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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.

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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.

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Cover letter to the editors addressing the following points:

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· The declaration of transparency from the corresponding author.

· Clinical trial registration, if applicable.

· Institutional review board (IRB) approval or exemption.

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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).

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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.

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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.

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Examples

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LETTER FROM THE PRESIDENT

Distinguished colleagues and Esteemed TJOD (Turkish Society of Gynecology and Obstetrics) family,

I am very happy to have the chance to address you with the December issue of TJOG, the scientific publication of the Turkish Society of Gynecology and Obstetrics, the largest and umbrella association of obstetricians and gynecologists working in Turkey.

This issue of our journal brought together up-to-date and scientific publications with high scientific value to you, our esteemed colleagues. As it is known, we have made new arrangements and assignments in the editorial board structure of our journal. There is a very serious working team known as the editorial team in the publication of periodic scientific journals.

I would like to thank Eray Çalışkan for his efforts and contributions in this handover ceremony, which we consider a relay race in order to achieve scientific popularity and actuality, and wish success to our new editors and editorial team.

Bulent Tiras, Prof. MD
President of TJOD

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EDITORIAL

Dear Colleagues,

We are greatly honored to present to you the December issue of our scientific journal of great value, as we believe, for the Obstetrics and Gynecology community. In the final issue of the year 2022, there are 11 articles that we believe our physicians working in the field of obstetrics and gynecology and value scientificness will regard as satisfactory in this aspect.

We firmly believe that we are going to reunite you with more scientific and up-to-date publications with our employees on the renewed and refreshed editorial board and we sincerely thank all the employees who contributed to our journal in reaching this level of achievement.

As we bid farewell to the year 2022 through this issue, we hope that the year 2023 brings love, joy, health, and success to all and we wish you peace and health upon noting that we are going to be in your presence again with the March issue.

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

Clinical Investigation / Araştırma

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Turk J Obstet Gynecol 2022;19:257-67

Evaluation of serum neopterin, periostin, Tenascin-C, tissue inhibitor of metalloproteinase-1 and matrix metalloproteinase-2 levels in obese pregnant women

Obez gebelerde serum neopterin, periostin, Tenascin-C, metalloproteinaz-1 doku inhibitörü ve matriks metalloproteinaz-2 düzeylerinin değerlendirilmesi

Rauf Melekoğlu[1], Songül Ünüvar[2], Neşe Başak Türkmen[2], Aslı Çetin[3], Nesibe Zeyveli Çelik[1], Hande Yüce[2], Şeyma Yaşar[4]

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Abstract

Objective: To investigate the role of extracellular matrix proteins in the molecular mechanism of inflammatory response in obese pregnant women by comparing serum levels of neopterin, periostin, Tenascin-C, tissue inhibitor of metalloproteinase-1, and matrix metalloproteinase-2 between obese and normal weight pregnant women in the third trimester.

Materials and Methods: A prospective cross-sectional study was conducted between April 2021 and December 2021. A total of 84 pregnant women were included and three groups were formed with 28 participants in each group.

Results: Serum levels of neopterin, periostin, Tenascin-C and tissue inhibitor of metalloproteinase-1 were significantly higher in class II-III obese pregnant women than in class I obese and normal-weight women ($p=0.002$, $p<0.001$, $p<0.001$, and $p<0.001$, respectively). There was no significant difference in serum matrix metalloproteinase-2 levels between the groups ($p=0.769$). Receiver operating characteristic curve analysis showed that Tenascin-C and periostin were effective in predicting pre-eclampsia [area under the curve (AUC)=0.82, 95% confidence interval (CI), 0.72-0.90, $p<0.001$ and AUC=0.71, 95% CI, 0.60-0.80, $p=0.007$, respectively].

Conclusion: This study demonstrated that class II-III obese pregnant women had significantly higher serum levels of neopterin, periostin, Tenascin-C, and tissue inhibitor of metalloproteinase-1 in the third trimester. These higher serum levels may be associated with the adverse perinatal effects of obesity during pregnancy.

