50 (dilution factor) that could be another cause for higher levels of serum fetuin B.

In this study, there was no correlation noted between serum fetuin B and gestational age at sampling, fetal complications, adverse composite maternal outcomes, adverse neonatal outcomes, and biochemical parameters of the LFT. These results match, to some extent, with the study by Koroglu et al.[12] This study is the first that has elucidated the relationship between fetuin B and adverse perinatal outcomes to date.

We could find a medium to strong correlation between serum TBAs and gestational age at sampling, birth weight of the baby, fetal complications, adverse composite maternal outcomes, adverse neonatal outcomes, and biochemical parameters of LFTs including serum fetuin B. This is quite similar to the study by Glantz et al.[23] There was a proportionate increase in fetal complications by one percent to two percent for every mmol/L rise in serum TBA levels. The authors also stated that the risk of MSAF was seen with serum TBA levels of 20 mmol/L and above, whereas serum bile acid levels beyond 40 mmol/L increased the risk of preterm delivery, asphyxia events, and green staining of the placenta and membranes[23].

Inflammation has also been implicated in the pathogenesis of IHCP, and this leads to the production of fetuin B from hepatocytes. It has been substantiated that increased levels were observed in IHCP in the prevailing study, however, it was not statistically significant. Increased fetuin B levels were also related to a disruption in the metabolism of glucose and lipid. The specific pathophysiological mechanism and functions of serum fetuin B remain unclear and further studies need to be conducted to validate such findings.

Study Limitations

This study has some limitations. The sample size is too small and as this is a single-center study, the results can have less generalizability. We also could not examine the serum TBAs and serum fetuin B levels at the different time periods during pregnancy because of cost and irregularities in antenatal check-ups by study participants. The strength of our study is that it is a prospective comparative . The criteria used for diagnosing IHCP were objective, we have used parameters like elevated serum bile acids, and serum transaminase levels combined pruritus during pregnancy. We excluded women tested for other causes of potential liver disease from the case group and we included a control group. We suggest further research with a robust sample size that can determine the definite pathophysiologic method of increased serum fetuin B levels in IHCP and its correlation with adverse maternal and perinatal outcomes.

Conclusion

Our study re-established the increased risks and poor perinatal outcomes among pregnant women with IHCP vis-a-vis pregnant women in good health. Maternal serum TBA levels are related to adverse perinatal outcomes in IHCP. The measurement of serum TBA levels with a cut-off of 10.0 µmol/L is very effective in the diagnosis of IHCP in this population. We did not detect any significant difference between the IHCP group and pregnant

women in the good health group in relation to the serum fetuin B levels. Similarly, we could not correlate its levels with adverse maternal and perinatal outcomes except with the birth weight of the baby. Thereby serum fetuin B is not an effective marker for establishing the pathophysiology of IHCP. The measurement of bile acids for diagnosis and clinical management of this condition remains a hallmark and there should be wider availability of laboratory facilities for measuring it.

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Ethics

Ethics Committee Approval: This study was approved by the All India Institute of Medical Sciences Bhubaneswar (approval number: T/IM-F/18-19/36, date: 16.01.2019).
Informed Consent: All patients who participated in the study signed a consent form.
Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: J.B., Design: J.B., S.S., M.K.P., Data Collection or Processing: J.B., G.K.S., Analysis or Interpretation: J.B., G.K.S., Literature Search: J.B., S.S., Writing: J.B., G.K.S.
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Genitourinary syndrome in menopause: Impact of vaginal symptoms

Menopozda genitoüriner sendrom: Vajinal semptomların etkisi

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Abstract

Objective: To describe the impact of genitourinary syndrome symptoms on daily activities and well-being in peri- and postmenopausal women living in an urban area.

Materials and Methods: Observational, prospective, and cross-sectional research in a population of peri- and postmenopausal women living in the Lima region. A non-probabilistic sample was used. The instrument used is "The Day-to-Day Impact of Vaginal Aging" questionnaire. It consists of four domains and its internal reliability is between 0.82 and 0.93. The questions were answered using a Likert scale. High values indicate a more severe impact. Statistical procedures were performed using SPSS version 26.

Results: One thousand seventy women participated; the mean age was 54±7.5 years. The results about the activities of daily living showed that 35% of women reported regular vaginal symptoms and 14.7% major symptoms. In the emotional well-being domain, 90% had minor symptoms. In the sexual function domain, 57.6% reported minor vaginal symptoms, and in the self-concept and body image domain, 60.9% reported minor symptoms and 20.7% major symptoms. According to the global score, 60.9% reported minor discomfort, 36.3% regular discomfort, and 2.8% major discomfort. The sexually active women declared an impact of severity in terms of their daily activities and sexual function ($p<0.05$).

Conclusion: There is a relationship between activities of daily living, sexual function, and women with sexual activity, causing a negative impact on social life and quality of life.

Keywords: Menopause, symptoms, vulvovaginal atrophy, postmenopause

Öz

Amaç: Kentsel bir bölgede yaşayan peri ve postmenopozal kadınlarda genitoüriner sendrom semptomlarının günlük aktiviteler ve iyilik hali üzerindeki etkisini tanımlamak amaçlanmıştır.

Gereç ve Yöntemler: Lima bölgesinde yaşayan peri ve postmenopozal kadın popülasyonunda gözlemsel, prospektif ve kesitsel bir araştırma yapılmıştır. Olasılığa dayalı olmayan bir örneklem kullanılmıştır. Kullanılan ölçek "Vajinal Yaşlanmanın Günden Güne Etkisi" anketidir. Dört alandan oluşmaktadır ve internal güvenirliği 0,82 ile 0,93 arasındadır. Sorular Likert ölçeği kullanılarak cevaplanmıştır. Yüksek değerler daha ciddi bir etkiyi göstermektedir. İstatistiksel analiz SPSS 26 versiyonu kullanılarak yapılmıştır.

Bulgular: Bin yetmiş kadın katılmıştır ve yaş ortalaması 54±7,5 olarak hesaplanmıştır. Günlük yaşam aktiviteleri ile ilgili sonuçlar, kadınların %35'inin düzenli vajinal semptomlar ve %14,7'sinin majör semptomlar bildirdiğini göstermiştir. Duygusal esenlik alanında, katılımcıların %90'ın minör semptomlar bildirmiştir. Cinsel işlev alanında katılımcıların %57,6'sı minör vajinal semptomlar ve benlik kavramı ve beden imajı alanında %60,9'u minör semptomlar ve %20,7'si majör semptomlar bildirmiştir. Global skora göre, %60,9'u minör rahatsızlık, %36,3'ü düzenli rahatsızlık ve %2,8'i majör rahatsızlık bildirmiştir. Cinsel olarak aktif kadınlar, günlük aktiviteleri ve cinsel işlevleri açısından semptomların şiddetin bir etkisi olduğunu bildirmişlerdir ($p<0,05$).

Sonuç: Günlük yaşam aktiviteleri, cinsel işlev ve kadın ile cinsel aktivite arasında ilişki vardır, bu da sosyal yaşamı ve yaşam kalitesini olumsuz etkiler.

Anahtar Kelimeler: Menopoz, semptomlar, vulvovajinal atrofi, postmenopoz

PRECIS: Genitourinary syndrome in menopause: Impact.

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Introduction

The Genitourinary syndrome of menopause is characterized by symptoms and signs resulting from the effect of estrogen deficiency on the female genitourinary tract. Symptoms associated with genitourinary syndrome are highly prevalent and affect approximately up to 84% of postmenopausal women worldwide[1-3]. Women often report genital and/or vulvovaginal symptoms, such as: Vaginal dryness or burning, urinary urgency symptoms, dysuria, and sexual symptoms such as: Pain during intercourse or loss of sexual desire[4,5]. The variety of symptoms often appear from the late menopausal transition onwards and persist for several years. These symptoms have been associated with poor sexual functioning, reduced quality of life, and poor emotional well-being[6-8]. Note that the genitourinary syndrome was previously known as vulvovaginal atrophy to which the urinary component was added to have a better clinical approach[9]. Research conducted in the United States reported that only 3% of women of reproductive age reported vaginal dryness, whereas 21% of women in late menopausal transition and 47% of women three years after menopause reported this symptom[10,11]. Another research conducted involving 4.000 postmenopausal women reported a prevalence of 39% for vaginal discomfort[12]. The Vaginal Health: Insights, Views & Attitudes survey conducted with the participation of 3.520 postmenopausal women from six countries reported that 45% of women have experienced vaginal symptoms and 75% stated that their symptoms had a negative impact on life[13]. Similar research conducted with 500 American women reported that 48% of women reported vaginal discomfort, and the most frequent symptoms were vaginal dryness and pain during intercourse[14]. Likewise, the same research reported several adverse events related to vaginal discomfort: Negative impact on their lives (80%), adverse effect on sexual intimacy (75%), feeling less sexual (68%), negative consequences on marriage (33%), and a negative effect on self-esteem (26%)[15].

If untreated, genitourinary syndrome will be progressive and will negatively affect sexual function and quality of life[16,17]. Recent research has shown that women not only recognize genitourinary syndrome as a chronic condition, but are reluctant to discuss their vaginal or sexual discomfort with their physician because of embarrassment or concern about the side effects of treatment[18]. Barriers to the identification and treatment of genitourinary syndrome include limited time during patient visits, lack of physician training regarding the diagnosis and treatment of this condition, and the misconception that genitourinary syndrome only affects sexually active women[19].

Currently, there are a growing number of hormonal and non-hormonal treatments to alleviate the various symptoms of genitourinary syndrome, although low-dose vaginal estrogens remain a safe and highly effective hormonal treatment option. However, many women and physicians have concerns about estrogen therapy, particularly in the context of a personal or high-risk history of breast cancer[20,21]. A systematic review demonstrated that no single treatment was completely effective in relieving genitourinary syndrome-related dyspareunia in women with female sexual dysfunction. Therefore, the implementation of a multidisciplinary approach to the management of genitourinary syndrome on an individual and personalized basis is recommended[22,23].

The growing literature on genitourinary syndrome is not without limitations. First, few questionnaires are validated to assess the various symptoms, so investigators develop their own questionnaires, making comparison between studies difficult. Second, research often measures the presence of genitourinary syndrome but not symptom interference. Third, most of the literature focuses on sexuality, and vaginal symptoms are highlighted more often than vulvovaginal symptoms and with less priority given to a comprehensive evaluation. Additionally, important information on symptoms unrelated to sexual function or symptoms experienced by women who are not sexually active is omitted[24]. Therefore, the aim of this study aimed to describe the impact of genitourinary syndrome symptoms on daily functioning and well-being in peri- and postmenopausal women living in an urban area.

Materials and Methods

The research is observational, prospective, and cross-sectional in a cohort of peri- and postmenopausal women living in the Lima region. It included the participation of older women aged ≥45 years, who attended the gynecological consultation for any reason in health facilities of the first level of care in the Lima region in 2021. A non-probabilistic convenience sample was used. The inclusion criteria were: women aged ≥45 years, absence of menstrual period for at least one year and without difficulty to read or write, and having given their consent to participate in the research.

The instrument used is a self-report questionnaire called The Day-to-Day Impact of Vaginal Aging (DIVA)[25]. The participants completed the questionnaire, which has previously demonstrated good face validity, construct validity, and internal reliability, with values for the four domains between 0.82 and 0.93. It includes four specific domains to assess the impact of symptoms: activities of daily living (5 questions), emotional well-being (4 questions), sexual function (7 questions), and self-concept and body image (5 questions). All questions have response scales using a Likert scale with values between 0 to 4; higher values indicate a more severe impact. The scores for each domain are calculated by averaging the scores of the individual questions in each domain.

The research procedures were performed in accordance with the Declaration of Helsinki and were approved by the Ethics Committee of the Peruvian Menopause Society, under letter 003-2021/SPC dated January 15th 2021.

Statistical Analysis

The statistical analysis for qualitative variables was expressed as absolute and relative frequencies, while quantitative variables

were expressed as mean and standard deviation. Mean scores were established for each domain in women with and without genitourinary syndrome. Bivariate analysis was performed using the chi-square test of independence. All statistical procedures were performed using SPSS version 26.

Results

A total of 1,100 women were invited to participate, of which 30 were excluded because they did not meet the inclusion criteria. A total of 1,070 women were included in the analysis, who agreed to participate after providing explanations and answering questions. Regarding the sociodemographic characteristics of the women, the average age was 54 years standard deviation (SD)±7.5. 68.2% were married and/or cohabiting, 12.9% were single, 11.4% were divorced and 7.5% were widowed. Eighty-two percent reported being sexually active, and 18% reported not being sexually active.

With respect to the results corresponding to the activities of daily living: 32.2% stated that vaginal symptoms interfered very little when walking and 28.6% reported that vaginal symptoms interfered very little when wearing clothes or underwear. 28.9% stated that there was no interference when using the toilet, 27.4% felt very little interference when sitting for more than one hour, and 30.7% reported that vaginal symptoms interfered very little with getting a good night's sleep. Likewise, in the emotional well-being domain, 63.5% stated that the presence of vaginal symptoms had very little influence on feeling depressed, 54.2% very little influence on feeling embarrassed, 55.5% very little influence on feeling frustrated or resentful, and 57.1% very little influence on feeling sad.

The results corresponding to the sexual function domain showed that: 34.9% stated that vaginal symptoms have very little impact of severity on the desire and interest in having sexual intercourse, 40.5% very little impact with respect to the frequency of sexual intercourse or other sexual activity, 29.8% very little impact, and 24.6% moderate impact with respect to the ability to become aroused during sexual activity. Likewise, the presence of vaginal symptoms and their severity impact on the experience of feeling pleasure during sexual activity was very little in 30.9% of women and the impact was moderate in 26.1%; 37% expressed very little impact regarding the desire or interest in being in a sexual relationship. However, 28.9% of women reported that vaginal symptoms have very little impact of severity on their confidence in sexual satisfaction, and 14% reported that there is usually a severe impact. Regarding the indicator of the presence of vaginal symptoms and overall satisfaction with sex life, 30.7% reported very little impact and 29% reported a moderate impact.

As for the last domain analyzed, corresponding to self-concept and body image, 33.5% of women stated that vaginal symptoms affected very little their feelings about feeling that they were growing old, while 17.6% stated that this was the case in an extreme form. 37.5% reported that they never felt unwanted because of their vaginal symptoms. Likewise, 33.3% reported that they had lost something in their bodies when they thought about vaginal symptoms, and 33% reported that vaginal symptoms made them feel that their body was deteriorating. 39.5% of the women stated that they never felt less sexually attractive due to the presence of vaginal symptoms, while 17.8% stated that they felt extremely less sexually attractive.

According to the assessments by domains, activities of daily living had an average value of 1.5 SD ±0.9, while in the dimension of self-esteem and body image, the average value corresponded to 1.4 SD ±1.2 (Table 1).

The analysis of the results corresponding to the score in the activities of daily living found that 35% of the women showed regular vaginal symptoms and 14.7% showed major symptoms. In the emotional well-being domain, 90% showed minor symptoms. In the sexual function domain, 57.6% showed minor vaginal symptoms, and in the self-concept and body image domains, 60.9% showed minor symptoms, and 20.7% showed major symptoms. According to the analysis of the global score, 60.9% showed minor discomfort, 36.3% showed regular discomfort, and 2.8% showed major discomfort (Figure 1).

Sexually active women had higher mean scores than non-sexually active women in the four domains studied (Figure 2).

The comparison of results in women according to sexual activity identified differences between women with minor discomfort in the domain linked to activities of daily living: 58% in women who are not sexually active and 48.2% for women who are sexually active. However, in the sexual function domain, 82.4% of women without sexual activity reported minor discomfort, whereas 52.1% of sexually active women reported minor discomfort (Table 2). According to the global score, 72.5% of women without sexual activity reported minor vaginal symptoms, while that figure was of 58.4% for sexually active women. The bivariate analysis identified a relationship between the following domains: activities of daily living, sexual function, and sexually active women ($p<0.05$).

Discussion

The research was conducted in compliance with the inclusion criteria, obtaining consent, explaining the research objectives to the women, and the correct completion of the questionnaire,

Table 1. Impact of vaginal symptoms on functioning and well-being by domains

Domain	Average value	Standard deviation
Activities of daily living	1.5	0.9
Emotional well-being	0.9	0.6
Sexual function	1.3	0.8
Self-concept and body image	1.4	1.2

Source: Own elaboration

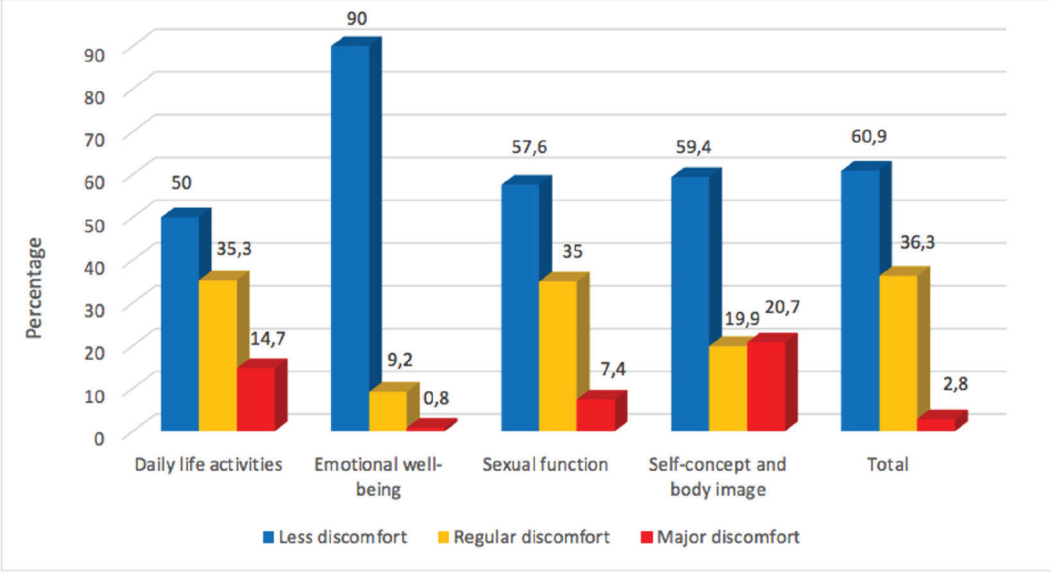

Figure 1. Global score of vaginal symptoms and their impact according to domains

Source: Own elaboration

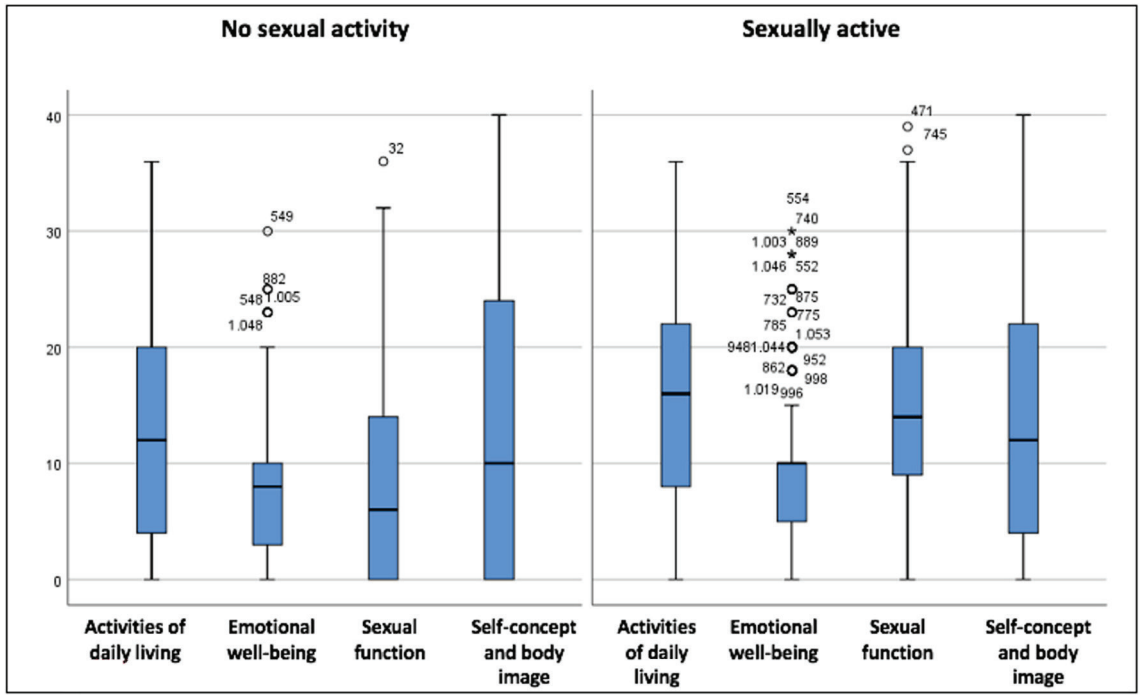

Figure 2. Results of vaginal symptoms by domains

which facilitated the analysis of the results according to the proposed methodology.

Middle age brings a series of personal and social changes to women. Generally, cultural and social beliefs influence the perception and interpretation of menopausal symptoms, as well as how much it affects daily activities[26]. Although 20% of women around the world consider menopause a disease, they are not completely sure of the effects it produces on their health[27]. Depending on the characteristics of each person and their occupation, the slightest symptoms can cause anxiety in some women, having difficulties in coping with their daily activities.

DIVA questionnaire was used to identify genitourinary syndrome in peri- and postmenopausal women[25]; however, the questionnaire does not consider the urinary component. Note that the urinary symptoms produced by the genitourinary syndrome continue to be less studied due to the direct influence of estrogen deficiency and require further study and analysis of

Table 2. Analysis of vaginal symptoms and sexual activity

Domain	Discomfort	No sexual activity	n%	Sexually activite	n%	Total	n%	p-value
Activities of daily living								
Minor discomfort		112	58	423	48.2	535	50	0.041
Regular discomfort		55	28.5	323	36.8	378	35.3	
Major discomfort		26	13.5	131	14.9	157	14.7	
Emotional well-being								
Minor discomfort		174	90.2	789	90	963	90	0.861
Regular discomfort		18	9.3	80	9.1	98	9.2	
Major discomfort		1	0.5	8	0.9	9	0.8	
Sexual function								
Minor discomfort		159	82.4	457	52.1	616	57.6	0.000
Regular discomfort		23	11.9	352	40.1	375	35	
Major discomfort		11	5.7	68	7.8	79	7.4	
Self-concept and body image								
Minor discomfort		114	59.1	522	59.5	636	59.4	0.151
Regular discomfort		31	16.1	182	20.8	213	19.9	
Major discomfort		48	24.9	173	19.7	221	20.7	
Total								
Minor discomfort		140	72.5	512	58.4	652	60.9	0.000
Regular discomfort		46	23.8	342	39	388	36.3	
Major discomfort		7	3.6	23	2.6	30	2.8	

Source: Own elaboration

this component[28-30]. Despite the reliability and validity of some questionnaires that evaluate urogenital and sexual symptoms in middle-aged women, self-report measures are not specifically focused on vulvovaginal symptoms in post menopause. According to the literature review, our results are the first to be conducted in a population from the region of Lima, Peru.