Keywords: Neopterin, obesity, periostin, pregnancy, Tenascin-C, tissue inhibitor of metalloproteinase-1

Öz

Amaç: Bu çalışmanın amacı, obez gebe kadınlarda ve normal kilolu gebe kadınlarda üçüncü trimester serum neopterin, periostin, Tenascin-C, metalloproteinaz-1 doku inhibitörü ve matriks metalloproteinaz-2 düzeylerini karşılaştırarak, ekstrasellüler matriks proteinlerinin obez gebe kadınlarda enflamatuvar yanıtın moleküler mekanizmasındaki rolünü araştırmaktır.

Gereç ve Yöntemler: Nisan 2021 ile Aralık 2021 tarihleri arasında yürütülen bu prospektif kesitsel çalışmaya toplam 84 hasta dahil edildi ve her grupta 28 katılımcı olmak üzere üç grup oluşturuldu.

PRECIS: Serum neopterin, periostin, tenascin-C, and TIMP-1 levels are significantly higher in class II-III obese pregnant women than in class I obese and normal weight women.

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Bulgular: Sınıf II-III obez gebelerde serum neopterin, periostin, Tenascin-C ve metalloproteinaz-1 doku inhibitörü seviyeleri, sınıf I obez ve normal kilolu kadınlara göre anlamlı olarak daha yüksek saptanırken (sırasıyla p=0,002, p<0,001, p<0,001, ve p <0,001), gruplar arasında serum matriks metalloproteinaz-2 seviyeleri açısından anlamlı fark izlenmedi (p=0,769). İşlem karakteristik eğrisi analizi, Tenascin-C ve periostinin preeklampsiyi öngörmede etkili olduğunu gösterdi [sırasıyla eğri altındaki alan (AUC)=0,82, %95 güven aralığı (GA), 0,72-0,90, p<0,001 ve AUC=0,71, %95 GA, 0,60-0,80, p=0,007].

Sonuç: Bu çalışma, sınıf II-III obez gebelerin üçüncü trimester serum neopterin, periostin, Tenascin-C ve metalloproteinaz-1 doku inhibitörü düzeylerinin anlamlı derecede yüksek olduğunu göstermiştir. Bu yüksek serum seviyeleri, gebelikte obezitenin olumsuz perinatal etkileri ile ilişkili olabilir.

Anahtar Kelimeler: Neopterin, obezite, periostin, gebelik, Tenascin-C, metalloproteinaz-1 doku inhibitörü

Introduction

Obesity, which is characterized by an excess of adipose tissue in the body, is a condition that affects an increasing number of people globally. Because of the numerous alterations in adipose tissue brought on by obesity, including significant changes in the structure and growth of adipocytes, the differentiation of preadipocytes into adipocytes, and the accumulation of inflammatory cells, obesity is thus considered a chronic inflammatory condition. As a result of obesity and pregnancy, pregnant obese women are prone to an increased inflammatory response with increased macrophage accumulation and synthesis of inflammatory mediators in maternal plasma[1]. Obesity during pregnancy is thought to alter metabolic, neuroendocrine, microbiological, and immunological pathways. Obesity is defined as having a body mass index (BMI) at the onset of pregnancy of 30 kg/m^2 or more; however, not all women fulfill these criteria. It has been reported that an estimated 18% of pregnant women are obese or overweight, and pre-pregnancy obesity is the greatest risk factor for obesity during pregnancy[2]. Several maternal and fetal adverse outcomes, such as pregnancy loss, fetal death, gestational diabetes, large for gestational age, gestational hypertension, venous thrombosis, increased rates of cesarean section, and fetal malformations, are associated with obesity during pregnancy and may be a consequence of the excessively inflammatory environment.

Remodeling of the extracellular matrix (ECM) in adipose tissue could alter adipocyte function and adipokine secretion profiles. The matrix metalloproteinase (MMP) family and the fibrinolytic plasminogen/plasmin system mediate the degradation of ECM components and regulate adipose tissue morphology. Alterations of adipose tissue in the ECM and inflammation associated with obesity have attracted considerable attention, highlighting the interaction of these processes in the transcriptome signature of adipose tissue in obese patients[3]. During the chronic inflammatory state associated with obesity, the ECM serves as a scaffold for cell infiltration and a reservoir for adipokines and growth factors[4]. A subset of the so-called damage-associated molecular patterns in the ECM can also directly activate the inflammatory response during tissue damage.