The results corresponding to the activities of the daily living domain showed higher values than other domains. The causes for this interpersonal variation in the conception of menopause may be linked to how intense the symptoms are. Although it could also depend on the interpretation and management of the symptoms, depending on their social and cultural situation[31]. However, an investigation carried out with postmenopausal women in Spain found that vaginal symptoms had less relevance in daily activities and linked them more with general aspects than with the genitourinary syndrome. The physical appearance or the work activity when having hot flashes are examples of this. In addition, women could have erroneously associated the state of emotional well-being with respect to their body perception and self-perception, since many postmenopausal women believe that vaginal symptoms are associated with age[32,33].

Several investigations indicate that one in four women with genitourinary syndrome of menopause reported emotional repercussions, the most frequent being: Concern about vaginal symptoms and the appearance of the vulva[34]. Contrarily, the results in this domain turned out to be lower and apparently do not have an impact on feeling depressed, embarrassed, or feeling sad. The differences with similar research may be due to the data being underestimated, as vaginal and/or genitourinary symptoms have become an uncomfortable topic of conversation for women with their partner, family members, and physician[7,8]. Note that the specialized literature emphasizes that vulvovaginal symptoms are associated with unfavorable emotional well-being scores (depression and anxiety) and these contribute to the presence of early vulvovaginal atrophy symptomatology[35,36].

Sexual function disorders are relatively common in postmenopausal women, but often go undiagnosed. They are defined "as the absence of sexual fantasies and thoughts and/or desire or receptivity to sexual activity that causes personal distress or relationship difficulties"[37]. Several studies indicated that 50% of postmenopausal women suffer from sexual symptoms, which include dryness, dyspareunia, or sexual

function, and these are related to sexual desire disorder, and the severity of these symptoms increases the rate of sexual dysfunction. Note that sexual function can be aggravated by emotional state or by chronic diseases, including metabolic syndrome[38-40]. Our findings allowed us to identify that vaginal symptoms had an unfavorable impact on arousal capacity, ability to feel pleasure, sexual satisfaction, and moderately affected sexual life. These findings appear to be supported by those described in the "Study of Women's Health Across the Nation," which found a significant decline in sexual desire as women moved through menopause. Likewise, the "Real Women's Views of Treatment options for Menopausal Vaginal Changes" study notes that sexual function is influenced by the confirmation of the diagnosis of vulvovaginal atrophy, mainly in the components linked to sexual arousal, lubrication, orgasm, and dyspareunia[41].

Concerning decreased sexual desire, research conducted in Australian women found a prevalence of 69.3%, and other research has reported a prevalence of 71% for decreased sexual desire[42-44]. The findings of the reviewed investigations are much higher than those of our study, so it is necessary that medical specialists pay more attention to the management of sexual function, considering aspects that favor a healthy sexual life in women with menopause, such as psychosocial, for example.

The self-concept and body perception domains showed an unfavorable impact due to the existence of vaginal symptoms. This could be influenced by the relationship between the presence of genitourinary syndrome and the quality of life of postmenopausal women, producing a negative impact on social life and quality of life in general[22,33,45].

This result is relevant because women with various vaginal symptoms must receive treatment, both to resolve their vaginal discomfort and to improve their self-esteem, sexual and emotional well-being, and other aspects related to their quality of life, which is the most important thing. Our findings coincide with those described by the "European Vulvovaginal Epidemiology Survey," which indicates that the self-concept and body perception dimensions had a negative impact since women feel less attractive and vaginal symptoms are considered part of aging and deterioration of health[46].

Likewise, some research indicated that women in southern European countries are more concerned about the long-term impact of vaginal discomfort, which negatively contributes to women's family and sexual lives[47].

The findings of our study showed that sexually active women have an unfavorable impact in all dimensions, and with greater relevance in the domains of daily activities and sexual function. This is reaffirmed by reports from the "Study of Women's Health Across the Nation" indicating that African American women place more importance on sex more frequently than white women; the importance of sexuality is explained in the frequency of sexual intercourse, sexual desire, arousal, and sexual functioning[48]. Likewise, North American women of Chinese and Japanese ancestry are reported to be less sexually active compared with Western women[48]. However, data from the "Pan-Asian REVIVE" study reveal in Asian women there is underdiagnosis and, therefore, undertreatment with genitourinary syndrome. When vaginal dryness and irritation occur, they negatively influence sexual enjoyment and intimacy for these women[49].

In our research, vaginal discomfort considered regular and major was lower than that described by the "Vaginal Health: Insights, Views, and Attitudes" study, where it was observed that 62% of women described moderate and severe vaginal discomfort[13]. Likewise, the European Vulvovaginal Epidemiology Survey noted that more than 65% of sexually active Italian women had vulvovaginal atrophy[46]. These differences are due to the type of population of participating women, cultural characteristics, and sexual behavior specific to European women. According to our results, it is confirmed that sexually active women are affected by vaginal symptoms and vulvovaginal atrophy, due to the relationship between the presence of vaginal symptoms, activities of daily living, and sexual activities.

Study Limitations

This research is not without limitations. Among the main limitations was the use of "The Day-to-Day Impact of Vaginal Aging" questionnaire, since this is little known and has not been used in Peru, which makes it difficult to compare results with research carried out in Peru and Latin America. Another limitation was the cross-sectional design, the lack of a control group, and possible selection bias because many women who present with vaginal symptoms do not visit a specialist for proper diagnosis and treatment. Although many women completed the questionnaire, the physicians did not have sufficient time to review and discuss the results with their patients at subsequent visits.

Conclusion

The domains analyzed in this investigation allowed identifying a relationship between activities of daily living, sexual function, and women who are sexually active ($p<0.05$). This produces a negative impact on social life and the quality of life in general.

Ethics

Ethics Committee Approval: The research procedures were performed in accordance with the Declaration of Helsinki and were approved by the Ethics Committee of the Peruvian Menopause Society, under letter 003-2021/SPC dated January 15th 2021.

Informed Consent: All participants gave their informed consent before the study was conducted.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: J.P.M.K., H.F.G.C., Design: J.P.M.K., H.F.G.C., R.A.R.A., Data Collection or Processing: J.P.M.K., H.F.G.C.,

R.A.R.A., I.A., Literature Search: I.A., Analysis or Interpretation: J.P.M.K., H.F.G.C., Writing: J.P.M.K., H.F.G.C.

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An evaluation of the effects on the ovaries of hyperbaric oxygen therapy in a rat model of premature ovarian failure created with cyclophosphamide

Siklofosfamid ile oluşturulmuş erken yumurtalık yetmezliği olan bir sıçan modelinde hiperbarik oksijen tedavisinin yumurtalıklar üzerindeki etkilerinin değerlendirilmesi

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Abstract

Objective: To evaluate hyperbaric oxygen therapy (HBO) based on ovarian histology, total antioxidant status (TAS), total oxidant status (TOS), and anti-müllerian hormone (AMH), in the ovarian insufiency (POI) model created with cyclophosphamide (CYP).

Materials and Methods: The rats were separated into 3 groups of the control group (n=6), the CYP group (n=6), and the CYP+HBO group (n=6). The rats in the CYP group and the CYP+HBO group were injected intraperitoneally with 200 mg/kg CYP on day 1, followed by 8 mg/kg/day for 14 days to create POI. From the 15th day onwards, the rats in the CYP+HBO group were placed in a hyperbaric cabin and exposed to 100% oxygen at 2.4 atm pressure for one h, and were then returned to their cages at the end of the hour.

Results: A statistically significant decrease was determined in the primordial and primary follicle counts in the CYP group compared with the control group ($p<0.05$). In the CYP+HBO group, a statistically significant increase was determined in the primordial and primary follicle counts ($p<0.05$). The serum AMH levels were seen to be significantly decreased in the CYP group compared with both the control group and the CYP+HBO groups. The HBO was seen to decrease TOS and increase TAS.

Conclusion: HBO could be an alternative treatment to minimize the effect of ovarian follicle loss caused by CYP, which is used for treating tumors that commonly occur in young females of reproductive age.

Keywords: Anti-müllerian hormone, cyclophosphamide, hyperbaric oxygen, ovarian failure

Öz

Amaç: Bu çalışmanın amacı siklofosfamid (CYP) ile oluşturulan over yetmezliği (POI) modelinde hiperbarik oksijen tedavisini (HBO) over histolojisi, total antioksidan durumu (TAS), total oksidan durumu (TOS) ve anti-müllerian hormon (AMH) bazında değerlendirmektir.

Gereç ve Yöntemler: Ratlar kontrol grubu (n=6), CYP grubu (n=6) ve CYP+HBO grubu (n=6) olmak üzere 3 gruba ayrıldı. CYP grubu ve CYP+HBO grubundaki ratlara 1. gün 200 mg/kg CYP, ardından 14 gün boyunca POI oluşturmak için 8 mg/kg/gün intraperitoneal olarak enjekte edildi. 15. günden

PRECIS: Hyperbaric oxygen therapy in premature ovarian failure.

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itibaren CYP+HBO grubundaki ratlar hiperbarik kabine alınarak 1 saat 2,4 atm basınçta %100 oksijene maruz bırakıldı ve 1 saatin sonunda ratlar tekrar kafeslerine alındı.

Bulgular: CYP grubundaki primordial ve primer folikül sayılarında kontrol grubuna göre istatistiksel olarak anlamlı azalma saptandı (p<0,05). CYP+HBO grubunda primordial ve primer folikül sayılarında istatistiksel olarak anlamlı artış saptandı (p<0,05). Serum AMH düzeylerinin CYP grubunda hem kontrol grubu hem de CYP+HBO grubuna göre anlamlı olarak düştüğü görüldü. HBO'nun TOS'yi azalttığı ve TAS'yi artırdığı görüldü.

Sonuç: Üreme çağındaki genç kadınlarda sık görülen tümörlerin tedavisinde kullanılan CYP'nin neden olduğu over folikül kaybının etkisini en aza indirmek için HBO alternatif bir tedavi olabilir.

Anahtar Kelimeler: Anti-müllerian hormon, siklofosfamid, hiperbarik oksijen, yumurtalık yetmezliği

Introduction

The total number of immature primordial follicles found in the ovaries is known as the ovarian reserve. Estimation of the ovarian reserve is accepted as a basic marker of fertility With a process known as "activation," the rate at which primordial follicles grow is an important point affecting fertility. From a total of approximately 1-2 million primordial follicles at birth, only approximately 400 will mature into primary oocytes with the capability for ovulation and fertilization in the reproductive years of a woman. The vast majority are victims of atresia.

When approximately 1.000 primordial follicles remain, this causes fertility to stop and menopause to start[1]. Information about the renewal of primordial follicles related to the depletion of the ovarian reserve has started to be discussed with the emergence of evidence related to the identification of oogonial stem cells, which can produce new oocytes in mouse ovaries[2], and their presence in human ovaries[3]. However, there is limited knowledge about the underlying molecules and biochemical processes of follicular activation in humans. Moreover, exposure to exogenous factors such as stress, radiation, anti-neoplastic agents, and cigarette smoke cause the formation of reactive oxgen species, which can develop in the body under physiological conditions. Oxidative stress occurs because of an increase in the oxidant level and/or a decrease in the antioxidant level in the body. Oxidative stress causes damage to cellular mitochondria, nucleus, and membranes. Free radicals cause irreversible damage in the organism with the effect of damage to deoxyribonucleic acid (DNA). It is thought that reactive oxygen species (ROS) plays an important regulatory role in folliculogenesis, oocyte maturation, and luteolysis.

Chemotherapeutic agents have a negative effect on the reproductive potential of young women, but the mechanisms through which this occurs are still unclear. Cyclophosphamide (CYP) is used to treat several cancer types, primarily leukemia and lymphoma, which are the most frequently seen cancers in adolescent females[4]. Alkylating agents, especially CYP, are extremely toxic for the gonads because the toxicities are independent of cell proliferation[5]. CYP directly damages DNA, induces follicular apoptosis, and produces ROS, which damage ovarian cells[6]. CYP also leads to over-activation of primordial follicles by actvating the P13K/AKT/mTOR pathway[7]. This is accepted as a factor that is effective in the early reduction of follicles.

Premature ovarian insufficiency (POI), which is a cause of female infertility, is defined as failure in ovarian functions for a period of longer than 4 months before the age of 40 years, and is characterized by amenorrhea, elevated gonadotropin levels, and hypooestrogenism[8]. This condition affects 0.3-1% of women[9]. POI can occur with the depletion of the ovarian reserve associated with acceleration in the rate of primordial follicle activation[10], primordial follicle loss[11], or iatrogenic causes such as chemotherapeutic agents[12]. These patients experience physiological symptoms associated with hypooestrogenism, infertility, and a series of psychological problems[13]. Simultaneously, there is an increased risk of cardiovascular disease, osteoporosis, urogenital atrophy, and neurodegenerative disease in these patients[14]. Therefore, the prevention of POI is important to protect women against infertility, and other systemic problems brought about by early menopause.

Hyperbaric oxygen therapy (HBO) is a treatment method in which 100% oxygen is breathed by exposure to increasing atmospheric pressure. Recently, it has been used as an effective adjuvant treatment method for treating several different pathologies. The oxygen present can re-oxygenate areas where hypoxia or hypoperfusion has occurred.

This study aimed to determine the possible effects that could formed after HBO therapy in rats applied with CYP to create a premature ovarian failure model, by examining ovarian histology and the serum parameters of Anti-müllerian hormone (AMH), total antioxidant status (TAS), and total oxidant status (TOS).

Materials and Methods

This study was approved by the Erciyes University Animal Experiments Ethic Committee (no: 21/168; date: 07.07.2021). The work was funded by the Erciyes University Scientific Research Projects Coordination Unit (TSA- 2022- 11550).

The study material comprised 18 adult, female Wistar rats, aged 10-12 weeks, each weighing 200±20 gr. The rats were kept under observation for 3 days, for adaptation, for signs of any health problems. Throughout the study, the animals were kept under stable conditions of 22 °C environmental temperature, 30-70% humidity, a 12-hour light/dark cycle, with food of dry pellets and tap water.

The rats were separated into 3 groups of 6 animals in each. The control group was named group 1, the CYP group, group 2,

and the CYP+HBO group, group 3. The control group rats were administered 1 mL/kg physiological saline intraperitoneally (IP) for 15 days. The rats in group 2 and group 3 were injected IP with 200 mg/kg CYP on day 1, based on the protocol in the study by Melekoglu et al.[15] followed by 8 mg/kg/day for 14 days to create POI. From the 15th day onwards, the rats in the CYP+HBO group were placed in a hyperbaric cabin and exposed to 100% oxygen at 2.4 atm pressure for one h, and were then returned to their cages at the end of the hour.

For the HBO therapy, the cabin was filled with 100% oxygen until the pressure reached 2.4 atmosphere at the rate of 0.1 bar/min in 15 min. At the end of 1 h of treatment, the air in the cabin was safely released until it fell to a normal pressure of 1 atmosphere at a rate of 0.1 bar/min. This procedure was continued at the same duration and pressure for 14 days.

On day 30, all the rats in all groups were administered general anesthesia of xylazine (10 mg/kg live weight, IP) and ketamine (60 mg ketamine hydrochloride/kg live weight, IP). First, an intracardiac blood sample of approximately 5 cc was taken. The serum was separated by centrifugation at 3.000 rpm for up to 10 min. While the animals were under deep anesthesia, a 2 cm skin incision was made over the linea alba in the abdominal region. The subcutaneous connective tissue and abdominal muscles were opened, and oophorectomy was performed by dissecting the left and right ovaries. The rats were then sacrificed with the cervical dislocation method.

Ovarian tissues were fixed in 10% formaldehyde solution for 48 h. The tissues were washed under running tap water, and then passed through increasing graded alcohol series. After being made transparent with xylene, the tissues were embedded in paraffin blocks. Slices 5 µm in thickness were cut from the paraffin blocks, stained with Hematoxylin and Eosin and Masson Trichrome, and then blinded examined under an Olympus BX51 microscope. Evaluations were made according to the determined criteria. The germinal epithelium and tunica albuginea were measured using ImageJ software.

Determination of TAS and TOS Levels

Serum TAS and TOS levels were determined using commercial Rel Assay kits (Rel Assay Kit Diagnostics, Turkey). Trolox was used as a calibrator for TAS tests, and the results were expressed in mmol Trolox equivalent/L. Hydrogen peroxide was used as a calibrator for TOS tests and the results were expressed in mmol H_2O_2 equivalent/L.

ELISA Analysis of AMH Levels

Serum samples were collected by centrifugation (5000 rpm for 10 min) and stored at -80 °C. The procedure was performed with an AMH ELISA Kit (AFG Bioscience, USA) following the manufacturer's instructions. Optical density (OD) values were measured at 450 nm using a spectrophotometer (BioTek, Synergy H, TX, USA). The concentrations of AMH in the serum samples were determined by comparing the OD values of the samples to the standard curve.

Statistical Analysis

The SPSS 22 software was used for statistical analysis. Statistical differences between groups were tested using One-Way ANOVA, and comparisons between the control and treated groups were performed using an unpaired Student's t-test. The data werre presented as mean ± standard error of the mean values. A value of $p<0.05$ was accepted as statistically significant.

Results

Serial slices (5 µm) were taken from the ovarian tissues obtained. One in five of the slices were stained, and follicles were counted. A statistically significant decrease was determined in the primordial and primary follicle counts in group 2 compared to group 1 ($p<0.05$). A statistically significant increase was determined in the primordial and primary follicle counts in group 3 compared to group 2 ($p<0.05$). When the groups were evaluated according to pre-antral and secondary follicle counts, the only statistically significant difference was between group 1 and group 2 ($p<0.05$). A statistically significant difference was determined between group 2 and both groups 1 and 3 with respect to secondary follicle count ($p<0.05$) (Table 1).

When the groups were evaluated with respect to the germinal epithelial thickness, the difference between group 1 and group 3 was statistically significant ($p>0.05$). An increase occurred in the mean germinal epithelial thickness of group 2, and this increase was determined to be statistically significant compared to groups 1 and 3 ($p<0.05$) (Table 1). In the evaluation of tunica albuginea thickness, a statistically significant difference was determined between all groups ($p<0.05$) (Table 1).

In group 1, tunica albuginea formed of fibrous connective tissue fibers and cells was observed below the germinal epithelium. Below the tunica albuginea was the cortex section, which was in the more peripheral part and contained various numbers and types of follicles, and the medulla, containing a rich vascular bed and with a loose connective tissue structure in the inner section. The germinal epithelium in group 3 was similar to that in group 1, but the tunica albuginea layer was seen to be thicker than in the control group. In group 2, in the whole tunica albuginea layer, including the cortex, there was more blood vessels and wider diameter of the vessels. In the primordial, primary, pre-antral, secondary, and tertiary follicles, the oocytes and the granulosa cells surrounding the oocytes were irregular. There was also observed to be an evident increase in the number of atretic follicles in group 2. The connective tissue sheath (theca) surrounding the follicle, and which differed from the stromal cells that began to be observed from the primary follicles, was enlarged and irregular. The granulosa lutein cells in the corpus luteum, which develops after ovulation, were observed to be irregular in shape with large gaps between them. There was a significant increase in the number of blood vessels in the medulla layer, and widening of the blood vessel diameters could be significantly differentiated. The histopathological findings in group 3 were close to those in group 1.

The number of blood vessels was decreased and the diameters of the vessels were narrower. Fewer atretic follicles were observed. The theca layer of the connective tissue surrounding the follicles was more regular in shape (Figure 1).

When the serum AMH levels of the groups were examined, the serum AMH levels in group 2 were seen to be significantly decreased compared with both group 1 and group 3 ($p<0.05$) (Table 2). The highest mean serum AMH level was determined in group 3 (8.83±0.87 ng/mL) but this was not at a statistically significant level compared to the control group (8.79±1.37 ng/mL). These results showed that CYP lowered the AMH level significantly, but the application of HBO therapy to these patients with CYP could prevent a fall in serum AMH levels.

When the TOS values were examined, there was determined to be statistically significantly higher oxidant status in group 2 than in group 1 (7.02±2.29 vs. 3.85±0.62, p=0.0083) (Table 2). In group 3, the HBO therapy was seen to have statistically significantly reduced the oxidant status (3.70±1.19, p=0.010). This status can be considered protective with respect to the ovarian follicles.

Table 1. Comparisons of the follicle counts between the groups

	Group 1	Group 2	Group 3	p
Primordial	102.80±8.57[a]	71.10±10.33[b]	81.80±8.91[c]	0.000
Primary	67.40±9.35[a]	49.90±6.33[b]	60.60±8.27[a]	0.000
Preantral	35.40±7.13[a]	26.90±7.06[b]	30.70±5.79[ab]	0.029
Secondary	18.00±2.44[a]	12.50±2.71[b]	16.40±3.59[a]	0.001
Tertiary	8.60±1.71[a]	6.40±2.11[b]	7.30±1.88[ab]	0.026
Germinal epithelial	5.81±1.58[a]	6.35±2.12[b]	5.70±1.72[a]	0.001
Tunica albuginea	15.60±5.07[a]	28.92±11.10[b]	17.52±5.13[c]	0.000

The same letters in the same rows indicate similarity between the groups, and different letters indicate a difference

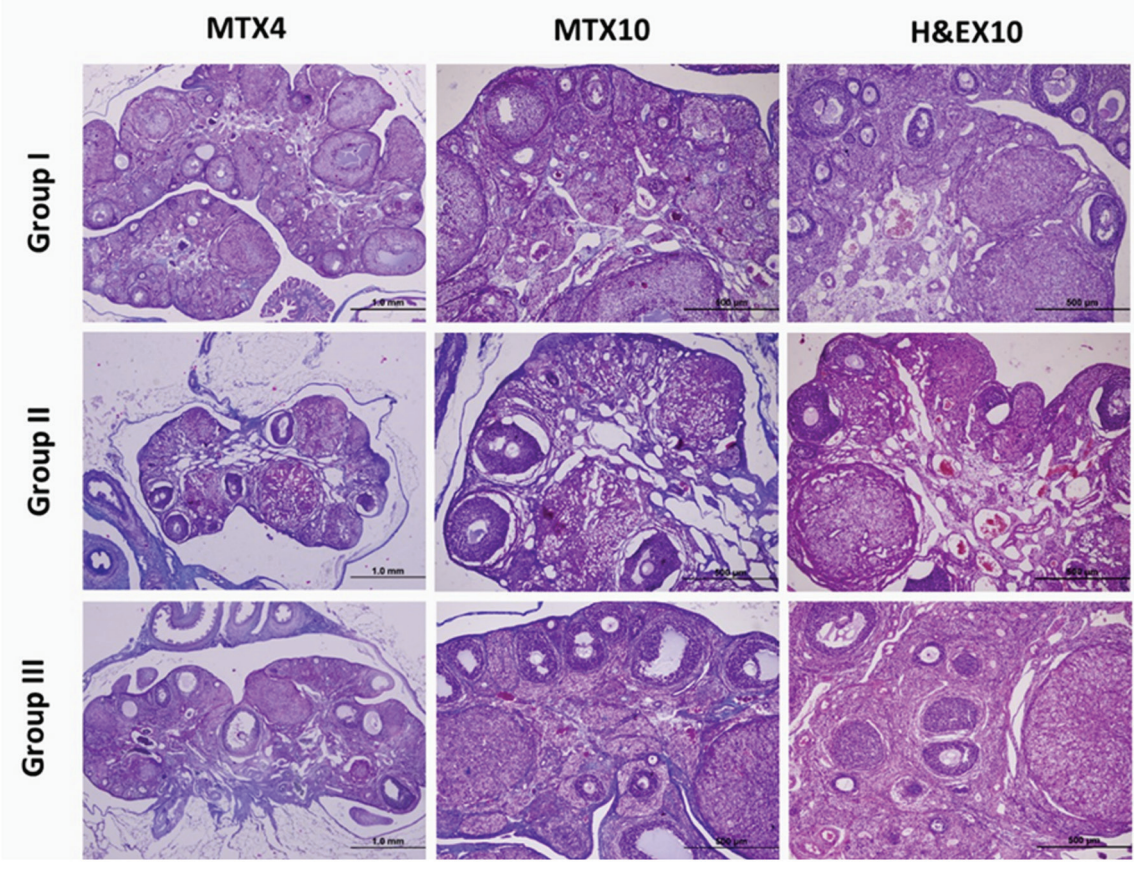

Figure 1. Light microscopic images of MT and H&E staining of all three groups

MT: Masson Trichrome, H&E: Hematoxylin and Eosin

The TAS levels were determined to be statistically significantly higher in group 1 than in group 2 (1.10±0.11 vs. 0.79±0.12, p<0.001) (Table 2). In group 3, HBO was determined to have statistically increased the TAS values (1.27±0.22, p=0.0008).

Discussion

In this study, the effect of HBO therapy was examined on the ovarian reserve in a rat model of ovarian failure created with CYP. The results of the study showed that there was a significant decrease in ovarian reserve with CYP, then with HBO therapy after CYP, and a statistically significant increase was determined histopathologically in the primordial and primary follicle counts in these rats. From an examination of the literature, it can be seen that the current study is the first to have evaluated the effects of HBO in preventing CYP-related ovarian damage, and to have shown that HBO has a therapeutic effect against ovarian failure of CYP origin.

Chemotherapeutic agents have a negative effect on the reproductive potential of young women, but the mechanisms through which this occurs are still unclear. Alkylating agents, especially CYP, are toxic for the gonads because they are independent of cell proliferation[5]. CYP is used to treat several cancer types, primarily leukemia and lymphoma, which are the most frequently seen cancers in adolescent females[4]. CYP directly damages DNA, induces follicular apoptosis, and produces ROS, which damage ovarian cells[6]. CYP also leads to over-activation of primordial follicles by activating the P13K/AKT/mTOR pathway[7].

Morgan et al.[16] concluded that acute loss of the growing follicle population during chemotherapy results in increased intake of primordial follicles to the growing follicle pool. This damage shows two clinical phenotype stages: 1) amenorrhea, which is generally reversible and usually occurs a short time after chemotherapy, and 2) early menopause, which is generally irreversible and related to over-activation of primordial follicles by chemotherapy. In this study, a statistically significant decrease was determined in the number of primordial and primary follicles in the group administered CYP compared with the control group.

There is a great deal of evidence linking exposure to CYP with follicle atresia and granulosa cell apoptosis[17,18]. It has also been reported that inflammation and vessel expansion with CYP create secondary vascular damage[19]. In this study, a thickened tunica albuginea, and increased vessel structure and vessel expansion in the germinal matrix were determined. This finding of increased vascularization and expansion is consistent with the literature. Considering that venous congestion is thought to cause ovarian insufficiency, free oxygen radicals and inflammatory cytokines can be considered to accumulate more intensively in the ovarian tissue.

Table 2. Comparisons between the groups of the AMH, TAS, and TOS levels

	Group 1	Group 2	Group 3	p
AMH (ng/mL)	8.79±1.37[a]	7.03±0.47[b]	8.83±0.87[a]	<0.05
TOS (µmol/L)	3.85±0.62[a]	7.02±2.29[b]	3.70±1.19[a]	<0.05
TAS (mmol/L)	1.10±0.11[a]	0.79±0.12[b]	1.27±0.22[a]	<0.05

AMH: Anti-müllerian hormone, TAS: Total antioxidant status, TOS: Total oxidant status. The same letters in the same rows indicate similarity between the groups, and different letters indicate a difference

When the literature is examined, it can be seen that there has been an investigation of potential protective agents (eg., Curcumin and capsaicin, Tamoxifen, Sphingosine1-phosphate) against the direct loss or accelerated activation of primordial follicles, increasing atresia of growing follicles, and damage to the vascular system in the ovaries, caused by CYP[15,20,21]. In the study by Melekoğlu et al.[15], it was revealed that CYP causes premature ovarian failure, and it was concluded in this study that Curcumin (CRC) and Capsaicin (CPS) can be used to prevent premature ovarian failure in rats. It was observed that follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels decreased in rats receiving CRC and CPS, and simultaneously, amh levels increased with estrogen.

The results of the current study clearly showed that CYP reduced the number of primordial and primary follicles in rat ovaries. The numbers of pre-antral and secondary follicles in the rats administered CYP were also observed to be lower compared with the control group and the rats that received HBO.

The ovaries express the vascularity of primordial and pre-antral follicles from the stromal blood vessels. Additionally, the growth of primary follicles leads to the development of the vascular network in the theca layer. Embryos developing from oocytes obtained from well -vascularised follicles have a higher implantation rate than embryos obtained from oocytes developing from follicles with poor vascularity[22].

The positive effects of HBO on wound healing have been documented in animal experiments. There are many studies showing that HBO increases collagen production, supports angiogenesis and leads to the development of the vascular network causing the formation of new capillaries by inducing the release of stem cells from the bone marrow into circulation, thereby promoting mechanisms that trigger regeneration and healing[23,24].

HBO therapy triggers the angiogenesis mechanism by providing the oxygen needed and with increased release of several cytokines such as vascular endothelial growth factor[25]. Angiogenesis is of critical importance for follicular development, oocyte quality, and early embro development. This showed that adjuvant HBO therapy after chemotherapy could regulate ovarian function and hormone levels. Mitrović et al.[26] showed that HBO therapy could be a potential treatment option for infertility by increasing endometrial receptivity through a change in blood vessel resistance. In this study, HBO therapy was seen to correct the abnormal expansions in the blood vessels created by CYP, reduced congestion, and provided a normal vascular structure

close to that of the control group. This correction formed in the vascular structure suggests that follicle loss is prevented by reducing oxidative stress and inflammatory cytokines in the ovary.

In addition to reducing follicle loss, that HBO showed an effect on serum AMH levels was among the main aims of this study. Yu et al.[27] examined the effect of HBO therapy on ovarian function following cystectomy. A total of 60 patients with ovarian cysts were treated with laparoscopic ovarian cystectomy. HBO was added to patients in the observation group in addition to the treatment in the control group. The AMH, FSH, LH, estradiol (E2), and antral follicle count (AFC) serum levels were detected in both groups before the operation and at the first and third menstrual cycles postoperatively to evaluate ovarian function. After the operation, AMH, E2, and AFC serum levels in the observation group were significantly higher than in the control group. FSH and LH serum levels were significantly lower than in the control group, and the differences were statistically significant[27]. In a study of 4 patients by Pineda et al.[28], the effect of HBO was examined on serum AMH levels. In 2 of the 4 patients, the serum AMH level showed an increase of 40% and 116%. In one patient, no change was observed in the serum AMH level, and the other patient became pregnant during the HBO therapy. When the serun AMH levels were examined in the current study, the AMH levels were found to be significantly higher in group 3 than in group 2, which was consistent with the literature[28].

Although some studies have shown that HBO-induced oxidative damage[29,30], several others have stated that HBO has a protective effect against oxidative damage[31,32]. The debate is ongoing on whether HBO acts as an oxidant promoter or as an antioxidant agent. It has been shown that ROS and mitochondrial DNA (mtDNA) affect cellular aging in the human body, including the female reproductive system. More importantly, studies have suggested that ovarian aging could be negatively affected by excessive ROS[33]. Van Blerkom et al.[34] showed the importance of oxygen in oocyte meiosis. It was reported in that study that a decrease in oxygen content in the ovarian follicular fluid in humans is associated with an increase in abnormalities in the organization of chromosomes on the metaphase spindle, and it was emphasized that a sufficient oxygen source is necessary to allow the acceleration of chromosomes and normal oocyte maturation[34,35]. In this study, TOS was seen to be increased in group 2 compared with group 1, and TAS was statistically significantly higher in group 3 than in group 2. These results suggest that follicle loss in the ovaries was reduced and the negative effects of CYP were prevented with HBO therapy.

Study Limitations

The strength of our study is that it evaluated both tissue oxidative stress markers, ovarian reserve markers, and histopathological parameters together. The limitation of our study is that this study is an animal experiment, and further clinical studies should be done to show its effect on humans.

Conclusion

CYP negatively affects the ovarian reserve and causes severe follicle loss. HBO therapy could be an alternative treatment to minimize the effect on ovarian follicle loss of these alkylating agents, which must be used for treating tumors that commonly occur in young females of reproductive age. Although different pressures and durations of HBO were not applied in this study, the dose applied suggests that HBO could be protective for the ovaries. There is a need for further clinical trials to provide more clarification on this subject.

Ethics

Ethics Committee Approval: This study was approved by the Erciyes University Animal Experiments Ethic Committee (no: 21/168; date: 07.07.2021).

Informed Consent: Not necessary.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: F.Ç., M.A.B., A.C., M.E., Concept: F.Ç., M.A.B., E.M.A., Design: F.Ç., M.A.B., M.D., Data Collection or Processing: E.B., A.C., B.Y., Analysis or Interpretation: E.B., A.C., B.Y., Literature Search: F.Ç., M.A.B., M.D., E.M.A., Writing: F.Ç., M.A.B., M.D., E.B., A.C., E.M.A.

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The investigation of cholinergic receptor muscarinic 1 activity in the rat ovary with induced ovarian hyperstimulation

Ovaryan hiperstimülasyonu oluşturulan ratların overlerinde kolinerjik reseptör muskarinik 1 aktivitesinin araştırılması

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Abstract

Objective: We look at the immunoreactivity of cholinergic receptor muscarinic 1 (CHRM1) in the ovarian tissues of rats with ovarian hyperstimulation syndrome (OHSS) considering the possibility that the muscarinic activity may contribute to the pathophysiology of OHSS.

Materials and Methods: In this study, 14 immature female Wistar Albino rats were divided into two groups at random. The rats were 22 days old. Rats in the control group (n=7) were 22 days old, while those in the OHSS group (n=7) received 10 IU follicle-stimulating hormone subcutaneously over the course of four days and 30 IU human chorionic gonadotropin (hCG) on the fifth day to induce ovarian hyperstimulation. All the rats were sacrificed after all the groups' ovaries and blood samples were collected at the conclusion of the experiment. The left ovarian tissues were kept in aluminum foil at -80 °C, while the right ovarian tissues were kept in 10% formalin. Tissue vascular endothelial growth factor (VEGF), interleukin (IL)-1β, IL-6, IL-10, tumor necrosis factor (TNF)-α and malondialdehyde (MDA) levels were measured by The Enzyme Linked Immunosorbent Assay technique in the ovarian tissues. CHRM1 immunoreactivity was scored immunohistochemically.

Results: Ovarian weight, tissue IL-10, TNF-α, VEGF and MDA levels, and CHRM1 immunoreactivity were significantly increased in the OHSS group.

Conclusion: Increased levels of CHRM1 activity may play a role in the pathophysiology of OHSS. With further studies, the effect of luteinizing hormone and hCG on the ovarian and hypothalamic cholinergic system can be further investigated, and useful information can be obtained in determining OHSS prevention strategies.

Keywords: Rat, OHSS, cytokine, CHRM1

Öz

Amaç: Bu çalışmada ovaryan hiperstimülasyon sendromlu (OHSS) ratların ovaryan dokularında kolinerjik reseptör muskarinik 1'in (CHRM1) immünreaktivitesine, muskarinik aktivitenin OHSS patofizyolojisine katkıda bulunma olasılığı ışığında bakmayı amaçlandı.

Gereç ve Yöntemler: Bu çalışmada 14 adet 22 günlük immatür Wistar Albino dişi rat rastgele iki gruba ayrıldı. Grup 1'deki (n=7) ratlar (n=7) 22 günlük iken, Grup 2'deki (n=7) ratlar dört gün boyunca subkütan 10 IU folikül stimülan hormone ve beşinci günde ovaryan hiperstimülasyonu indüklemek için 30 IU insan koryonik gonadotropin (hCG) aldı. Deney bitiminde tüm grupların ovaryumları ve kan örnekleri alındıktan sonra tüm ratlar sakrifiye edildi. Sol over dokuları -80 °C'de alüminyum folyo içinde, sağ over dokuları ise %10'luk formalinde saklandı. Ovaryan dokuda vasküler endotelyal büyüme faktörü (VEGF), interlökin (IL)-1β, IL-6, IL-10, tümör nekroz faktör (TNF)-α ve malondialdehid (MDA) seviyeleri Enzyme Linked Immunosorbent Assay tekniği ile ölçüldü. CHRM1 immünoreaktivitesi, immünohistokimyasal olarak skorlandı.

Bulgular: Kontrol grubu ile karşılaştırıldığında OHSS grubunda over ağırlığı, doku IL-10, TNF-α, VEGF ve MDA seviyeleri ve CHRM1 immünreaktivitesi açısından istatistiksel olarak anlamlı bir artış vardı.

PRECIS: CHRM1 activity may play a role in the pathogenesis of OHSS.

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Sonuç: Artmış CHRM1 aktivitesi OHSS'nin patofizyolojisinde rol oynayabilir. Daha ileri çalışmalar ile lüteinizan hormonu ve hCG'nin over ve hipotalamik kolinerjik sistem üzerindeki etkisi daha fazla araştırılabilir ve OHSS önleme stratejilerinin belirlenmesinde faydalı bilgiler elde edilebilir.

Anahtar Kelimeler: Rat, OHSS, sitokin, CHRM1

Introduction

The ovarian hyperstimulation syndrome (OHSS), a typically fatal and iatrogenic side effect of fertility treatments, is connected to an acute, intense fluid migration from the vascular space to the third space because of elevated capillary permeability. In OHSS, there is edema in the ovarian stroma along with luteal follicle cysts, necrotic foci, and neovascularization. The risk of OHSS was dramatically decreased by employing a gonadotropin releasing hormone (GnRH) antagonist protocol instead of a GnRH agonist technique, inducing ovulation with GnRH agonists, and using vascular endothelial growth factor (VEGF) receptor blockers like cabergoline[1,2]. Even with these protective measures, severe OHSS still occurs, and doctors continue to struggle with determining the best therapeutic strategy[3]. The etiology of OHSS is significantly influenced by the shift in vascular permeability brought on by human chorionic gonadotropin (hCG). In addition to angiotensin II and other cytokines involved in angiogenesis, many vasoactive substances, including angiopoietin fibroblast growth factor, hypoxia-inducible factor, plasminogen activator, platelet-derived growth factor, protein kinase converting growth factor-, VEGF receptor, and urokinase-type plasminogen activator, play a role in the development of OHSS[4,5].

Vascular growth regulators, like VEGF, contribute significantly to inflammation through changes in vascular permeability, control over blood flow, and localized edema. The transfer of circulating immune cells to the ovulatory follicle as a classic inflammatory response is facilitated by changes in angiogenesis and vascular permeability along with increased follicular blood flow. However, it's important to terminate inflammation and vascular alterations as soon as possible, especially in the ovulatory follicle. Rapid angiogenesis and luteinization in the ovulatory follicle are made possible by tightly controlled vascular growth, which also prevents over vascularization of the corpus luteum. This might occur because angiopoietins and other vascular growth regulators have growth-restricting properties[4]. Angiogenin, some interleukins (IL) and tumor necrosis factor (TNF)-α and some cytokines increase capillary permeability and ovarian neovascularization and inflammatory response and trigger inhibition of hepatic albumin production. This results in many of the symptoms of OHSS[6,7].

The ovary maintains a balance between oxidative and anti-oxidative states. Theca cell proliferation is stimulated by mild oxidative stress (OS), but when OS increases, theca cell proliferation is inhibited[8]. Overgrown ovaries are linked to severe OHSS[9]. Pala et al.[10] suggested that hypoxia in the ovaries may increase VEGF levels in OHSS. It has also been suggested that rapid follicle growth despite moderate vascularization of granulosa cells during the ovulation stage create a hypoxic environment in the preovulatory follicle[4]. Therefore, in our study, we also evaluated tissue malondialdehyde (MDA) levels in relation to oxidative stress and some cytokines that may be associated with OHSS.

M1 receptors interact with Gq/11 type G proteins and activate phospholipases and calcium channels. It has been reported that acetylcholine (ACh), produced by granulosa cells (GCs) in human and rat oocytes, acts through muscarine (M)1, M3, and M5 receptors in their GCs[11]. Additionally, GCs form the ovarian cholinergic system by expressing muscarinic receptors for ACh. ACh, which is a part of this cholinergic system, has also been shown to stimulate ovarian growth[12,13].

In this experimental model, we investigated the role of cholinergic receptor muscarinic 1 (CHRM1) activity in the pathophysiology of OHSS.

Materials and Methods

Local Animal Ethics Committee of Fırat University approval was obtained for this experimental study (date: 16.01.2019, session no: 2019/01, decision no: 14). Many 14 immature female Wistar albino rats were obtained from Fırat University Experimental Investigations Center, Elazığ, TUrkey. All procedures performed on animals during the experiment were treated in accordance with the experimental animal care guide (NIH Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, National Research Council, Washington, D.C.). Rats were fed ad libitum with standard diet and tap water throughout the experiment in appropriate cages, in the laboratory with a 12 h light cycle (lights from 8 am to 8 pm).

Experimental Protocol

For the OHSS model, immature rats were used as stated in previous studies. The purpose of using immature rats is that in this model the ovarian physiology can be simplified and immature rats are not affected by the corpus luteum produced in the previous cycle[14].

The Control Group (n=7): The rats in the control group were given 0.1 mL of 0.9% saline every day for 5 days (22-26 days) and were decapitated on the 27th day.

OHSS Created Group (n=7): 10 IU of pregnant mare serum gonadotropin was injected subcutaneously for four days and 30 IU of hCG on day five to initiate OHSS. Rats were decapitated on the 27th day.

Rats were monitored daily for body weight, food consumption, behavioral changes, and signs of toxicity.

The rats were anesthetized by intramuscular administration of ketamine 80 mg/kg (Ketalar, Eczacıbaşı, İstanbul, Turkey) and xylazine 10 mg/kg (Rompun Vet, Bayer AB, İstanbul, Turkey).

The rats in both groups were decapitated by the cervical dislocation method at the end of the experimental period (on the 27th day). After anesthesia was provided, the abdomen of the rats was opened and the presence of ascitic fluid in the abdomen was checked. After that, both ovarian tissues were removed, quickly cleaned from the surrounding adipose tissue and dried on filter paper. After drying, the ovaries were weighed on an analytical balance and the organ weight, expressed in mg, was recorded. Left ovarian tissue was wrapped in aluminum foil and stored at -80 °C. The right ovarian tissue was placed in 10% formol. It was then embedded in paraffin blocks.

Biochemical Examination

Biomarkers were determined with the ELISA method on a BioTek EPOCH 2 Instrument using Elisa Kits by SunRed for such factors as tissue IL-1β (SunRed Biotechnology Company Katalog No: 201 11 0120), IL-6 (SunRed Biotechnology Company, Shanghai, China, Katalog No: 201 11 0136), IL-10 (SunRed Biotechnology Company Katalog no: 201 11 0109), TNF-α (SunRed Biotechnology Company, Katolog no: 201 11 0765), MDA (SunRed Biotechnology Company, Katolog no: 201 11 0157), VEGF (VEGFR-2 ELISA kit, SunRed Biotechnology Company, Katolog no: 201 1636). The absorbance was read spectrophotometrically at 450 nm on the ELX800 ELISA reader. Bio-tek ELX50 (BioTek Instruments, USA) was used as an automatic washer for plate washing. The results obtained are shown in pg/mL unit. The dilution factor multiplied by the dilution ratio method was used to calculate the results.

Immunohistochemical Examination

Sections of 4-6 µm thick from paraffin blocks were taken on slides and deparaffinized. Then, the sections passed through the alcohol series were boiled in citrate buffer solution at pH: 6 in a microwave oven (750W) for 12 min. After boiling, the tissues kept at room temperature for cooling were washed with phosphate buffered saline (PBS), and endogenous peroxidase activity was inhibited by applying hydrogen peroxide solution for 6 min. Block solution was applied for 5 min to the tissues washed with PBS for 3x5 minutes. Then, it was incubated with primary antibodies (CHRM1 polyclonal antibody, E-AB-14000, Elabscience, China) diluted at a ratio of 1/200 for 60 min at room temperature in a humid environment. After the primary antibody application, the tissues were washed with PBS for 3x5 minutes and incubated with secondary antibody compatible with the primary antibody for 30 min at room temperature in a humid environment. Tissues were washed with PBS for 3x5 minutes after secondary antibody application, incubated with Streptavidin for 60 min at room temperature and then taken into PBS. 3-amino-9-ethylcarbazole (AEC) Substrate + AEC Chromogen solution was dripped onto the tissues. Then, after the image signal was obtained under the light microscope, all groups were washed with PBS simultaneously. Tissues that were counterstained with Mayer's hematoxylin were passed through PBS and distilled water and closed with a water-based closure solution. Preparations were evaluated and photographed using the Leica DM500 microscope (DFC295; Leica, Wetzlar, Germany). Histoscore was established based on the extent and extent of immunoreactivity in staining[15]. Histoscore = prevalence x severity [The extent of immunoreactivity (0.1: <25%, 0.4: 26-50%, 0.6: 51-75%, 0.9: 76-100%); the intensity of immunoreactivity (0: no, + 0.5: very little, +1: less, +2: moderate, +3: severe)].

Statistical Analysis

SPSS version 22 program was used for statistical analysis of the data. Rats' body weights in grams (g) and ovary weights in milligrams (mg), tissue cytokines, VEGF and MDA levels were given as pg/mL, and CHRM1 immunoreactivity was given as histoscore. Mean relative ovarian tissue weights were obtained from the ratio of mean ovarian tissue weights to mean body weight of mice in the same group ([Overweight/Whole body weight ($\times 10^{-3}$)]. Quantitative data were expressed as median, minimum and maximum values. Kolmogorov-Smirnov method was used to determine the normal distribution of quantitative data. Mann-Whitney U test was used for comparison between groups in non-normally distributed quantitative data. $p < 0.05$ was considered statistically significant.

Results

Ovarian Weight: The proportion of ovarian weight to whole body weight increased in the OHSS group compared with the control group (p=0.001), (Table 1).

CHRM1 Immunoreactivity: CHRM1 immunoreactivity statistically significantly increased in the OHSS group compared with the control group (p=0.001), (Table 1, Figure 1).

Biochemical Findings: According to the results of the ELISA study performed on ovarian tissues; IL-10, TNF-α, VEGF and MDA levels were significantly increased in the OHSS group compared to the control group. IL-1β and IL-6 levels were similar in both groups (Table 2).