Before its reclassification as a matricellular protein, periostin was initially characterized as a secreted cell adhesion protein and was previously known as osteoblast-specific factor 2[5]. Periostin is produced by various cytokines and may contribute to the maintenance or exacerbation of inflammation. Additionally, periostin is secreted as a direct result of inflammatory reactions instead of acting to regulate the activities of these responses. The pathophysiology of fibrosis, arthritis, osteoarthritis, and atherosclerosis, as well as carcinogenesis and metastasis, including breast, colon, pancreatic, and ovarian cancers, has been linked to periostin, which was originally cloned from a mouse osteoblast cell line. Multiple signaling pathways, such as Wnt/b-catenin and PI3K/AKT, have also been associated with periostin. Periostin has also been linked to metabolic disorders by suppressing hepatic fatty acid oxidation via the JNK pathway[6]. It is not yet known what role periostin plays in lipid metabolism and obesity in pregnancy.

Tenascin-C (TnC) is also an ECM protein that is significantly expressed throughout organogenesis and is involved in cell interactions during proliferation, migration, epithelial-mesenchymal transition, and parenchymal-mesenchymal interactions[7]. In animal models of diet-induced obesity, increased tissue inflammation was associated with increased expression of TnC and Toll-like receptor 4 in stellate liver cells[8]. TnC has also been shown to be overexpressed in human preadipocytes in response to stimuli secreted by macrophages. TnC is usually expressed in association with MMP, and suppression of MMP inhibits the expression of TnC. MMPs, which are endoproteinases that require zinc and calcium to function, can break down the ECM. Because MMPs perform such a wide variety of tasks, there is growing evidence to suggest that they may play a role as either activators or inhibitors in the processes of tissue remodeling, cardiovascular disease, and obesity. Recent research has established a close relationship between adipose tissue inflammation and ECM, in which MMP and TnC expression may play a critical role. However, to our knowledge, there are no data on the level of TnC in the serum of obese pregnant women or in its putative involvement in adipose tissue inflammation.

The pteridine derivative neopterin is generated by dendritic cells, macrophages, activated monocytes, and endothelial cells. It has been found in many bodily fluids, including plasma, urine, saliva, synovial fluid, and cerebrospinal fluid. The level of neopterin in body fluids is used to assess the systemic immunological and inflammatory responses in various diseases[9]. Macrophages are the most abundant type of immune cells in a healthy placenta. The T helper type 2 immune response appears to be represented in the placenta by a predominance of alternatively activated macrophages. Obesity during pregnancy results in an enhanced inflammatory response in the placenta, characterized by accumulating numerous macrophage subsets

and the production of proinflammatory mediators, a hallmark of chronic villous inflammation[10]. Before pregnancy, chronic inflammation in obese women triggers a cascade of events leading to an inflammatory environment in the uterus. However, no studies have been conducted on the association between pregnant obesity, inflammation, and serum neopterin levels[11]. Tissue Inhibitor of Metalloproteinase-1 (TIMP-1) is the major regulatory peptide for extracellular protease enzymes, including MMPs and disintegrin metalloproteinases (ADAMs and ADAMTSs), which are responsible for ECM degradation. It is also secreted by adipocytes, which are increased in obesity, and it supports the formation of adipose tissue. MMPs and TIMPs have recently been linked to obesity-induced adipose tissue enlargement, and TIMP-1 appears to be an interesting candidate for increased fat formation for several reasons. The fat-derived protein TIMP-1 promotes adipose tissue growth when weight gain occurs[12]. However, the exact mechanisms by which obesity-induced TIMP-1 production is triggered in adipocytes remain a mystery. To date, no human studies have been performed to examine blood levels of MMP and TIMP-1 in obese pregnant women, although animal studies have shown alterations in these markers in cardiac and cartilage tissue of offspring of obese mothers[13].

While a few studies have found increased serum levels of neopterin, periostin, TN-C, TIMP-1, and MMP-2 in patients with perinatal complications such as preeclampsia, no studies have examined these ECM protein levels in pregnant women with obesity. Also, the usefulness of these markers in predicting adverse perinatal outcomes has not been extensively studied. Here, we investigated the role of ECM proteins in the molecular mechanism of the inflammatory response in obese pregnant women by comparing serum levels of neopterin, periostin, TnC, tissue inhibitor of metalloproteinase-1, and MMP-2 in obese and normal-weight pregnant women in the third trimester.