Discussion

In this experimental study, we showed that CHRM1 immunoreactivity was significantly increased in OHSS-induced

Table 1. The ratio of ovarian tissue to body weight and CHRM1 immunoreactivity histoscore. Values are shown as median (minimum-maximum)

Groups	Ovarian weight/total body weight ($x10^{-3}$) Median (minimum- maximum)	CHRM1
Control group	0.45 (0.28-0.59)	0.20 (0.10-0.45)
OHSS group	1.15 (0.89-1.66)*	0.90 (0.60-1.20)*
P-value	0.001	0.001

*: Compared to control group (p<0.05), CHRM1: Cholinergic receptor muscarinic 1, OHSS: Ovarian hyperstimulation syndrome

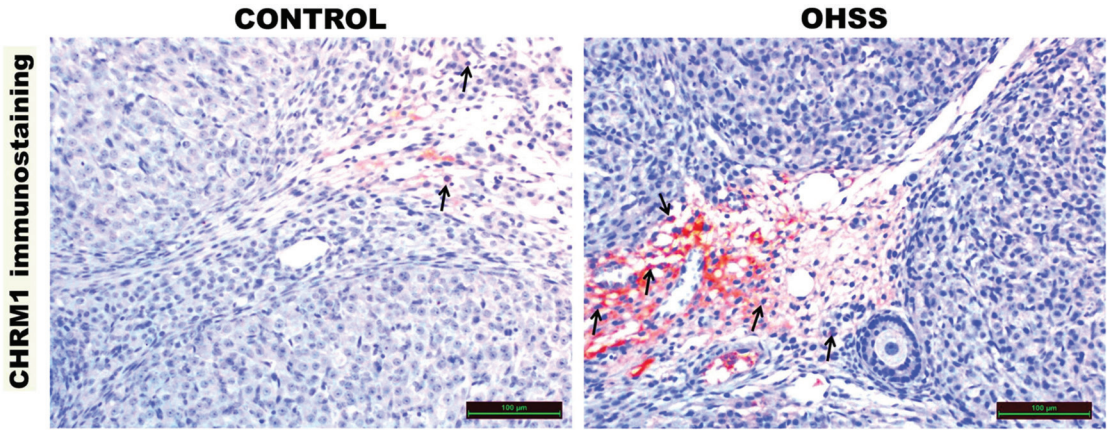

Figure 1. CHRM1 immune positive cells (black arrow) is observed

CHRM1: Cholinergic receptor muscarinic 1, OHSS: Ovarian hyperstimulation syndrome

Table 2. The tissue IL-1β, IL-6, IL-10, TNF-α, VEGF and MDA levels of all groups (pg/mL), values are shown as median (minimum-maximum)

Parameters	Control group	OHSS group	p-value
IL-1β	57.48 (43.43-140)	82.02 (56.27-351)	0.259
IL-6	22.42 (9.92-27.29)	28.88 (16.75-48.89)	0.165
IL-10	16.81 (11.29-27.47)	23.89 (19.03-41.14)	0.038*
TNF-α	39.50 (23.37-56.26)	61.84 (41.24-79.41)	0.007*
VEGF	1.12 (0.85-1.42)	1.75 (1.11-4.06)	0.017*
MDA	2.59 (1.73-4.35)	4.67 (3.42-6.85)	0.004*

*: Compared to control group, ($p<0.05$), OHSS: Ovarian hyperstimulation syndrome, IL: Interleukin, TNF: Tumor necrosis factor, MDA: Malondialdehyde, VEGF: Vascular endothelial growth factor

rats. This increase of CHRM1 immunoreactivity may indicate that CHRM1 may be a component of the mechanism that leads to excessive ovarian response in the clinical course of OHSS. Our study is the first to investigate CHRM1 immunoreactivity in the pathophysiology of OHSS.

Generally, in OHSS, the ovaries are highly enlarged and have a multi-cystic appearance[9]. We also observed significant growth and weight gain in the ovaries in our OHSS group. There is a highly active process of angiogenesis in the development of the corpus luteum. This angiogenesis process is controlled by autocrine, endocrine and paracrine factors. More than half of the total cell number of the corpus luteum is composed of endothelial cells. This makes the corpus luteum a tissue in the body where angiogenesis is most intense[16]. The high VEGF values in our study support the presence of increased angiogenesis in the OHSS group. Interestingly, in our study, we found an increase in MDA levels with the increase in VEGF levels. Based on this result, we thought that supraphysiological steroid hormone levels have harmful effects on ovarian tissue as MDA levels increase. It is unclear whether this is a consequence or a cause. TNF-α is cytotoxic for endothelial cells of the corpus luteum[17]. Therefore, we thought that high TNF-α levels in our OHSS group might cause endothelial damage in ovarian follicles. IL-10 is an anti-inflammatory cytokine. Therefore, it has been shown that IL-10 inhibits the production of many inflammatory cytokines such as IL-1, IL-2 and IL-6 by inhibiting TNF-α in monocytes[18,19]. Tissue IL-1β and IL-6 levels were similar in both groups in our study. However, we showed that IL-10 levels were increased in our OHSS group. This finding may suggest that a compensatory mechanism in ovarian tissue tries suppressing inflammation.

Endogenous acetylcholine can induce angiogenesis by acting on nicotinic and muscarinic receptors. M1 and M3-mAChR, which are found in most of the vessels, stimulate nitric oxide release because of muscarinic stimulation of the endothelium. However, overstimulation or disruption of the non-neuronal cholinergic system decreases endothelial barrier function. This reduction in the endothelial barrier creates a hyperpermeable environment for signaling molecules from the endothelium and for migrating immune system cells. This results in inflammation and an imbalance between proliferation and cell death may occur[20]. In our study, we found a significant increase in TNF-α levels, which is an inflammation marker, and MDA levels, which is an indicator of oxidative damage, with increased CHRM1 activity in our OHSS group. Additionally, we found significantly higher VEGF levels in relation to vascular permeability and neovascularization. These findings may suggest that OHSS has an inflammatory process in the ovarian tissue and that the increase in muscarinic stimulation may also contribute to this process.

Acetylcholine (ACh), produced by granulosa cells (GC) in rats and humans, has important functions in regulating ovarian

functions as part of an intraovarian system. ACh acts via M1, M3 and M5 muscarinic receptors in the GC[11]. Urra et al.[21] showed in their study that inhibition of acetylcholinesterase (AChE) with a specific AChE inhibitor, Huperzine A (Hup A), for 4 weeks, strongly changed follicular growth and reduced ovarian cyst formation due to intraovarian ACh increase. In our study, increased CHRM1 activity in the OHSS group may be associated with multifollicular development.

It has been shown that mammalian CHRMs modulate adenylate cyclase activity[22] and cAMP plays a role in the differentiation of GCs[23]. It has been shown that cAMP is central to the response to FSH by binding to the FSH receptors of newly formed primary follicles. Follicles that start to grow with neurotransmitter activity in more intensely innervated ovarian regions can enter gonadotropin control more rapidly by showing selective superiority compared to those not exposed to the effect of cAMP[24]. The stimulation we induced with FSH in our study may increase CHRM1 activity by some mechanisms.

On the day of proestrus, a signal produced by M1R-dependent acetylcholine in the follicular cells of the left ovary regulates GnRH secretion stimulates LH secretion and triggers ovulation[25]. Ovulation is a physiological process defined by the LH surge, rupture of the dominant follicle and release of the oocyte into the fallopian tube[4]. Some neurotransmission systems, similar to the cholinergic system, are involved in the increase in estradiol concentration and the regulation of the preovulation LH peak[25-28]. In an experimental study, it was shown that ovulation could be induced by injecting LHRH at 14.00 in rats treated with atropine during proestrus, while ovulation could not occur in rats with atropine implants[28]. Cruz et al.[29] showed that animals treated with atropine sulfate had a 24-hour delay in the preovulatory LH surge. In an experimental PCOS model, however, it was shown that the cholinergic system regulates steroid hormone secretion and the occurrence of ovulation depends on the presence of muscarinic receptors[30].

Our current study was not designed to describe in all detail the possible mechanisms between OHSS and cholinergic activity. In our study, we wanted to emphasize that the cholinergic system can be considered in the physiopathology of OHSS. Based on the above information, the cholinergic system has important roles in follicular growth, ovulation and ovarian steroid hormone regulation. For this reason, an antagonistic effect such as the relationship of the cholinergic system with agonist and antagonist drugs, reduction of estradiol production, suppression of ovulation can be obtained, especially in protocols in which controlled ovarian hyperstimulation is applied, and the OHSS formation can be reduced. However, this theory needs to be investigated with advanced and multidisciplinary experimental studies.

Study Limitations

The weaknesses of our study are that the population is small due to the limitations in the number of animals to prevent animal rights violations. Another weakness is that it is impossible to prove the relationship between the cholinergic system and OHSS only with our current parameters. It should not be overlooked that the results obtained from experimental studies may not match exactly with human results.

The strength of our study is that it is the first study to investigate CHRM1 activity in OHSS and the pathophysiology of OHSS with a different approach, as an example for future studies that will fill this gap in the literature. Since it is very difficult ethically to conduct studies on the ovary in humans, animal experiments are very valuable for such studies and form the basis for future clinical studies.

Conclusion

We showed that ovarian CHRM1 activity is also increased in OHSS. The cholinergic system may play a role at every stage from multifollicular development, increased angiogenesis, inflammation and vascular permeability to ovulation, which is responsible for developing OHSS. In this context, our study can investigate the use of anticholinesterase and muscarinic receptor blockers as an antagonist agent in reducing the formation of OHSS or in controlled ovarian stimulation.

*This experimental study is derived from Dr. Cengiz Şanlı's specialty thesis in medicine.

Ethics

Ethics Committee Approval: Local Animal Ethics Committee of Fırat University approval was obtained for this experimental study (date: 16.01.2019, session no: 2019/01, decision no: 14).

Informed Consent: All participants gave their informed consent before the study was conducted.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: C.**Ş**., R.A., N.İ., Concept: R.A., Design: R.A., T.K., N.İ., Data Collection or Processing: C.**Ş**., T.K., Ş.P., Analysis or Interpretation: R.A., T.K., Ş.P., N.İ., Literature Search: C.**Ş**., Ş.P., Writing: R.A., N.İ.

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Oocyte vitrification for oncological and social reasons

Onkolojik ve sosyal nedenlerle oosit vitrifikasyonu

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Abstract

The aim of this review is to present information related to oocyte cryopreservation, and particularly oocyte vitrification, performed to preserve fertility in oncologic and social indications. The success rates of oocyte cryopreservation have increased with the widespread use of the vitrification technique and are currently similar to those of in vitro fertilization performed with fresh oocytes. Vitrification is the most successful technique for oocyte cryopreservation. The most important factors that influence the success rate are the patient's age at the time of vitrification and the number of mature oocytes frozen. Thus, live birth rates differ for each age depending on the number of oocytes thawed and the freezing method. The American Society of Reproductive Medicine and the American Society of Clinical Oncology recommend presenting the option of oocyte cryopreservation for fertility preservation in cancer patients. Besides cancer patients, use of oocyte vitrification is increasing in women who wish to postpone pregnancy age and to have reproductive freedom with the development of the cryopreservation technique and the achievement of pregnancy rates similar to the use of fresh oocytes. Patients are provided consultancy service in terms of indication, the success rates by age, and the total number of oocytes frozen. It should be emphasized that this procedure is not a type of insurance policy for fertility, especially in elective oocyte cryopreservation.

Keywords: Oocyte vitrification, fertility preservation, oocyte cryopreservation

Öz

Bu derlemenin amacı onkolojik ve sosyal endikasyonlarla fertilitenin korunması amacıyla oosit kriyoprezervasyonu özellikle de oosit vitrifikasyonu ile ilgili güncel bilgileri sunmaktır. Oosit kriyoprezervasyon başarı oranları, vitrifikasyon tekniğinin yaygınlaşması ile birlikte artmış ve günümüzde taze oositlerle yapılan in vitro fertilizasyon gebelik oranlarına benzerdir. Vitrifikasyon oosit kriyoprezervasyonunda en başarılı tekniktir. Başarı oranını etkileyen en önemli faktör vitrifikasyon sırasında hastanın kaç yaşında olduğu ve kaç olgun yumurtasının dondurulduğudur. Dolayısıyla, çözülen yumurta sayısı ve dondurma yöntemine göre de her yaş için canlı doğum oranları farklıdır. Amerikan Üreme Sağlığı Birliği ve Amerikan Klinik Onkoloji Birliği, kanser hastalarında fertilite prezervasyonu için oosit kriyoprezervasyon seçeneğinin sunulmasını önermektedir. Kanser olguları dışında dondurma tekniğinin gelişmesi ve taze oositlerle benzer gebelik oranlarının elde edilmesiyle, gebelik yaşını ertelemek isteyen ve üreme özgürlüğünü kaybetmek istemeyen kadınlarda oosit vitrifikasyonunun kullanımı artmaktadır. Hastalara endikasyon, yaş ve toplam dondurulan oosit sayısına göre başarı oranları ile ilgili danışmanlık verilmelidir. Özellikle, elektif oosit kriyoprezervasyonunda bu işlemin fertilite için bir tür sigorta poliçesi olmadığı gerçeği vurgulanmalıdır.

Anahtar Kelimeler: Oosit vitrifikasyonu, fertilite prezervasyonu, oosit kriyoprezervasyonu

Introduction

A wide range of advancements have occurred in the area of assisted reproductive technology (ART) since the birth of Louise Brown in 1978[1]. In this area, maximum improvement has been observed in fertility preservation. Fertility preservation is a method of giving individuals the right to have their own genetic offspring by preserving the germ cells (oocytes, sperms) and the gonadal tissues (testicles, ovaries). Each condition that can create a risk of reduction in reproductive capacity in women and men is an indication for fertility preservation. The most common reasons for patients presenting for fertility preservation are as follows: will to have a surgical operation or receive chemotherapy because of cancer; they have medical conditions that could lead to premature menopause; or they wish to postpone pregnancy because of social reasons[2].

Oocyte and embryo cryopreservation has been one of the most important advancements in ART in the last 20 years. Although vitrification is entitled a novel technology, it was successfully used to freeze mouse embryos 35 years ago[3]. However, the use

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of vitrification in human oocytes and embryos did not become widespread until the end of the 1990s. Oocyte cryopreservation, which was previously considered experimental, was accepted as a method that should be routinely presented to patients who had indications for fertility preservation and was no longer considered an experimental method after a journal published in 2013 by the American Society of Reproductive Medicine (ASRM)[4].

The aim of this review is to present information related to oocyte cryopreservation, and particularly oocyte vitrification, performed for fertility preservation in oncologic and social indications.

Oocyte Vitrification

The first birth following the use of cryopreserved oocytes occurred at the end of the 1980s[5]. In all the studies after that, researchers have attempted to establish an ideal oocyte cryopreservation protocol. However, the expected advancements in this area could not be achieved due to technical issues and low success rates[6]. This was associated with difficulties related to the use of the slow-freezing technique. It is considerably difficult to freeze oocytes because of their large size and low surface area-to-volume ratio[7]. In oocyte cryopreservation, the large amount of water in oocytes leads to intracellular ice formation, chilling injury, and osmotic injury. Additionally, studies have shown that cryopreservation has negative effects on microtubule and microfilament stability, which are essential for normal chromosome segregation in mammalian oocytes[8,9]. Other difficulties related to cryopreservation include the hardening of the zona pellucida and the low fertilization rates related to this[10]. In later studies, it was shown that human oocytes regained morphology and chromosomal integrity following cryopreservation[11,12]. The number of studies related to oocyte cryopreservation has increased, especially in countries where embryo cryopreservation is illegal[13]. The use of the vitrification technique instead of slow freezing enabled both the reduction of injury in the internal structures of oocytes and higher pregnancy rates[14,15]. Oocyte cryopreservation, which was previously considered experimental, was accepted as a method that should be routinely presented to patients who had indications for fertility preservation, and it was no longer considered an experimental method with the journal published in 2013 by the ASRM[4]. Fertilization and pregnancy rates for in vitro fertilization (IVF) performed using vitrified/warmed oocytes have been reported to be similar to those using fresh oocytes[4].

Cryopreservation is the complete stopping of biological reactions by storing cells and tissues at temperatures below zero degrees Celsius for long periods. Cryoprotectant additives (CPA) are used to prevent ice formation and cryoinjury. They are classified as permeating or non-permeating CPAs depending on their ability to permeate the cellular membrane[16]. Various combinations of permeating and non-permeating CPAs can be used. Two techniques are used in the cryopreservation of human oocytes: Slow-freezing and ultrarapid cooling with vitrification.

In the slow-freezing technique, oocytes are exposed to low concentrations of CPAs and the temperature is reduced slowly. Cooling to -5 to -7 °C, at which point balance and seeding occurs, is conducted primarily. Subsequently, cooling at a slow rate (0.3-0.5 degrees/min) continues until a temperature of (-30)-(-60) °C is achieved. Afterwards, liquid nitrogen is added for storing[17]. Studies comparing slow-frozen and fresh oocytes have shown that the results are worse with frozen/thawed oocytes[18,19].

Higher concentrations of CPA are used in vitrification and this reduces the risk for crystallization and ice nucleation. Additionally, the cooling rate is 100s-10.000 °C/minute[17]. In the early days of vitrification, high concentrations of CPA were used for longer periods, and this used led to osmotic stress. In later studies, the use of CPA mixtures was initiated to reduce this osmotic stress. A combination of ethylene glycol-dimethyl sulfoxide (1:1) is considerably efficient[20]. A high number of studies have shown that vitrification is superior to slow-freeze protocols. Although a low number of pregnancies were obtained with cryopreserved oocytes in this period, a meta-analysis emphasized that better pregnancy rates were achieved with oocytes frozen by way of the vitrification method[21]. When the IVF results obtained with slow-frozen and vitrified oocytes were compared, better survival, fertilization, and pregnancy rates were shown with vitrification[15,22]. There is accumulated evidence showing that the results of IVF performed with vitrified oocytes are similar to the results of IVF performed with fresh oocytes[23]. Clinical pregnancy rates range between 35.5% and 65.2% per transfer[24,25]. A meta-analysis found that the fertilization, embryo cleavage, high-quality embryo, and continuing pregnancy rates with the vitrification method were similar to the rates obtained with the use of fresh oocytes[2]. These studies concluded that the appropriate technique for oocyte cryopreservation was vitrification and the 2013 National Institute for Health and Care Excellence guideline reported that the vitrification technique should be used instead of controlled-rate freezing in oocyte and embryo cryopreservation if the required equipment is available[26]. In a retrospective cohort study, which compared 96 frozen embryos obtained from frozen oocytes with 4.394 frozen embryos obtained from fresh oocytes, no significant difference was found between embryo viability rates following thawing and live birth rates per cycle (97.2% vs. 95.7%, $p<0.005$, survival rate; 33.8% vs. 30.9%, $p<0.005$, live birth rates)[27].

Two classifications are related to the vitrification technique: open and closed vitrification. In open vitrification, there is direct contact between oocytes and liquid nitrogen, and low-volume devices, including capillary glass, cooper devices, pulled straws, and loops, are used[28]. In closed vitrification, there is indirect contact between oocytes and liquid nitrogen because tubing systems are used[28].

Oocyte Cryopreservation Results

In studies evaluating long-term obstetric and perinatal outcomes related to vitrification, negative obstetric and perinatal outcomes related to vitrification have not been reported[29]. The mean birth weight and frequency of congenital anomalies in infants produced by oocyte vitrification are not different from those of spontaneous pregnancies or IVF[29]. In another study, the frequency of congenital anomalies (1.3%) was found to be the same in pregnancies obtained by cryopreservation performed with slow-freezing and vitrification[30]. In conclusion, more than 5.000 live births have occurred with oocyte freezing up to the present, and the rate of congenital anomalies reported for these births is not different from the rate reported for ART and the normal population. However, no data related to long-term follow-up with these children have been published yet.

Fertility Preservation in Cancer Patients

Approximately 10% of women diagnosed with cancer are 45 years old or younger[31]. Requests for fertility preservation are increasing with an increase in the survival rates of cancer[30]. Gonadal failure and related infertility are among the long-term negative effects of radiotherapy and chemotherapy.

In 2006, the ASRM presented an opinion that oncologists should also discuss potential infertility problems and fertility-preserving approaches when informing and counseling patients who are at the reproductive age before cancer treatment and refer to these patients to reproductive health specialists if needed[32]. Although awareness is greatly increased, fertility-preservation approaches are not being applied at an adequate level. Strengthening multidisciplinary cooperation and the widespread use of fertility-preservation services will increase the number of patients.

In oncologic patients, embryo, ovarian tissue, and oocyte cryopreservation are the available options for fertility preservation[31]. Although embryo cryopreservation is the best option, the need for a partner and separations that could be experienced during treatment processes are the disadvantages[33]. The most appropriate option for single women to have a chance of getting pregnant with their own gametes is oocyte cryopreservation. With the advances in the oocyte cryopreservation technique, oocyte cryopreservation is being routinely recommended in fertility preservation. The most important disadvantage in oocyte cryopreservation in these patients is the need for controlled ovarian stimulation to collect oocytes. This leads to a delay in cancer treatment for weeks and poses a risk related to high levels of estrogen in hormone-receptive cancers[34]. However, these problems have been solved, particularly with the use of protocols that are independent of the menstrual cycle, shortening the delay period in initiating treatment, and the use of anti-oestrogens in stimulation in women with breast cancer[35,36].

Social Oocyte Cryopreservation

Worldwide, women are postponing pregnancy to later ages. Currently, oocyte cryopreservation is considered an acceptable method for age-related fertility reduction[37,38]. The popularity of social egg freezing is gradually increasing.

It is a well-known phenomenon that fertility rapidly decreases in women after the age of 35 years[37]. With oocyte freezing, women attain reproductive freedom later in life, like men. The two most important factors that determine the possibility of live birth with cryopreserved oocytes are the total number of mature oocytes and the age of the woman at the time of oocyte collection. Although the primary studies showed that collecting at least eight oocytes increased the rates of live birth at all ages from 22% to 46%, later studies found that this effect was lower in older women[39]. At the age of 35 years and below, the rates of live birth per patient are approximately two-fold higher compared to 36 years and above, and it was concluded that oocyte cryopreservation should be recommended to women aged 36 years and below for maximum success rates[40]. The possibility of live birth per thawed oocyte, which is known as oocyte efficiency rate, was 6.5%. As expected, the oocyte efficiency rate decreases with age, and this decrease is more prominent after the age of 37 years (7.4% for <30 years, 7% for <35 years, 6.5% for 35-37 years and 5.2% for 38-40 years)[41]. In conclusion, women should be informed accurately about the oocyte cryopreservation technique and success rates, and the fact that this procedure is a medical procedure rather than being an insurance policy should be emphasized. Another concern is the high cost of oocyte cryopreservation. Physiologically, the most appropriate age range for oocyte cryopreservation is the early- and middle-thirties. However, the most cost-effective strategy is still unclear[42]. Nevertheless, it was shown that the age range between 35 and 37 was the most appropriate period in terms of cost-effectiveness, and cryopreservation could be performed up to the age of 40 years[42].

Conclusion

The oocyte vitrification technique is efficient and safe for oocyte cryopreservation. The fertilization, embryo development, and pregnancy rates are similar compared to fresh oocytes. In oncologic patients, oocyte cryopreservation is the only chance for fertility preservation for these patients. Women who wish for elective oocyte cryopreservation should be informed about success rates by age and the number of mature oocytes, and it should also be emphasized that long-term outcomes of babies obtained by way of cryopreserved oocytes are not known.

Ethics

Peer-review: Internally and internally peer-reviewed.

Authorship Contributions

Concept: N.K., Design: N.K., Analysis or Interpretation: N.K., T.A., Literature Search: N.K., T.A., Writing: N.K., T.A.

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A meta-analysis of fertility and adverse outcomes in oil- and water-based contrast for hysterosalpingography

Histerosalpingografide kullanılan yağ ve su bazlı kontrast maddenin doğurganlık ve olumsuz sonuçlar üzerine etkisinin meta-analizi

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Abstract

Infertility is the inability to conceive after one year of regular unprotected intercourse. There is a debate about the therapeutic effect of hysterosalpingography (HSG) and whether the selection of contrast materials makes a difference in the chance of subsequent conception. In this study, we aimed to compare the fertility-enhancing outcomes and adverse effects of oil and water-based contrasts in patients who underwent HSG. This systematic review and meta-analysis was conducted following the PRISMA guidelines. We searched the Web of Science, PubMed, and Scopus until September 2022. We included all primary randomized controlled trials evaluating the fertility-enhancing benefits of HSG in oil-based versus water-based contrast media in women of childbearing age with infertility. Eleven studies with 4,739 patients were selected. The pregnancy rate in the oil group was significantly higher than that in the water group [odds ratio (OR)=1.51 (1.23, 1.86), p<0.0001]. Our meta-analysis favored the oil group in abdominal pain and vaginal bleeding with the odd ratios of 0.73 (0.58, 0.91), (p=0.006) and 0.91 (0.46, 1.81), (p=0.79), respectively. Water-based contrast was associated with less intravasation [OR=2.09 (1.09-4.02), p=0.03]. There were no differences between the contrasts for miscarriage [OR=1.02 (0.71, 1.46), p=0.92], and ectopic pregnancy [OR=0.84 (0.27, 2.63), p=0.77]. HSG with oil-based contrast was related to a higher pregnancy rate, live birth rate, and intravasation rate. While HSG using a water-based contrast medium was associated with increased abdominal discomfort, vaginal bleeding, and the visual-analogue scale pain score.

Keywords: Hysterosalpingography, infertility, contrast media, pregnancy outcome

Öz

Kısırlık, bir yıl düzenli korunmasız ilişkiden sonra gebe kalamama durumudur. Histerosalpingografinin (HSG) terapötik etkisi ve kontrast madde seçiminin sonraki gebe kalma şansı üzerinde bir fark yaratıp yaratmadığı konusunda bir tartışma vardır. Bu çalışmada, HSG uygulanan hastalarda yağ ve su bazlı kontrastların doğurganlığı artırıcı sonuçlarını ve yan etkilerini karşılaştırmayı amaçladık. Bu sistematik inceleme ve meta-analiz, PRISMA yönergeleri izlenerek yapılmıştır. Eylül 2022'ye kadar Web of Science, PubMed ve Scopus'ta arama yapılmıştır. Kısırlığı olan doğurganlık çağındaki kadınlarda su bazlı ve yağ bazlı kontrast maddelerin kullanıldığı HSG uygulamalarının doğurganlığı artıran faydalarını karşılaştıran tüm primer randomize kontrollü çalışmalar dahil edilmiştir. Dört bin yedi yüz otuz dokuz hasta ile 11 çalışma dahil edilmiştir. Yağ grubundaki gebelik oranı, su grubundan anlamlı olarak yüksekti [tahmini rölatif risk (RR)=1,51 (1,23, 1,86), p<0,0001]. Meta-analizimiz, sırasıyla 0,73 (0,58, 0,91), (0,006) ve 0,91 (0,46, 1,81), (p=0,79) tahmini RR değerleri ile karın ağrısı ve vajinal kanama açısından yağ grubu lehine sonuçlandı. Su bazlı kontrast daha az intravazasyon ile ilişkilendirildi [RR=2,09 (1,09-4,02), p=0,03]. Düşük [RR=1,02 (0,71, 1,46), p=0,92] ve dış gebelik [RR=0,84 (0,27, 2,63), p=0,77] açısından kontrastlar arasında fark yoktu. Yağ bazlı kontrastlı HSG, daha yüksek gebelik oranı, canlı doğum oranı ve intravazasyon oranı ile ilişkiliydi. Su bazlı bir kontrast madde kullanan HSG, artmış karın rahatsızlığı, vajinal kanama ve görsel-analog skala ağrı skoru ile ilişkilendirildi.

Anahtar Kelimeler: Histerosalpingografi, infertilite, kontrast madde, gebelik ile sonlanım

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Introduction

Infertility is the term used to describe a patient who fails to conceive after one year of regular unprotected intercourse. Infertility affects 12% of reproductive-aged women worldwide. Female factors represent about 46% of infertility causes[1,2]. Fertilization occurs in the fallopian tubes. Hence, functioning fallopian tubes are essential for conception[3]. One-third of infertility cases are attributable to fallopian tube obstruction. Tubal damage frequently a results from adhesions, where proximal tubal occlusion is associated with endometriosis, while distal tubal occlusion is commonly caused by pelvic inflammatory disease[4].

Laparoscopy is the gold standard investigation for the diagnosing of tubal diseases, whereas minimally invasive Hysterosalpingography (HSG) is the first line of radiological evaluation for tubal patency. HSG detects tubal blockage using a contrast medium to visualize the endometrial cavity and fallopian tubes[5]. The sensitivity and specificity of HSG in detecting tubal obstruction are 65% and 83%, respectively, with an accuracy rate of 71%[3,4].

HSG is often conducted using either water-soluble or oil-soluble contrast as a medium. Although HSG is a diagnostic procedure, there is continuing debate about its therapeutic effect and whether the selection of contrast materials makes a difference in the chance of subsequent conception. Previous randomized controlled trials (RCT) suggested that an oil-based contrast medium is more favorable than a water-based contrast medium due to its fertility-enhancing effects and good image quality[6,7]. However, an oil-based contrast medium takes longer to deliver, causing prolonged discomfort and posing a theoretical risk of intravasation and embolism[8]. A systematic review with meta-analysis comparing the therapeutic effects of oil-based versus water-based contrast mediums in HSG was published in 2018. This review, with six trials and a total of 2,562 patients, concluded that an oil-based contrast medium has a higher pregnancy rate with an odd ratio of 1.47 compared with a water-based contrast medium[9]. However, there are three trials with an unknown bias profile. Since then, several RCTs with sample sizes greater than 1,000 and longer post-HSG follow-ups have been published.

The primary objective of this study was to conduct high evidence systematic review and meta-analysis of the scientific literature to determine the fertility-enhancing outcomes and adverse effects of oil-soluble contrast media versus water-soluble contrast medium in patients undergoing HSG.

Materials and Methods

This systematic review and meta-analysis was prepared based on the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA)[10].

Literature Searchs and Information Sources

Searches were carried out in the following major electronic databases: Web of Science, PubMed, and Scopus till Sept 2022, using the following strategy "hysterosalpingography" or "HSG" or "salpingogram" or "hysterosalpingogram" AND "Water-soluble contrast media" or "water-based contrast material (WBCM)" or "oil-soluble contrast media", or "oil-based contrast material (OBCM)" or "lipiodol" or "ethiodol", "Ethiodized" or "iotrolan" or "Tubal flushing". There were no search filters or language limitations.

Selection Criteria and Eligibility Criteria

We conducted the selection and inclusion process for the study in two stages. We screened the titles and abstracts in the first stage to identify potentially relevant articles. In the second stage, we evaluated relevant articles and included them based on our inclusion criteria. We included all primary RCTs comparing the enhancing-fertility effects of HSG in oil-based contrast medium against a water-based contrast medium in children-bearing aged women with infertility. Any RCTs, which did not evaluate the therapeutic effects of fertility were excluded. We also excluded studies that evaluated the effectiveness of HSG using a single contrast agent without any comparison. Any studies other than RCTs, such as case reviews, case reports, and case series were excluded.

Data Extraction

We extracted data from the included RCTs and plotted them on an extraction sheet. Other objective outcomes, such as pregnancy outcomes, discomfort, and adverse effects, were recorded. We also collected data on pregnancy rate, live birth, miscarriage, ectopic pregnancy, abnormal pain, vaginal bleeding, intravasation, pain VAS score, and duration between HSG and pregnancy. We also extract relevant data for quality assessments according to the Cochrane assessment tool[11].

Outcomes

The primary outcome is ongoing pregnancy, which is a positive fetal heartbeat on ultrasound at 12 weeks of gestation. The secondary outcome was the successful conception, which includes (1) gestation sac detection on ultrasonography, (2) live birth (defined as the birth of an infant with the signs of life after 24 weeks of gestation), (3) Miscarriage (defined as no evidence of foetal heartbeat detected on ultrasound or spontaneous loss of pregnancy before 20 weeks of gestation), and (4) ectopic pregnancy (defined as implantation occurs outside the uterus). The degree of pain after HSG is measured by the visual-analogue scale on a scale between 0 and 10, where a high value represents more severe pains.

Quality Assessment

Only RCTs were included in this study. Thus, they were assessed using the Cochrane risk of bias assessment tool[11]. We examined each study for identifiable biases, which are listed as follows: (1) no random sequence generation, (2) no blinding of participants and personnel, (3) no allocation concealment, (4) no blinding of outcome assessment, (5) incomplete outcome data, (6) selective reporting, and (7) other biases. For each

domain, trials could be classified as low, unclear, or high risk of bias.

Statistical Analysis

Statistical analyses were performed with RevMan 5.4.1 software to assess the retrieved data. Our study included continuous and dichotomous outcomes. We used the inverse variance method to analyze the continuous data using mean difference (MD) and 95% confidence intervals (CI), while dichotomous data were analyzed using Mantel-Haenszel method which were calculated using odds ratio (OR) and 95% CIs. The presence of heterogeneity among the studies was measured by the I^2 and the p-value of the chi-square test. Values of $p<0.1$ or $I^2>50\%$ were significant indicators of heterogeneity. We tried solving the inconsistency among data using the Cochrane leave-one-out method[12].

Results

Search Results and Characteristics of the Included Studies

The search results are illustrated in the PRISMA flow diagram (Figure 1). We included 11 studies[6,13,14-22], which met our inclusion criteria. We analyzed 4,739 patients who underwent HSG either by OBCM or WBCM. The average age of the included patients from both groups was 28.48 years.

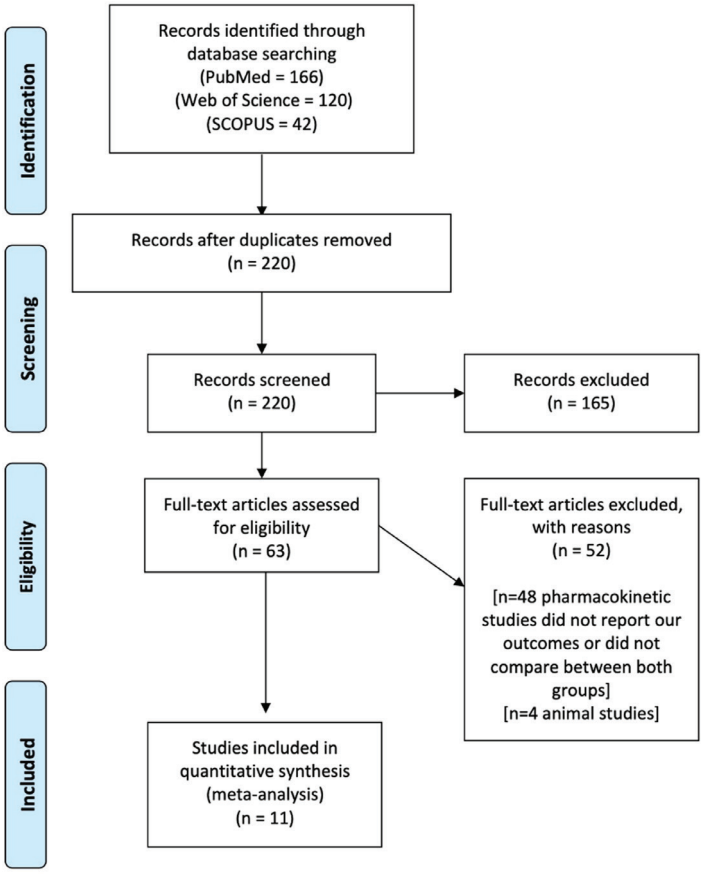

Figure 1. Shows the PRISMA flow diagram

Table 1 shows the baseline characteristics of the included studies.

Results of the Risk of Bias Assessment

All studies were evaluated according to Cochrane's tool[23]. Regarding randomization, six studies[6,13,15,16,21,22] reported proper randomization and were categorized as low risk of bias, while the other five studies[14,17-20] reported insufficient details regarding the randomization domain therefore they were categorized as unclear risk of bias. Concerning the performance bias, only Dreyer et al.[15] were categorized as high risk of bias, the remaining studies were categorized as unclear risk of bias. In detection bias, all studies were categorized as unclear risk of bias, except Zhang et al.[6] who reported adequate blinding of the outcome investigators. Figure 2 shows a detailed illustration of the risk of bias of the included studies.

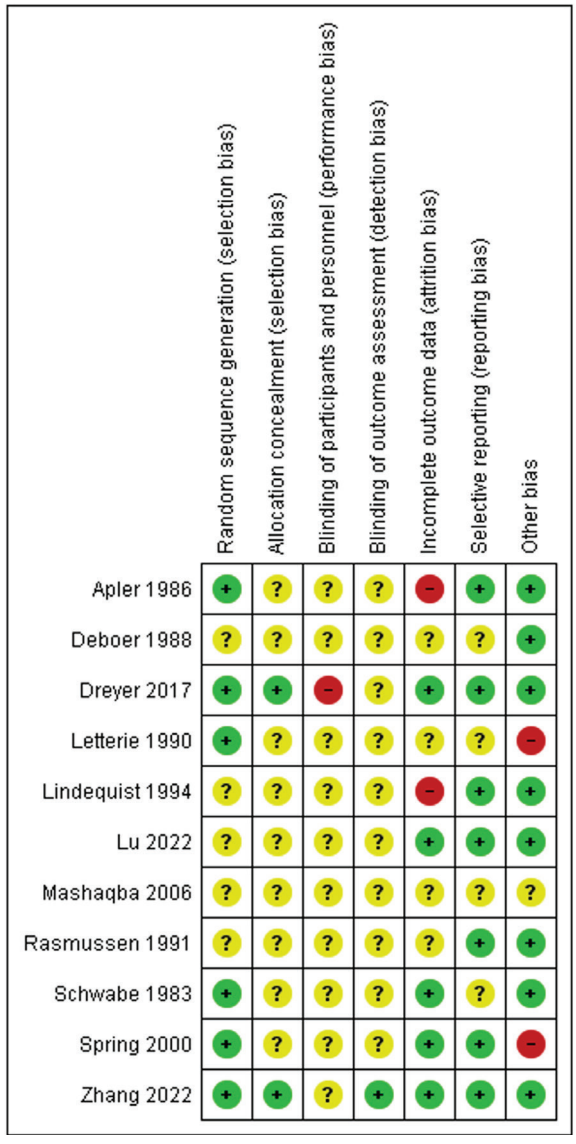

Figure 2. Shows a detailed illustration of the risk of bias of included studies

Analysis of Outcome

1. Pregnancy Rate

Eleven studies[6,13,14-21,22] reported this outcome. The overall analysis showed that the pregnancy rate was significantly higher in the oil group than in the water group [OR=1.51 (1.23, 1.86), (p<0.0001)]. Data were heterogeneous (p=0.05); I^2=46% (Figure 3A). We solved the heterogeneity by excluding Spring et al.[22] (p=0.85); I^2=0%. The combined estimate after solving the heterogeneity also favored the oil group [OR=1.64 (1.43, 1.89), (p<0.00001)] (Figure 3B).

2. Live Birth

This outcome was reported by four studies[6,15,20,22]. We divided the four studies into two subgroups. The first subgroup included two studies that used HSG for therapeutic reasons[6,15]. The overall OR in this subgroup favored the oil group significantly [OR=1.55 (1.28, 1.86), (p<0.00001)]. data were homogeneous (p=0.58); I^2=0%.

Regarding the second subgroup, which included two other studies[20,22] that used HSG for diagnostic reasons, there was no significant variation between both groups [OR=1.76 (0.48, 6.44), (p=0.39)]. We faced a significant heterogeneity in this subgroup (p=0.0002); I^2=93%.

The overall analysis of the four studies showed that live birth is significantly higher in the oil group than in the water group [OR=1.59 (1.09, 2.33), (p=0.02)] (Figure 4).

3. Miscarriage

2,668 patients were analyzed from four studies[6,15,19,22], which reported the incidence of miscarriage. The combined estimate showed very similar values [OR=1.02 (0.71, 1.46), (p=0.92)]. We found a moderate heterogeneity among studies (p=0.10); I^2=56% (Figure 5).

4. Ectopic Pregnancy

Our analysis of data retrieved from three studies[15,19,22] showed that both groups are associated with similar ectopic incidence [OR=0.84 (0.27, 2.63), (p=0.77)]. Our results were homogeneous (p=0.54); I^2=0% (Figure 6).

5. Abnormal Pain

This outcome was reported by two studies[6,18]. The overall OR favored the oil group over the water group [OR=0.73 (0.58, 0.91), (p=0.006)]. Data were homogeneous (p=0.31); I^2=3% (Figure 7).

6. Vaginal Bleeding

Three studies reported vaginal bleeding[6,17,18]. We found no variation between both groups [OR=0.91 (0.46, 1.81), (p=0.79)]. Although we found heterogeneity among studies (p=0.01); I^2=77% (Figure 8A), we could solve this heterogeneity by excluding Lindequist et al.[17] (p=0.88); I^2=0%. The overall analysis after solving heterogeneity showed that the oil group had less incidence of vaginal bleeding [OR=0.67 (0.52, 0.86), (p=0.002)] (Figure 8B).

Table 1. Shows the baseline characteristics of the included studies

Study	Country		Sample size		Age, years mean (SD or IQR)		Duration of infertility		Intervention	
	OBCM	WBCM	OBCM	WBCM	OBCM	WBCM	OBCM	WBCM	OBCM	WBCM
Alper[13]	Canada		46	60	29.3 (4.6)	29.1 (2.9)	NR	NR	Lipiodol	Renographin
de Boer[14]	Netherlands		87	88	29 (19-44)	29 (19-44)	37 (26.2)		Lipiodol	Iopamidol
Dreyer[15]	Netherlands		554	554	32.8 (30-36)	33.0 (30-36)	19.8 (16.0-26.3)	19.6 (15.4-27.4)	Lipiodol	Telebrix
Letterie[16]	USA		15	14	27 (3.5)	25 (4.1)	NR	NR	Ethiodized oil	Conray-60
Lindequist[17]	Denmark		121	121	29.9 (21-43)	29.5 (20-40)	40	41	Lipiodol	Iotrolan
Lu[18]	China		500	500	29.0 (24.3-32.0)	27.0 (24.0-32.0)	24 (12-36)	24 (12-36)	Ethiodized poppy seed oil	Ioversol
Mashaqba[19]	Jordan		35	40	28 (3)	28 (4)	NR	NR	NR	NR
Rasmussen[20]	Denmark		98	300	22.0 (4.5)	22.4 (5.5)	NR	NR	Lipiodol	Iohexol, ioxaglate, diatrizoate
Schwabe[21]	USA		56	65	NR	NR	NR	NR	Ethiodol	Sinografin
Spring[22]	USA		273	260	29.3 (4.6)	29.1 (2.9)	37.8 (38.1)	37.5 (36.3)	Lipiodol	Diatrizoate, iodipamide
Zhang[6]	China		473	479	30.5 (3.7)	30.8 (3.6)	20.4 (13.32)	20.24 (19.93)	Ethiodized poppyseed oil	Iohexol, Iopromide, Ioverol

OBCM: Oil-based contrast material, WBCM: Water-based contrast material, NR: Unreported, SD: Standard deviation, IQR: Interquartile range.

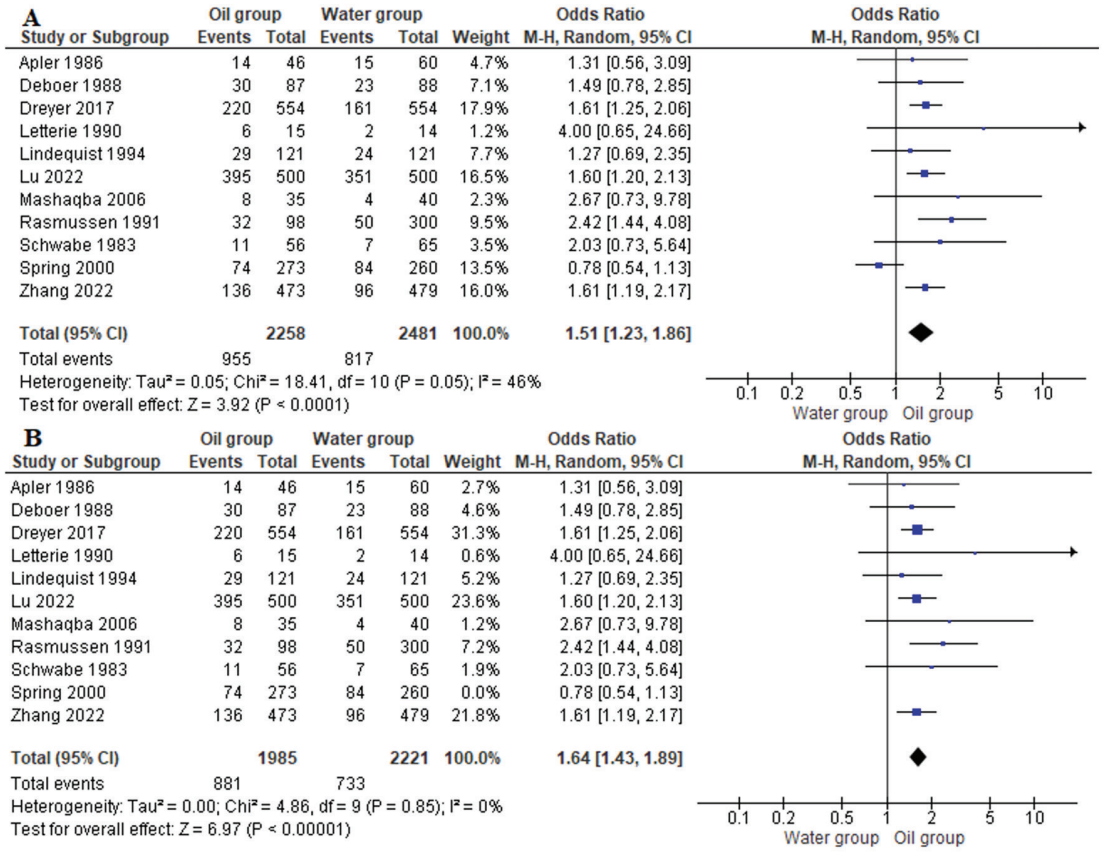

Figure 3. Shows the outcome of pregnancy rate-part A includes 11 studies[6,13,14-22] & part B excludes Spring et al.[22]

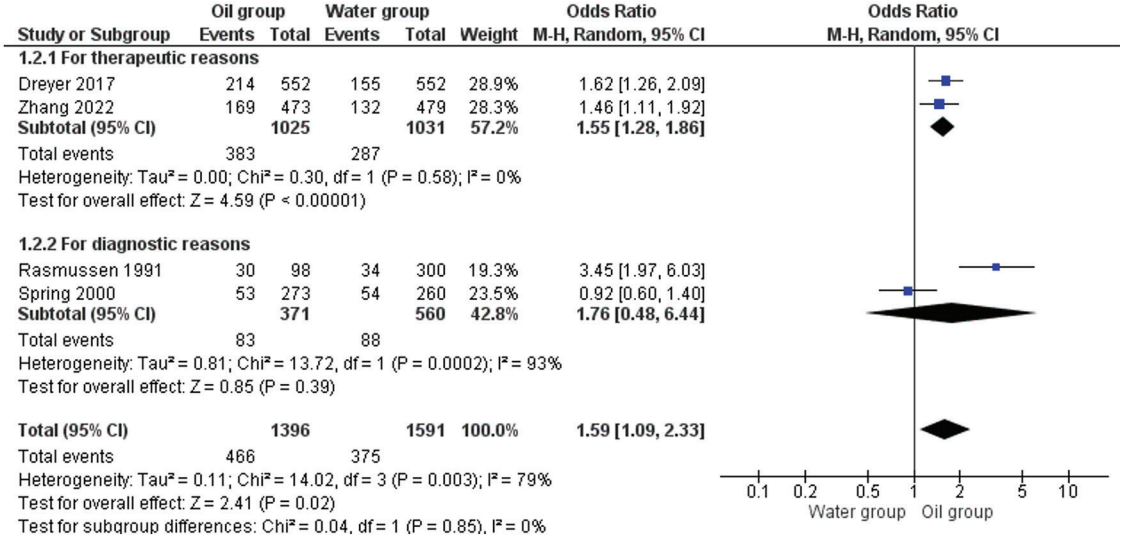

Figure 4. Shows the outcome of live birth

7. Intravasation

2,516 patients were analyzed from five studies[6,13,15,17,19] that investigated this side effect. We found that HSG by water-based contrast was associated with a lower incidence of intravasation than oil-based contrast [OR=2.09 (1.09, 4.02), (p=0.03)]. The overall analysis was homogenous (p=0.33); I^2=12% (Figure 9).

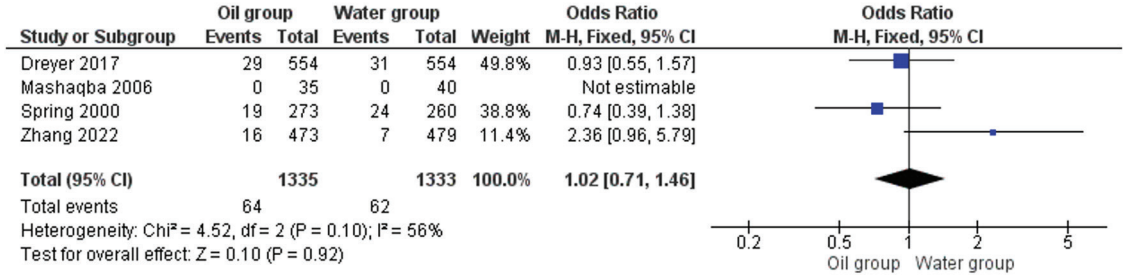

Figure 5. Shows the outcome of miscarriage

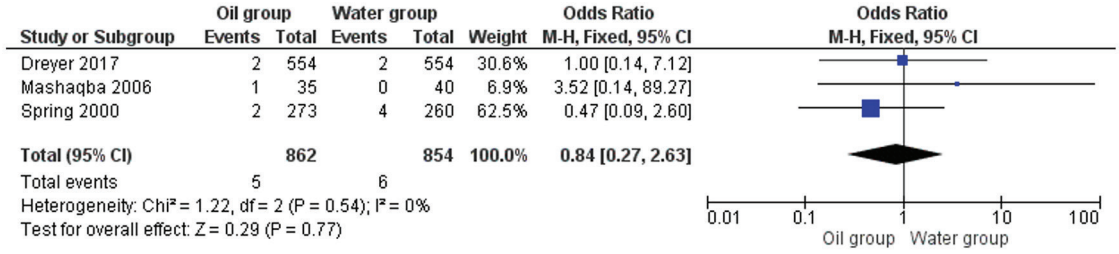

Figure 6. Shows the outcome of ectopic pregnancy

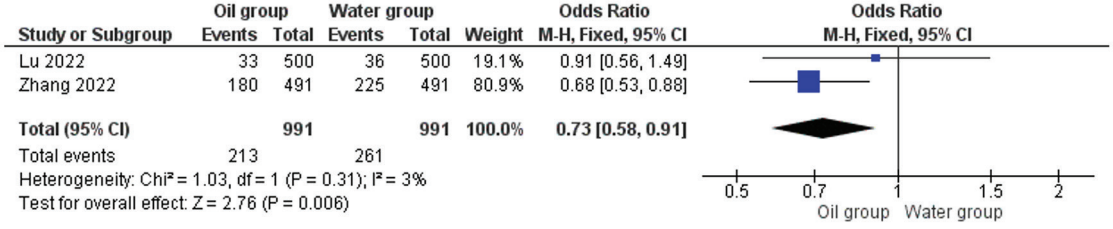

Figure 7. Shows the outcome of abnormal pain

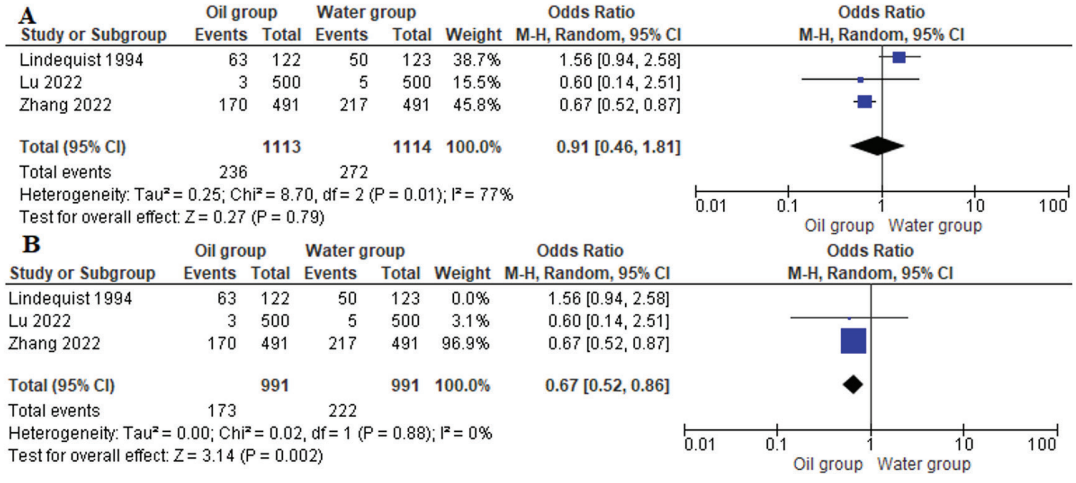

Figure 8. Shows the outcome of vaginal bleeding-part A includes three studies[6,18,19] & part B excludes Lindequist et al.[17]

8. Pain VAS Scores

Three studies[6,13,15] assessed the pain VAS score among the included patients. The overall mean difference showed that the pain VAS score was significantly lower in the oil group than in the water group [MD=-0.40 (-0.56, -0.24), (p<0.00001)]. We found no heterogeneity among data (p=0.25); I^2=28% (Figure 10).

9. Duration Between HSG and Pregnancy (Weeks)

This outcome was reported by four studies[6,15,18,19]. The combined estimate showed no difference between both groups [MD=-1.08 (-3.43, 1.28), (p=0.37)]. The analysis showed major heterogeneity (p=0.0002); I^2=85% (Figure 11A). We could solve this heterogeneity by excluding Zhang et al.[6] (p=0.19); I^2=41%. The overall analysis after solving this heterogeneity also showed similar values in both groups [MD=0.41 (-0.72, 1.55), (p=0.48)] (Figure 11B).

Discussion

This is the most recent meta-analysis comparing the results of HSG performed with OBCM and WBCM. Our meta-analysis revealed that the pregnancy rate in patients who had HSG with OBCM was 1.51 times greater than in those who had WBCM. This agrees with previous studies. In terms of pregnancy outcome, patients receiving OBCM are more likely to deliver a live birth than those receiving WBCM. There were no statistically significant differences between these two contrast materials for patients with miscarriage and ectopic pregnancy. There were

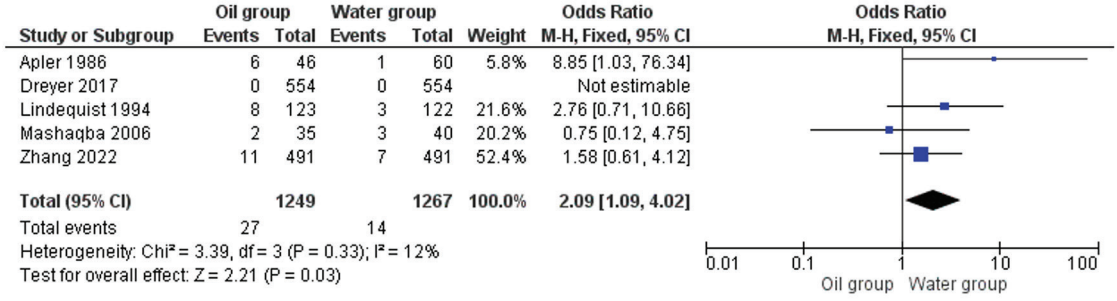

Figure 9. Shows the outcome of intravasation

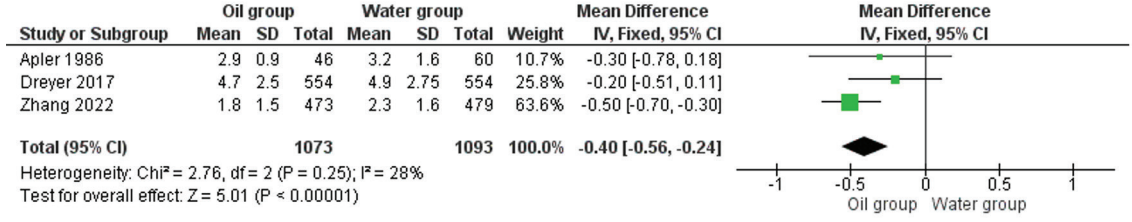

Figure 10. Shows the outcome of pain VAS score

VAS: Visual analog scale

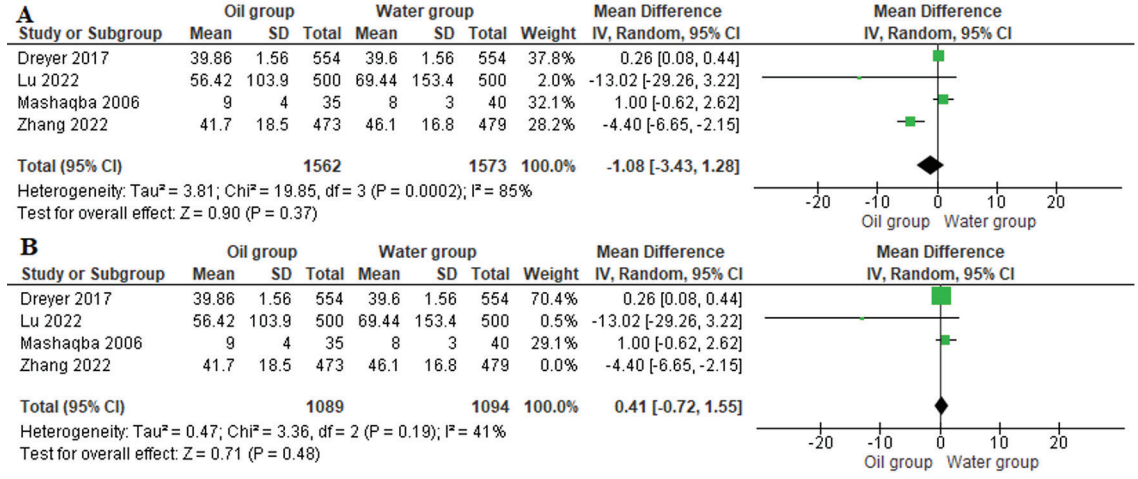

Figure 11. Shows the outcome of duration between HSG and pregnancy (weeks)-Part A includes four studies[6,16,19,20] & Part B excludes Zhang et al.[6]

HSG: Hysterosalpingography

diverse outcomes when it came to side effects. The oil group had a lower incidence of vaginal bleeding and abdominal pain than the water group, although OBCM was associated with more incidence of developing intravasation than WBCM.

A previously published meta-analysis, which was conducted in 2018, included six RCTs and 2,564 patients[9]. They showed that women who received HSG with OBCM had a greater pregnancy rate than women who underwent HSG with WBCM, but there were no statistically significant differences between patients with miscarriage and ectopic pregnancy. However, the population size was insufficient for evaluating the risk of publication bias and rare pregnancy outcomes, such as miscarriage and ectopic pregnancy. Two studies also included patients with co-treatment, which may have contributed to pregnancy outcome measurements. Another meta-analysis released in 2019 investigated the effectiveness of HSG on fertility outcomes using different materials[24]. However, most RCTs compare the fertility outcome of a single contrast medium to control. There are only five RCTs that directly compare WBCM and OBCM.

Early studies in the 1980s revealed that patients who underwent HSG with OBCM had a higher pregnancy rate than those who received HSG with WBCM. However, no statistically significant variations in pregnancy outcomes were found until two RCTs in the 1990s[16,20]. These findings are consistent with our meta-analysis finding of an odd ratio of 1.51 in OBCM versus WBCM. The mechanisms of fertility-enhancing effects in an oil-based contrast medium remain unknown. It is theorized that the bacteriostatic and fibrinolytic properties of oil-based contrast media minimize edema on the mucus membrane. In addition to the stimulation of ciliary activity, mechanical cleansing of the uterine cavity and fallopian tubes makes the environment more conducive to conception and spermatozoa penetration.

Despite its therapeutic potential, OBCM is associated with a higher risk of overall side effects. The introduction of foreign substances into the bloodstream via blood or lymph vessels is known as intravasation. Previous studies have shown that the risk of intravasation in OBCMs is higher than in WBCMs[25]. This is consistent with our research, which found an odd ratio of 2. Embolism is one of the most serious complications of intravasation. A systematic review of 31 studies involving 19,339 people[8] showed that only 18 women experienced oil embolism, with four cases including embolism to the brain and retina. None of the patients ended up with long-term complications.

The primary objective of pregnancy is a live birth. However, there are other possible pregnancy outcomes, such as miscarriage and ectopic pregnancy. A five-year follow-up study showed that OBCM improves live birth by 7.5% compared to WBCM (OR=1.11), and our findings support this with a stronger association (OR=1.51). Patients who received HSG for infertility have a baseline risk of miscarriage and ectopic pregnancy[26]. The same study with five years follow-up also showed that the association between miscarriage and ectopic pregnancy in the OBCM group was not statistically significant compared with the WBCM group[27]. OBCM could increase the rate of maternal subclinical hypothyroidism (SCH) because of its high iodine content. A large dose of OBCM is also related to thyroid dysfunction in Neonates[28]. However, another RCT on 140 neonates found no difference in thyroid function between OBCM and WBCM[29]. Women in early pregnancy with SCH had a higher chance of miscarriage[30,31]. A study suggested that up to 25% of HSG patients with OBCM-developed SCH, compared with 10% of those with WBCM[32]. The risk factors for ectopic pregnancy vary by the patient, including a history of pelvic inflammatory disease or surgery. Literature on ectopic pregnancy following HSG is limited, and our analysis showed that both materials are associated with the same ectopic incidence. The prevalence of miscarriage and ectopic pregnancy following HSG requires further research.

Most studies examined pregnancy or conception at a specific time but not cumulatively. An RCT of 5 years follow-up confirmed that the OBCM group had a higher cumulative spontaneous pregnancy rate than the WBCM group[27]. Another RCT concluded that the median time between HSG and pregnancy for OBCM and WBCM is 13 and 16 months, respectively[18]. However, our analysis with four RCTs found no statistically significant differences between OBCM and WBCM for the duration from HSG until pregnancy. The fertility-enhancing effect of HSG in the OBCM lasts for at least a year and is reduced over time. The therapeutic effects are expected to return to baseline in 2 years. The diminishing therapeutic effects in OBCM after an HSG may be attributed to other measures taken by patients to address their infertility problems, such as weight loss, starting IVF, or smoking cessation[18].

Lower abdomen pain and vaginal bleeding are other significant complications of HSG[33]. Only half of the HSG patients complained of abdominal pain and vaginal bleeding. Most pain is resolved within 24 h, and the amount of blood is typically less than menstruation[17]. No pre-procedural risk factors, including volume of contrast used, osmolality, or viscosity of contrast, are identified with worsening pain during HSG[34]. The expansion following contrast administration causes visceral sensory nerve stimulation, release of local prostaglandin and, subsequently, uterine contraction[35]. However, women with an abnormal HSG result reported more pain during and 30 min following treatment[36]. Previous literature suggested that OBCM resulted in less pain throughout the procedure. The incidence of delayed pain following HSG is lower in the OBCM group, which is consistent with our findings. In terms of vaginal bleeding, previous studies have shown that the occurrence and duration of vaginal bleeding are more significant in HSG patients with WBCM[17]. Our analysis supports this finding. The cause of vaginal bleeding after HSG still requires additional investigation. One explanation is that the overflow of OBCM in

the uterine umbrella tip region is gentler and less stimulating the peritoneum, resulting in less pain and vaginal hemorrhage[35]. Multiple RCTs support the use of ethiodized poppyseed oil-based contrast due to its potential therapeutic effects and common adverse effects, which is the material of choice for HSG[6,17]. Hysterosalpingo-foam sonography (Hyfosy) is a newly evolving alternative to HSG for determining tubal patency. The sensitivity of Hyfosy is similar to that of HSG, whereas one of the primary advantages of Hyfosy over HSG is the absence of radiation exposure, which removes patient anxiety and the risk of undetected early pregnancy[37,38]. However, no therapeutic effects of Hyfosy on infertility have yet been identified.

Study Limitations

The main limitation of our study was the heterogeneity found in some outcomes. However, we could solve them either by the leave-one-out method or by conducting a subgroup analysis. Five new RCTs with a total of 2,177 individuals have been included in our meta-analysis, including three and five-year follow-up studies in our qualitative synthesis and more recent studies with participants greater than 1,000. A larger population size enables us to provide a more accurate evaluation for uncommon pregnancy outcomes, such as miscarriage and ectopic pregnancy, and rare adverse effects, such as intravasation and embolism. This also allowed us to examine publication bias. There are several confounding factors during pregnancy. Increasing the number of RTCs will enable us to examine the influence of each variable and better understand its adverse effects.

Conclusion

To conclude, HSG using OBCM was associated with a higher incidence of pregnancy rate, live birth, and intravasation. While HSG using WBCM was associated with more abdominal pain, vaginal bleeding, and the overall VAS pain score. We found no significant difference between the groups regarding miscarriage, ectopic pregnancy, and the duration of HSG and pregnancy.

Ethics

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: A.A.S., Design: A.A.S., Data Collection or Processing: A.A.S., Analysis or Interpretation: A.A.S., Processing and Interpretation: S.T., Writing: S.T.

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Comparison of polyvinyl alcohol particles and tris-acryl gelatin microspheres embolic agents used in uterine artery embolization: A systematic review and meta-analysis

Uterin arter embolizasyonunda kullanılan polivinil alkol partikülleri ve tris-akril jelatin kaplı mikroküreleri karşılaştırılması: Sistematik bir derleme ve meta-analiz

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Abstract

Objective: To identify the preferred agent by comparing the therapeutic efficacy, degree of infarction, and side effects of polyvinyl alcohol particles (PVA) and tris-acryl gelatin embolization (TAGM) agents in uterine artery embolization.

Materials and Methods: We included available articles comparing PVA with TAGM embolization agents in the management of fibroids. The primary outcomes included the decrease in uterine volume (%), decrease in dominant tumor volume (%), fibroid infarction rate, complete infarction fibroid, complications, pain score after 24 h, procedure time (minutes), duration of hospital stay, fluoroscopy time (minutes), and the change in symptom severity score.

Results: Eight articles that met our inclusion criteria were included in this study. Our analysis yielded an overall superiority of PVA compared to TAGM regarding complete fibroid infarction rate at the first 24 h. However, TAGM was better than PVA concerning <90% infarction rate outcome. While both embolization techniques showed similar effects regarding the change in symptom severity score, the percentage of decrease in uterine volume, percentage of decrease of dominant tumor volume, 90-99% infarction rate, complete infarction rate when assessed after the first 24 h, pain score after the first 24 h, procedure time, fluoroscopy time, minor, and major complications.

Conclusion: Both PVA and TAGM embolization agents are effective and safe modalities in treating patients with fibroids, with no significant variation of both agents in most outcomes.

Keywords: Polyvinyl alcohol particles, tris-acryl gelatin microspheres, uterine fibroid, uterine artery embolization

Öz

Amaç: Bu çalışmanın amacı, uterin arter embolizasyonunda polivinil alkol partikülleri (PVA) ve tris-akril jelatin kaplı embolizasyon (TAGM) ajanlarının terapötik etkinliğini, enfarktüs derecesini ve yan etkilerini karşılaştırarak tercih edilecek ajanı belirlemektir.

Gereç ve Yöntemler: Miyomların tedavisinde PVA ile TAGM embolizasyon ajanlarını karşılaştıran mevcut makaleleri derledik. Birincil sonlanımlar arasında uterus hacminde azalma (%), baskın tümör hacminde azalma (%), fibroid enfarktüs oranı, tam enfarktüs fibroid, komplikasyonlar, 24 saat sonra ağrı skoru, işlem süresi (dakika), hastanede kalış süresi, floroskopi süresi (dakika) ve semptom şiddeti skorundaki değişiklik yer almaktadır.

PRECIS: There is no significant difference between polyvinyl alcohol particles and tris-acryl gelatin microsphere embolic agents in treating fibroid patients with uterine artery embolization.

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Bulgular: Dahil edilme kriterlerimize uyan sekiz makale bu çalışmaya dahil edildi. Analizimiz, ilk 24 saatte tam fibroid enfarktüs oranı açısından TAGM'ye kıyasla PVA'nın genel bir üstünlüğünü ortaya çıkardı. Ancak TAGM, <%90 enfarktüs oranı sonucu açısından PVA'dan daha iyiydi. Her iki embolizasyon tekniği semptom şiddeti skorundaki değişim, uterus hacmindeki azalma yüzdesi, dominant tümör hacmindeki azalma yüzdesi, %90-99 enfarktüs oranı, ilk 24 saatten sonra değerlendirildiğinde tam enfarktüs oranı, ilk 24 saatten sonraki ağrı skoru, işlem süresi, floroskopi süresi, minör ve majör komplikasyonlar açısından benzer özelliklere sahipti.

Sonuç: Hem PVA hem de TAGM embolizasyon ajanları, miyomlu hastaların tedavisinde etkili ve güvenli modalitelerdir ve çoğu sonlanım açısından her iki ajan arasında önemli bir fark görülmemektedir.

Anahtar Kelimeler: Polivinil alkol partikülleri, tris-akril jelatin kaplı mikroküreler, uterin fibroid, uterin arter embolizasyonu

Introduction

Uterine artery embolization (UAE) is a minimally invasive radiological procedure used to treat various gynecological conditions, such as fibroids and adenomyosis[1]. Additionally, UAE could also be used to control bleeding in obstetric emergencies, such as postpartum obstetric hemorrhage. Uterine fibroid is a benign tumor that affects up to 70% of fertility-seeking women, causes menorrhagia and dysmenorrhea and results in a lower quality of life[2].

During a UAE procedure, a catheter is advanced from the femoral artery to the uterine artery using fluoroscopy and contrast dyes; then, embolic agents are injected into the uterine arteries that supply fibroids, causing the formation of a clot that obstructs blood flow and eventually results in fibroid shrinkage. This raises the question of whether different embolic agents have varying efficacy in treating fibroids[3,4].

Despite a higher reintervention rate and severe post-procedural pain, UAE remains a popular option for fibroid management compared with conservative surgery because of its minimal blood loss, reduced recovery time, and lower operation risks[1,2,5]. Multiple studies have demonstrated the efficacy and safety of UAE in treating fibroid symptoms and reducing uterine volume[6,7]. Post-embolization pain is commonly noted due to ischemia resulting in significant inflammatory reactions during UAE, which can be severe and necessitate hospitalization with intravenous analgesics[8].

Various embolic agents have distinct properties and absorption rates. The shape of embolic agents also affects their difficulty in administration. Spherical agents are easier to administer, whereas irregularly shaped agents may clump and block microcatheters[9]. Additionally, temporary agents such as Gelfoam is the preferable choice for treating obstetric hemorrhage because they can quickly reabsorbed[2]. Other longer-lasting agents, such as Polyvinyl alcohol (PVA) particles or tris-acryl gelatin microspheres (TAGM), are more effective at reducing uterine volume. Conversely, an embolic agent with lasting effects may weaken the potency of the uterine artery and hence reduce fertility. Thus, Gel-bead is a bioresorbable embolic agent in calibrated spheres of varying sizes that is resorbable within 12 weeks and has a high infarct rate for fibroid reduction[10].

In this study, we conducted a meta-analysis of available studies to identify the preferred agent by comparing the therapeutic efficacy, degree of infarction, and side effects of PVA and TAGM embolization agents.

Materials and Methods

The PRISMA protocol was used as a guideline in performing our meta-analysis[11].

Searchs and Information Databases

We used the following search strategy in our search until Oct 2022: ("uterine artery embolization" OR "uterine artery occlusion" OR UAE) AND (fibromyoma OR leiomyomata OR myoma OR leiomyoma OR fibroid) AND (polyvinyl alcohol OR PVA OR tris acryl OR "tris-acryl gelatin microspheres" OR TAGM OR "gelatin sponge particles" OR gelfoam OR Embosphere OR Embosphere OR "embolic agents"). PubMed, Web of Science, Cochrane library, and Scopus were the main used databases.

Selection Criteria and Eligibility Criteria

We selected our included articles in two steps. First, we depended on titles and abstracts to identify relevant articles, which were then were further evaluated to reach the final included studies according to our eligibility criteria. We included all articles comparing the efficacy and safety of PVA versus TAGM embolization agents in UAE for uterine fibroids. We excluded any studies which did not report our assessed outcomes, single-arm studies, and secondary studies such as systematic reviews and meta-analyses.

Data Extraction

Data from the included studies were retrieved and plotted on an excel sheet. We extracted the general information data about studies such as study design, used imaging techniques, and follow-up period. We also extracted baseline characteristics of included patients, including age, preprocedural uterine volume, preprocedural dominant tumor volume, embolization agent volume, and health-related quality of life. Then we extracted data of our main outcomes, including decrease in uterine volume (%), decrease in dominant tumor volume (%), fibroid infarction rate, complete infarction of fibroid, complications, pain score after 24 h, procedure time (minutes), duration of hospital stay, fluoroscopy time (minutes), and change in symptom severity score. Finally, we collected the data required for the quality assessment.

Quality Assessment

We included both randomized controlled trials (RCTs) and observational studies. Therefore, we used two different tools for the quality assessment of the included studies. Concerning the clinical trials' quality assessment, we used the Cochrane assessment tool[12]. RCTs were categorized as high, moderate, or low quality according to the state of randomization, allocation concealment, sequence generation, adequate blinding, if the trial was free of selective reporting, and if the missing outcome data were adequately addressed. Regarding the quality assessment of the included observational studies, we used the National Heart, Lung, and Blood Institute (NHLB) tool[13].

Statistical Analysis

We analyzed data of our main outcomes using RevMan 5.4.1. We used the inverse variance and Mantel-Haenszel analysis methods for continuous and dichotomous data, respectively. Continuous outcomes were analyzed using mean difference (MD) or standardized mean difference (standard MD) and 95% confidence intervals (CIs). Dichotomous outcomes were analyzed using the risk ratio (RR) and 95% CIs. The p-value of the chi-square test and the I^2 assessed the heterogeneity among the studies. The outcome is considered heterogeneous if $p<0.1$ or $I^2>50\%$. We tried solving the inconsistency among data using subgroup analysis and the Cochrane leave-one-out method[14].

Results

Search Results and Characteristics of the Included Studies

The results of our search are shown in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) diagram (Figure 1). Eight articles[9,10,15-20] met our eligibility criteria and were included in our meta-analysis. We evaluated 403 women who underwent UAE for uterine fibroid. One hundred ninety-seven women underwent PVA embolization, while TAGM embolization agent was used in 206 women. The mean age of all the included women was 44.11 years. Table 1 shows the general characteristics of the included studies. Table 2 shows the baseline data of the included patients.

Results of the Quality Assessment

According to Cochrane's tool[21], all trials[9,15,16,18-20] reported proper randomization, blinding of outcomes assessment, blinding of participants and personnel, and selective reporting bias was not detected; therefore, they were categorized as low-risk in the previous domains. Regarding selection bias, all studies reported proper allocation concealment except Han et al.[19], which did not report sufficient details, so it was categorized as an unclear risk of bias. Four studies concerning attrition bias[9,16,18,20] were categorized as high risk due to inadequate handling of incomplete outcome data. In contrast, the other two trials[15,19] were categorized as low risk of bias. A detailed illustration of the quality assessment is shown in Figure 2. The quality assessment of the included observational studies yielded an average score of 9.5 out of 14, according to NHLB[13]. A detailed summary of the quality assessment of our included studies is illustrated in Figure 2 and Table 3.

Analysis of Outcome

Decrease in Uterine Volume (%)

Five of our included studies reported a decrease in uterine volume percentage[9,10,15,16,18]. Our final results demonstrated a similar decrease in both groups MD=-1.29 (-5.44, 2.86), p=0.54. Data were homogenous (p=0.12); I^2=46% (Figure 3).

Decrease in Dominant Tumor Volume (%)

We conducted a subgroup analysis of this outcome according to the imaging method used to assess dominant tumor volume. The first subgroup included five studies[9,10,15,16,18] that used magnetic resonance imaging (MRI). The combined analysis demonstrated that patients allocated to PVA showed a comparable decrease in the dominant tumor volume with patients allocated to TAGM MD= 9.04 (-4.20, 22.27), p=0.18. We faced heterogeneity among studies in this subgroup (p=0.03); I^2=66% (Figure 4a). The second subgroup includes one study[20] that assessed the

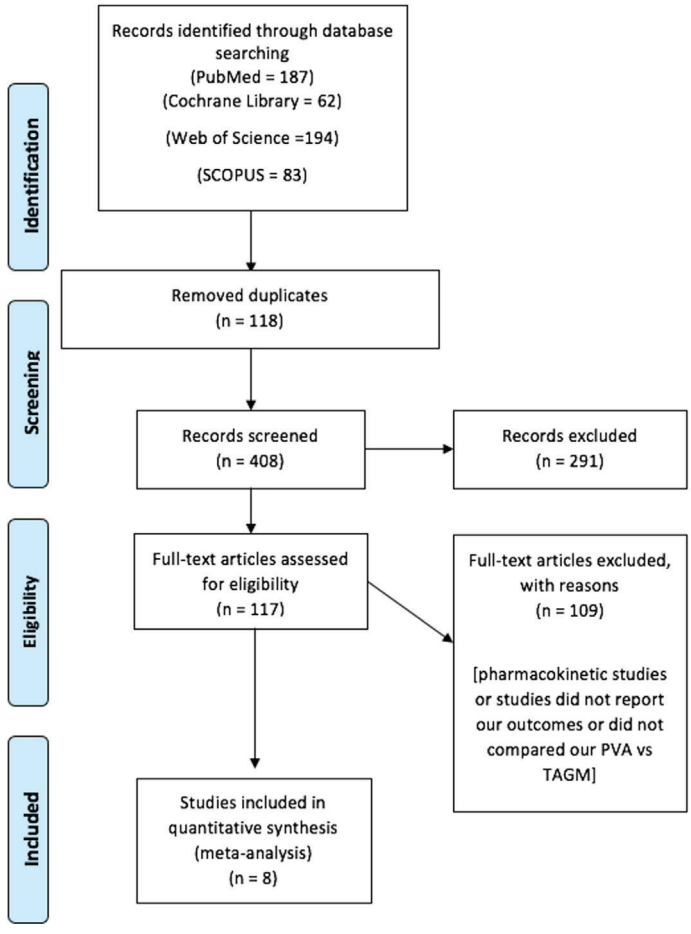

Figure 1. Shows a PRISMA flow diagram of our literature search

Table 1. Shows general characteristics of the included studies

Study name	Study Design	Used imaging techniques	Follow-up after procedure
Galvez et al.[17]	Observational cohort	MRI with gadolinium	1, 3, and 6 months
Han et al.[19]	RCT	Contrast-enhanced MRI (gadolinium chelate)	Three months
Maclean et al.[10]	Retrospective cohort	MRI/MRA	Three months
Shlansky-Goldberg et al.[28]	RCT	Contrast-enhanced MRI	The first day, three months, and one year
Siskin et al.[16]	RCT	MRI	Four weeks
Spies et al.[15]	RCT	Contrast-enhanced MRI	Three months
Spies et al.[15]	RCT	Contrast-enhanced MRI	Three months
Yu et al.[20]	RCT	Ultrasonographic with Doppler	Two years

RCT: Randomized controlled trial, MRI: Magnetic resonance imaging

Table 2. Shows the baseline data of the included patients

Study name	Age (year)				Preprocedural uterine volume (cm^3)				Preprocedural dominant tumor volume (cm^3)			
	PVA		TAGM		PVA		TAGM		PVA		TAGM	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Galvez et al.[17]	NR	NR	NR	NR	656 mL	440	757 mL	680	NR	NR	NR	NR
Han et al.[19]	45	5	44	4	406	227	423	216	6.2 cm	1.6	6.8	1.8
Maclean et al.[10]	47.1	6.3	46.2	4.7	674	498	401.9	288	352.2	421	136.1	158
Shlansky-Goldberg et al.[28]	43.9	5	41.7	5.4	1536.7	937.3	1491.6	1456.5	203.3	275.1	141.1	179.6
	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Siskin et al.[16]	44.9	NR	45.1	NR	518.2	477.4	611.6	281.575	190.6	167.57	196.9	130.625
Spies et al.[15]	42.5	5	43.4	5.4	603.9	343.3	648.7	326.7	162.4	169.3	138.4	139.5
	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Spies et al.[15]	44.9	6.2	45.9	4.4	510.5	314.8	618.8	305.1	142.4	126.6	150.1	178.9
	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Yu et al.[20]	42.7	5.15	40.3	5.1	14.18	3.29	14.2	3.2	197.7	179	181.26	139.5

Study name	Embolization agent volume (cm^3)				Scale	Health-related quality of life			
	PVA		TAGM			PVA		TAGM	
	Mean	SD	Mean	SD		Mean	SD	Mean	SD
Galvez et al.[17]	NR	NR	NR	NR	NR	NR	NR	NR	NR
Han et al.[19]	NR	NR	NR	NR	HRQOL	62	14	65	8.25
Maclean et al.[10]	NR	NR	NR	NR	NR	NR	NR	NR	NR
Shlansky-Goldberg et al.[28]	12.8 mL	9.4	12.6	7.2	UFSQOL symptom score	64.2	20.6	65.1	20.3
	NR	NR	NR	NR	UFSQOL subscores	42.1	20.2	42	23.7
Siskin et al.[16]	8.4	12.50	11.6	4	NR	NR	NR	NR	NR
Spies et al.[15]	3 cm^3	1.6	9.4	5.7	Fibroid-specific QOL symptom score	50.2	23.2	57.4	19.8
	NR	NR	NR	NR	Fibroid-specific QOL total score	57.8	22.5	47.6	21.1
Spies et al.[15]	5.3 mL	3.6	7.8	6.3	UFSQOL symptom score	57.4	22.4	61.5	19.3
	NR	NR	NR	NR	UFSQOL total score	51.6	20.3	43.5	26.3
Yu et al.[20]	NR	NR	NR	NR	NR	NR	NR	NR	NR

PVA: Polyvinyl alcohol particles, TAGM: Tris-acryl gelatin microspheres, SD: Standard deviation, NR: Unreported, UFSQOL: Uterine fibroid symptom and quality of life

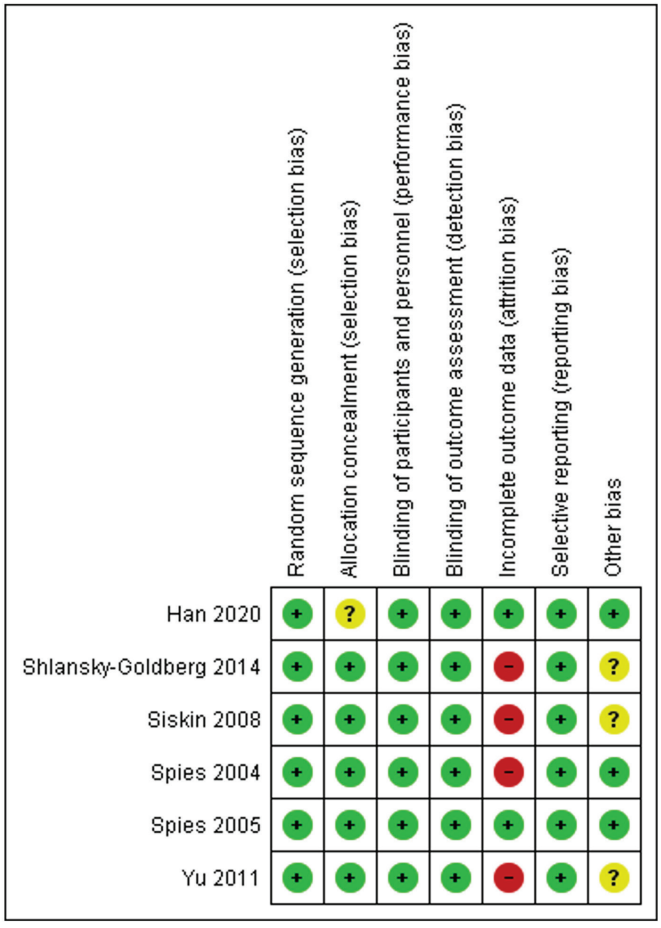

Figure 2. Shows a detailed illustration of the risk of bias of included studies

decrease in dominant tumor volume using ultrasound. We found similar results in both groups MD= 1.30 [-18.49, 21.09], p=0.90 (Figure 4b).

Fibroid Infarction Rate

Regarding the 90-99% infarction rate, four studies[9,10,15,16] reported this outcome. The overall RR was similar in both groups RR=0.70 [0.36, 1.37], p=0.30. Data were homogenous (p=0.35); I^2=9% (Figure 5a).

Three studies[10,15,16] reported <90% infarction outcome. The combined analysis showed a significant favoring of the TAGM group RR=2.92 [1.26, 6.73], p=0.01. Analysis was homogenous (p=0.25); I^2=28% (Figure 5b).

Complete Infarction of a Fibroid

Two studies[9,19] reported the rate of complete infarction outcome in the first 24 h after UAE. We found that the percentage of complete infarction was significantly lower in the TAGM group RR=1.16 [1.00, 1.33], p=0.04. We found no heterogeneity among data in this subgroup (p=0.78); I^2=0% (Figure 6a).

Five studies[9,10,15-17] assessed this outcome in the period after the first 24 h. There was no significant variation between the groups RR=0.86 [0.67, 1.10], p=0.22. We faced heterogeneity among studies in this subgroup (p=0.04); I^2=61% (Figure 6b).

Complications

Data of minor complications were retrieved from four studies[16,18-20]. The percentage of minor complications was similar in both groups RR= 0.81 [0.62, 1.07], p=0.14. The overall analysis was homogenous (p=0.87); I^2=0% (Figure 7a).

Three studies[16,18,20] compared major complications between both groups. The overall RR showed no significant difference between both embolization agents RR=2.42 [0.73, 8.03], p=0.15. Analysis was homogenous (p=0.99); I^2=0% (Figure 7b).

Pain Score After 24 h

The pain score was assessed in four studies[9,15,18,19]. The pain score in both groups was comparable SMD=-0.06 [-0.31, 0.19], p=0.66. Data were homogenous (p=0.50); I^2=0% (Figure 8).

Procedure Time (Minutes)

This outcome was reported in three studies[9,15,19]. The combined mean difference did not favor any group over the other MD=-0.16 [-3.54, 3.23], p=0.93. There was no heterogeneity among studies (p=0.59); I^2=0% (Figure 9).

Duration of Hospital Stay

Two studies[9,20] reported the duration of hospital stay. Patients in both groups showed approximately similar duration of hospitalization MD= 0.00 [-0.10, 0.10], p=0.98. Data were homogenous (p=0.79); I^2=0% (Figure 10).

Fluoroscopy Time (Minutes)

Four studies[9,15,18,19] evaluated the fluoroscopy time. The overall mean difference did not show any difference between both groups MD=-0.21 [-1.34, 0.93], p=0.72. Analysis was homogenous (p=0.15); I^2=43% (Figure 11).

Change of Symptom Severity Score

Data of symptom severity score were retrieved from four studies[9,15,18,19]. The combined analysis did not favor any embolization agent MD=-2.81 [-13.88, 8.26], p=0.62. We faced heterogeneity among data (p=0.02); I^2=69% (Figure 12a). We could decrease the heterogeneity by excluding Spies et al.[18] I^2=52%; (p=0.12). The overall mean difference after solving the heterogeneity showed a comparable change in both groups MD=1.31 [-9.96, 12.59], p=0.82 (Figure 12b).

Discussion

Uterine fibroids are present in about 60% of females during their reproductive period[22]. Leiomyomas are associated with various adverse events that affect the physical, psychological, and social functions of women[23]. Thus, the presence of a complicated uterine fibroid is a reliable indication for performing hysterectomy[24]. To avoid the complications of such invasive procedures and preserve women fertility, alternative minimally invasive techniques have been developed. UAE effectively

Table 3. Shows the quality assessment of observational studies

Study name	Galvez et al.[17]	Maclean et al.[10]
1. Was the research question or objective in this paper clearly stated?	Yes	Yes
2. Was the study population clearly specified and defined?	Yes	Yes
3. Was the participation rate of eligible persons at least 50%?	Yes	Yes
4. Were all the subjects selected or recruited from the same or similar populations (including the same period)? Were inclusion and exclusion criteria for being in the study specified and applied uniformly to all participants?	Yes	Yes
5. Was a sample size justification, power description, or variance and effect estimate	No	Yes
6. For the analyses in this paper, were the exposure (s) of interest measured before the outcome(s) being measured?	No	Yes
7. Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?	Yes	Yes
8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?	No	No
9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	No	Yes
10. Was the exposure(s) assessed more than once over time?	NA	NA
11. Were the outcome measures (dependent variables) learly defined, valid, reliable, and implemented consistently across all study participants?	Yes	Yes
12. Were the outcome assessors blinded to the exposure status of the participants?	No	No
13. Was the loss to follow-up after baseline 20% or less?	Yes	Yes
14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?	Yes	Yes
Total score (out of 14)	8	11

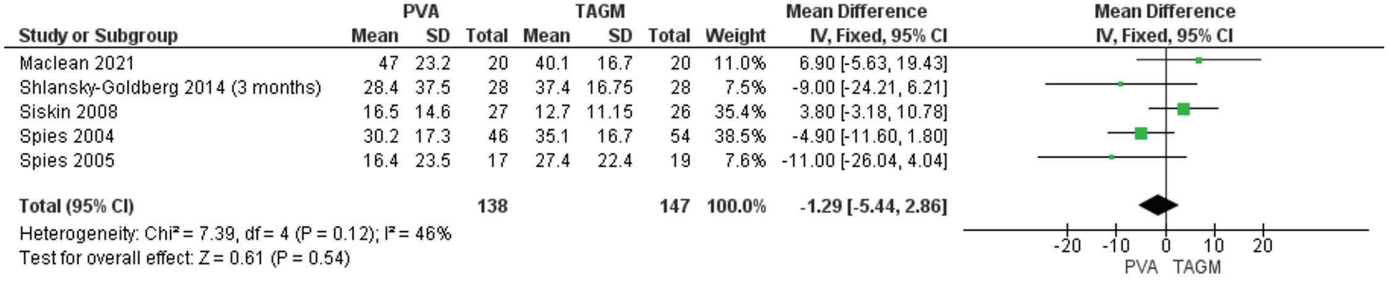

Figure 3. Shows the decrease in uterine volume (%)

PVA: Polyvinyl alcohol particles, TAGM: Tris-acryl gelatin embolization, CI: Confidence interval, SD: Standard deviation

reduces fibroid symptoms, volume, and complications since it was first conducted in 1995[3]. PVA, a non-biodegradable agent, is the first and most prevalent used embolization agent in UAE[25]. While Microporous cross-linked acrylic beads are used to create the calibrated microspheres known as TAGM[20]. In this systematic review and meta-analysis, we compared the post-procedural outcomes of PVA versus TAGM in treating uterine fibroids. Our combined analysis demonstrated that PVA was superior to TAGM in terms of complete fibroid infarction rate when assessed at the first 24 h. However, TAGM was better than PVA, concerning a less than 90% infarction rate outcome. While both embolization techniques showed similar effects regarding the change in symptom severity score, the percentage of decrease in uterine volume, percentage of decrease of dominant tumor volume, 90-99% infarction rate, complete infarction rate when assessed after the first 24 h, pain score after the first 24 h, procedure time, fluoroscopy time, minor, and major complications.

In 2013, Das et al.[26] conducted a meta-analysis, which compared embospheres (TAGM) with spherical PVA (sPVA),

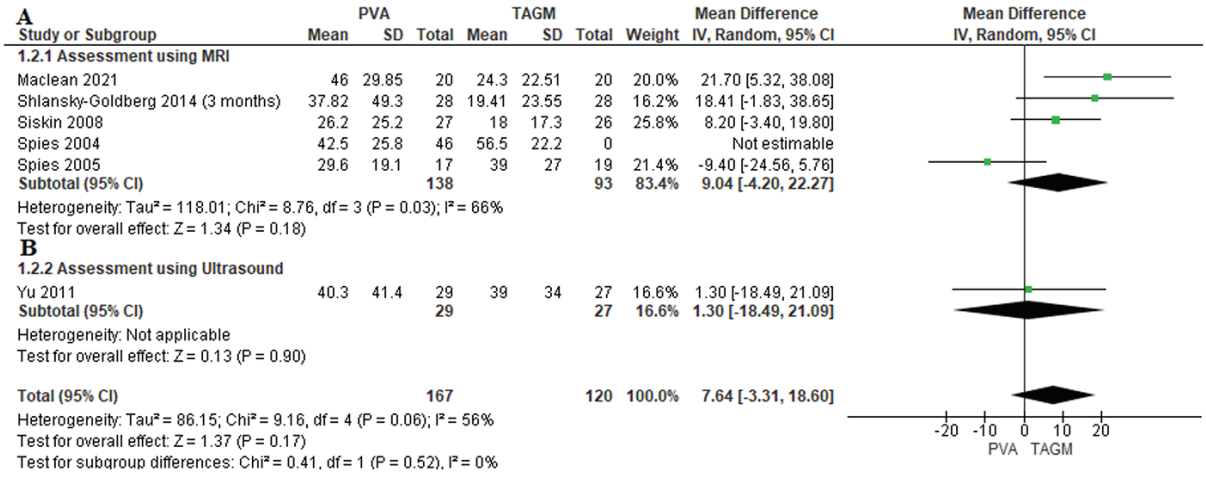

Figure 4. Shows the decrease in dominant tumor volume (%)-Part A includes five studies[9,10,15,16,18] using MRI & Part B includes one study[20] using ultrasound

MRI: Magnetic resonance imaging, PVA: Polyvinyl alcohol particles, TAGM: Tris-acryl gelatin embolization, CI: Confidence interval, SD: Standard deviation

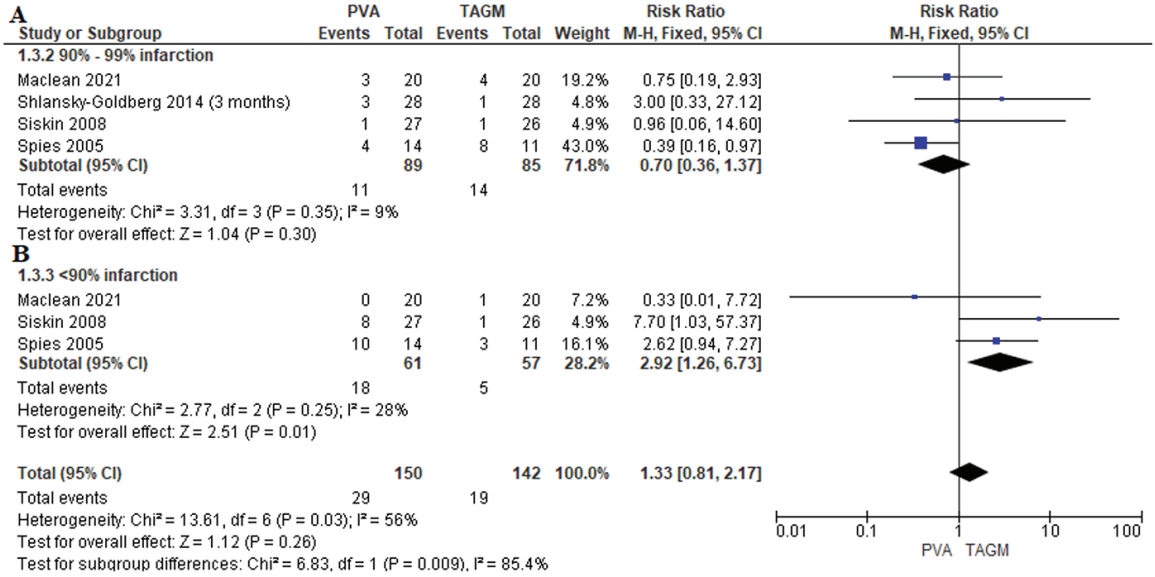

Figure 5. Shows the outcome of fibroid infarction rate-Part A includes four studies[9,10,15,16] with 90-99% infarction rate & Part B includes three studies[10,15,16] with <90% infarction outcome

PVA: Polyvinyl alcohol particles, TAGM: Tris-acryl gelatin embolization, CI: Confidence interval

non-spherical PVA, or acrylamido PVA in terms of post-procedural MRI assessment of both uterine and tumor volume and the quality of life of treated women. The overall analysis between TAGM and sPVA showed that TAGM was associated with a significantly more fibroid devascularization percentage. However, similar to our findings, both embolization agents showed a similar reduction of the uterine and dominant fibroid volume. The lack of randomized trials in both groups was the main limitation of this study.

Galvez et al.[17] evaluated the MRI infarction rate of 101 patients who underwent UAE using sPVA, non-spherical PVA, or TAGM. They found that sPVA was associated with a less complete fibroid infarction rate and more incidence post-procedural residual enhancement. These results are similar the to previously published studies[27,28] that demonstrated the superiority of non-spherical PVA over sPVA. The inability of the spherical-shaped PVA to occlude the blood vessels because it is created from compressible materials, which may allow its distal migration through the blood circulation to reach small vessels may explain the superiority of non-spherical PVA over sPVA. Moreover, to get the spherical shape of sPVA, it is created in a specific technique that increases its *in vivo* dissolution

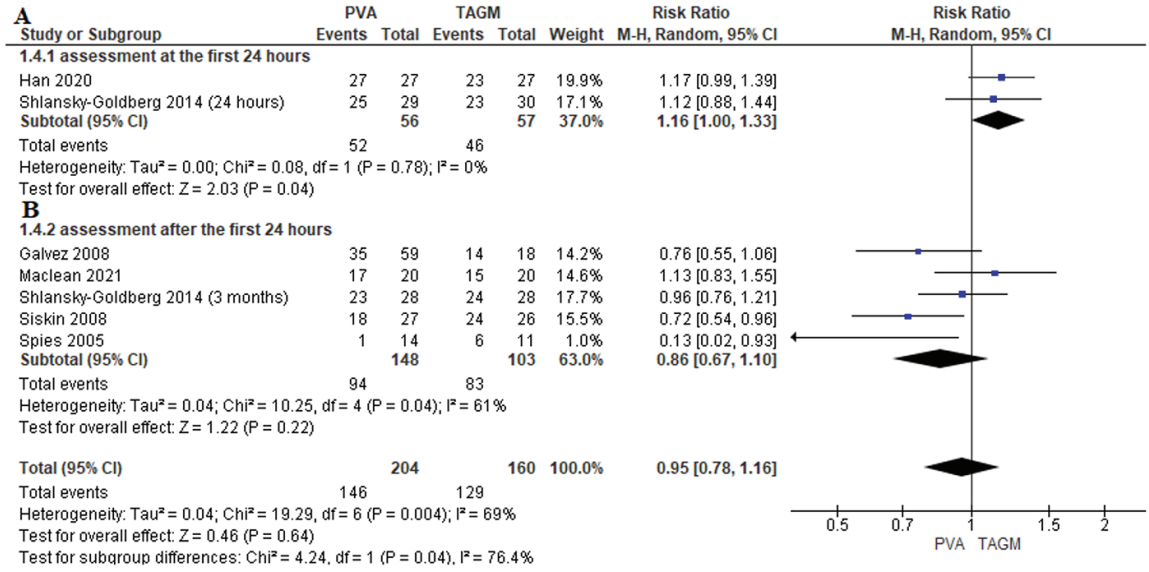

Figure 6. Shows the outcome of complete infarction of fibroid-Part A includes two studies[9,19] that assessed the outcome within the first 24 hours after UAE & Part B includes five studies[9,10,14-16] that assessed the outcome after the first 24 hours

UAE: Uterine artery embolization, PVA: Polyvinyl alcohol particles, TAGM: Tris-acryl gelatin embolization, CI: Confidence interval

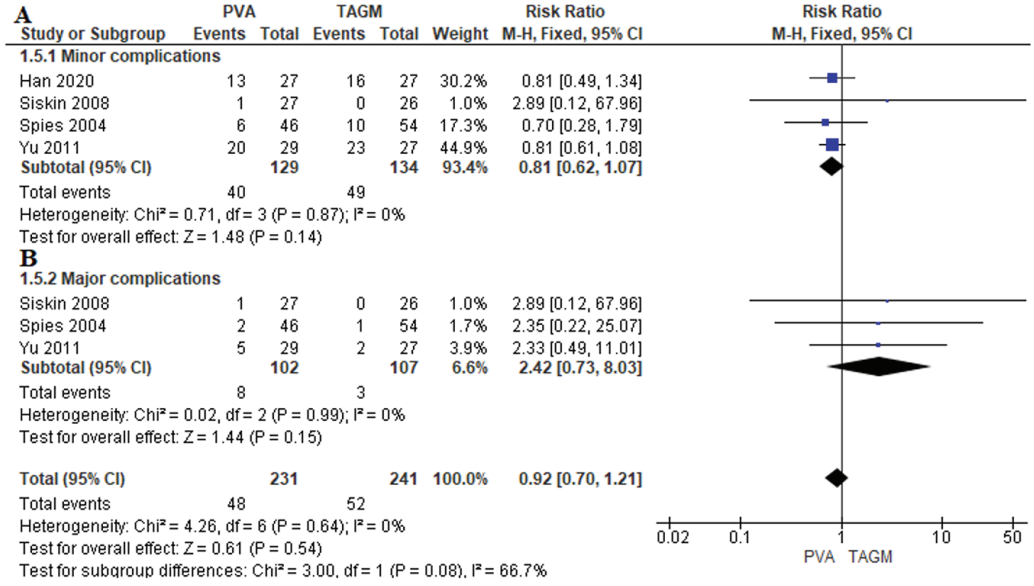

Figure 7. Shows the outcome of complications-Part A includes four studies[16,18-20] with minor complications & Part B includes three studies[16,18,20] with major complications

PVA: Polyvinyl alcohol particles, TAGM: Tris-acryl gelatin embolization, CI: Confidence interval, SD: Stardard deviation

more than conventional PVA[29]. To overcome some of these disadvantages, Pelage et al.[30] used larger size sPVA (700-900 µm), which caused complete fibroid infarction in 83% of patients. Additionally, they suggested that five minutes waiting period after each arterial embolization would confirm the appropriate vascular occlusion before catheter removal. The manufacturer of these microspheres has validated this protocol and included it in the guidelines for using the product[16].

Another previously published meta-analysis of five included studies[31] comparing PVA with TAGM showed similar results regarding the mean change in pain score and the average reduction in uterine volume, which is consistent with our findings. However, Contrary to our results, their analysis yielded an overall superiority of TAGM in terms of average fibroid-volume change, symptom, and quality of life change. Besides the clinical and radiological differences in both groups,

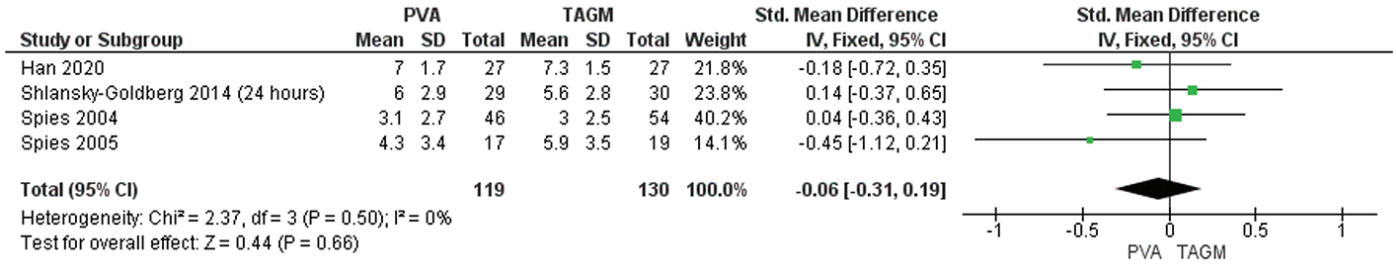

Figure 8. Shows the pain score after 24 hours

PVA: Polyvinyl alcohol particles, TAGM: Tris-acryl gelatin embolization, CI: Confidence interval, SD: Stardard deviation

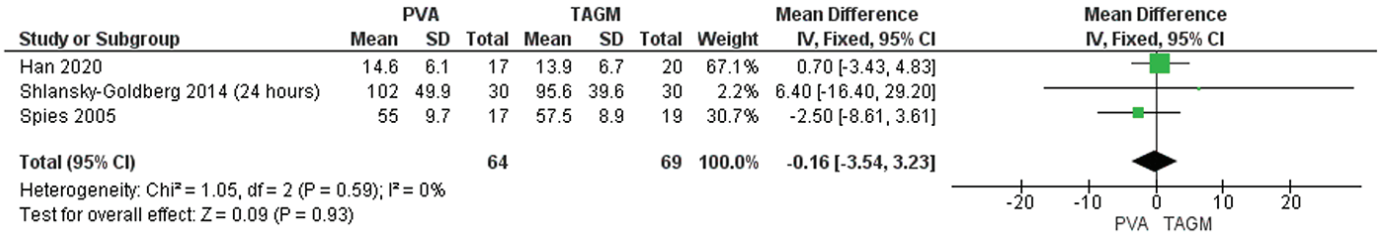

Figure 9. Shows the outcome of procedure time (minutes)

PVA: Polyvinyl alcohol particles, TAGM: Tris-acryl gelatin embolization, CI: Confidence interval, SD: Stardard deviation

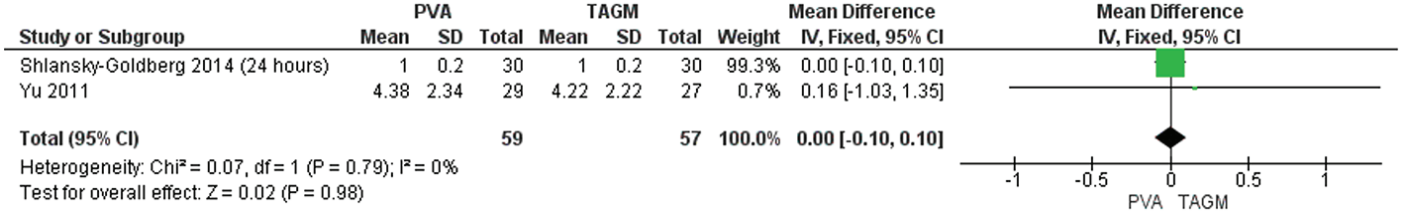

Figure 10. Shows the duration of hospital stay

PVA: Polyvinyl alcohol particles, TAGM: Tris-acryl gelatin embolization, CI: Confidence interval, SD: Stardard deviation

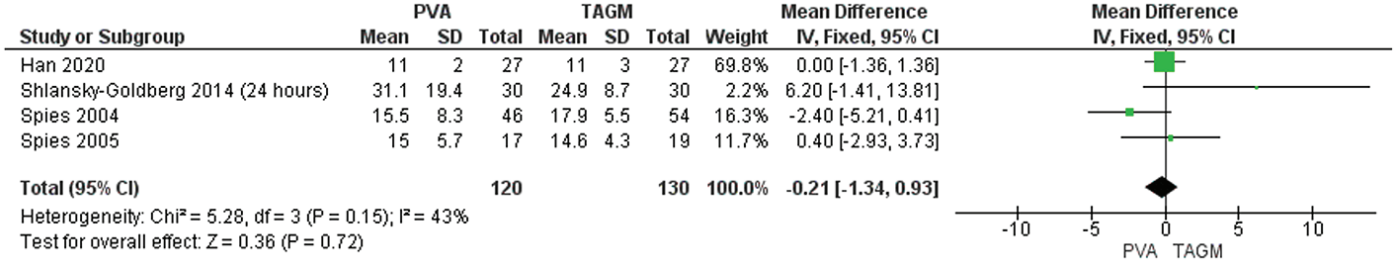

Figure 11. Shows the fluoroscopy time (minutes)

PVA: Polyvinyl alcohol particles, TAGM: Tris-acryl gelatin embolization, CI: Confidence interval, SD: Stardard deviation

the choice of an embolic agent depends on other factors, including the availability, ease of preparation, and the cost of these agents. Fortunately, both agents are available in most places. UAE requires more TAGM vials than PVA, and the overall cost of TAGM is higher. TAGM is easier to inject with less possibility of catheter occlusion[18].

Three hundred fifty one women with uterine leiomyomas were treated with either PVA or TAGM embolization agents in a recent systematic review[32]. Their final results favored the TAGM embolization agent concerning the overall quality of life, uterine volume, tumor volume, and less than 90% infarction leiomyoma infarction rate. They found no significant variation

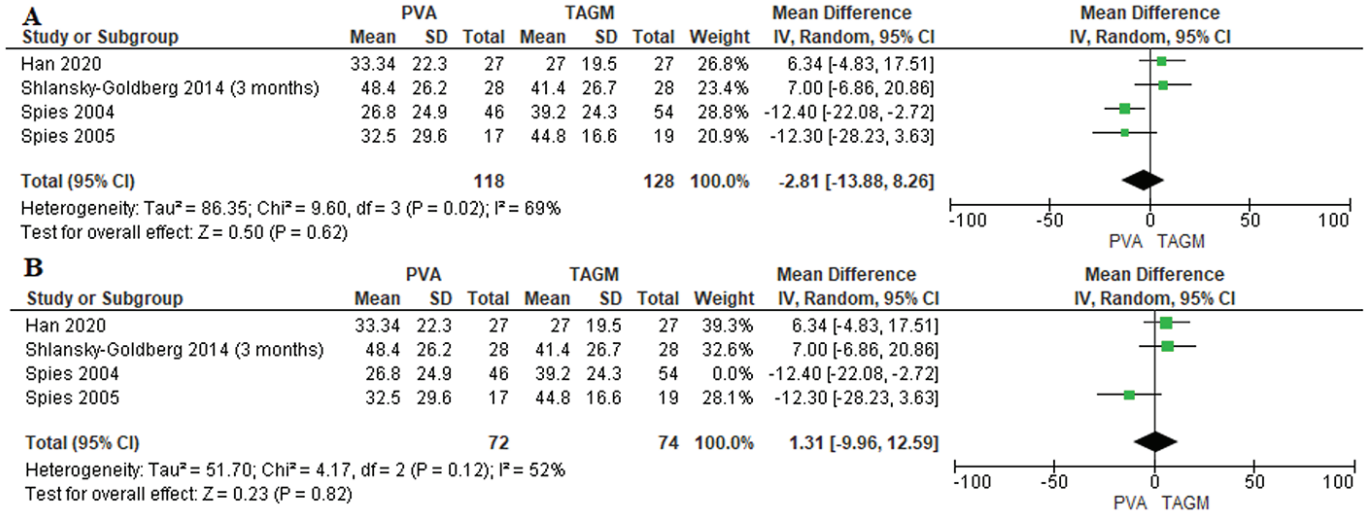

Figure 12. Shows the change of symptom severity score - Part A includes four studies[9,15,18,19] & Part B excludes Spies et al.[18]

PVA: Polyvinyl alcohol particles, TAGM: Tris-acryl gelatin embolization, CI: Confidence interval, SD: Stardard deviation

between the groups in the reduction of symptom severity, 90-99% infarction rate, complete infarction rate, minor, and major complications. This study has some limitations. They included data from Yu et al.[20] in the analysis of imaging-assessed uterine and fibroid changes. Yu et al.[20] used ultrasound while the remaining studies used MRI assessment.

The Fibroid Registry for Outcomes Data (FIBROID) was developed for women undergoing UAE in treating fibroids. They demonstrated that UAE was a reliable method yielding an overall improvement in the quality of life and long-term treatment from uterine fibroids. Additionally, their findings support the broad spectrum practice of this technique in such conditions by interventional radiologists, which is consistent with our results[33]. After approximately three decades of continuous clinical investigations and long-term follow-up of patients who underwent UAE from many previously published studies, UAE has shown promising results in short-term and long-term follow-up, which are similar to the results of conventional surgical intervention. Besides, almost all patients are fit for UAE. When performed by an experienced interventional radiologist, UAE will have the advantage of having a low-cost profile, early recovery, and early return to work. Thus, UAE should be considered as the first-line method in managing uterine leiomyomata[34].

Study Limitations

Our study has some limitations, such as the relatively low number of assessed patients, the inclusion of retrospective studies in our analysis, and the high risk of attrition bias among the included clinical trials. Therefore, in the future, more clinical trials with large sample sizes and long-term follow-up are needed to provide stronger evidence regarding the usage of different embolic agents in treating uterine fibroids. Additionally, we need more future studies that compare all available embolic agents to determine the best option regarding safety and efficacy.

Conclusion

To conclude, most UAE techniques show successful results in almost all patients with minimal side effects and very efficient outcomes. In our study, we suggest that both PVA and TAGM embolization agents are effective and safe modalities in treating patients with fibroids, with no significant variations of both agents in most outcomes. Interventional radiologists can choose the available and cheaper embolic agent to treat women with uterine leiomyoma.

Ethics

Ethics Committee Approval: This is a meta-analysis and systematic review of publicly available data and other materials. For systematic review and meta-analysis studies, the institutional review board (IRB) is exempted.

Informed Consent: Clinical trial registration and informed consent are not applicable.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

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

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

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

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Turk J Obstet Gynecol 2023;20:85

Copeptin, the prediction of poor ovarian reserve and the infertile women: Correspondence

Copeptin; infertil kadınlarda kötü over rezervinin ön görülmesi: Uygunluk

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Keywords: Copeptin, ovarian, reserve, infertile
Anahtar Kelimeler: Copeptin, yumurtalık, rezerv, kısır

Dear Editor,

We wants to share ideas on the publication "A potential marker for predicting poor ovarian reserve (POR) in the infertile women[(1)]." To determine the association between the level of serum copeptin and the presence of POR in infertile women, Görkem and Yıldırım[(1)] conducted their research. According to Görkem and Yıldırım[(1)], this investigation verified that the infertile women with POR had a higher blood copeptin concentration and that copeptin may have a predictive value for developing POR. To elucidate the potential impacts of copeptin in the POR pathogenesis, future large-scale prospective investigations are necessary[(1)]. We both agree that copeptin may be effective for ovarian reserve prediction. But it's important to be aware of any confounding factors. The interpretation needs to be careful in areas where hemoglobinopathy is prevalent. High levels of copeptin have been linked to thalassemia[(2)].

Ethics

Peer-review: Internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: R.M., V.W., Concept: R.M., V.W., Design: R.M., V.W., Data Collection or Processing: R.M., V.W., Analysis or Interpretation: R.M., V.W., Literature Search: R.M., V.W., Writing: R.M., V.W.

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

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

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