Materials and Methods

Study Design and Setting

This prospective cross-sectional study was conducted between April 27, 2021 and December 01, 2021 in the Gynaecology Department of Inonu University School of Medicine. A total of 84 pregnant women were enrolled and three groups were formed, each with 28 participants. The groups were divided according to the World Health Organization BMI classification: Control (BMI 18-24.9 kg/m^2), Class I obesity (BMI 30-34.9 kg/m^2), and Class II-III obesity (BMI ≥35 kg/m^2). The following are the criteria by which patients were enrolled in the study: (1) a viable singleton pregnancy; (2) 37^{+0} to 40^{+6} weeks of gestation (confirmed by first trimester ultrasound); (3) normal fetal anatomy; and (4) patients who understood both the oral and written instructions in Turkish and gave their written consent to participate. Exclusion criteria were as (1) coexisting medical conditions (diabetes, hypertension, renal failure, chronic hepatic disease, pulmonary or cardiovascular disease); (2) multiple pregnancy; (3) fetal death; (4) severe anomalies (fatal or those requiring prenatal or postnatal surgery) or chromosomal abnormalities; and (5) tobacco and alcohol use. All participants gave their informed consent before the study was conducted. This research was carried out in accordance with the principles outlined in the Declaration of Helsinki, and it received ethical approval number 2021/138 from the Clinical Research Ethics Committee of Inonu University.

Serum Analysis

Every pregnant woman who participated in the study had a small amount of blood drawn from a peripheral vein into biochemical tubes (2-3 mL) upon admission to the hospital. Serum was separated from blood samples by centrifuging them at 3500 rpm for 15 min at room temperature, and then placed in microcentrifuge tubes for long-term storage at -80 °C. Neopterin, periostin, TnC, TIMP-1, and MMP-2 were quantified in the serum samples using kits for enzymatic immunoassays (E3155Hu, E2063Hu, E3226Hu, E1414Hu, and E0796Hu; Bioassay Technology Laboratory Ltd, Birmingham, UK). The assay ranges for neopterin, periostin, TnC, TIMP-1, and MMP-2 were 0.1-38.0 nmol/L, 1-400 ng/mL, 0.5-150 ng/mL, 20-6.000 ng/L, and 0.3-90.0 ng/mL, respectively. The coefficients of variation for inter- and intra-assay precision were less than 10% and less than 8%, respectively, for all enzyme-linked immunosorbent assay (ELISA) kits. All serum samples were kept in the refrigerator and the ELISA tests were run in the laboratories at the School of Pharmacy. For each patient, age, BMI (weight in kilograms divided by square of height in meters), BMI before pregnancy, gestational week, gravidity, parity, adverse perinatal outcomes, mode of delivery, cord blood pH, cord blood base deficiency, histological results, neonatal outcomes, and serum levels of protein markers including neopterin, periostin, TnC, TIMP-1, and MMP-2 were recorded.

Histological Evaluation

Samples of placental tissue fixed in 10% formalin were examined under a light microscope. For the preparation and paraffin embedding of the placental tissue samples, the usual methods of tissue processing were used. To examine the tissue, 5-mm-thick sections were cut from the paraffin blocks, placed on slides, and hematoxylin and eosin (H-E) staining was performed. A Leica DFC280 microscope with a Leica Q Win and an image analysis system from Leica Micros Imaging Solutions Ltd (Cambridge, U.K.) was used to analyze the tissue sections. Histopathologic examination of damaged tissue was performed for each parameter, such as fibrin deposition, villous stroma disruption, villous basement membrane thickening, syncytial knots, and inflammatory cell infiltration in the placental samples. At least five microscopic regions were examined to evaluate the samples semiquantitatively. A three-point scale was used to score each specimen, indicating 0 none, 1 mild, 2 moderate, and 3 severely. The histopathologic score was calculated based on these parameters.

Power Analysis

Because of the power analysis performed, with a predicted effect size of 0.40 (moderate), the minimum sample size per group required to compare the changes in neopterin levels between the control group, the class I obesity group, and the class II-III obesity group was calculated to be 28 subjects with a power of 90% at a 95% confidence level[1].

Statistical Analysis

Mean ± standard deviation (SD), median (minimum-maximum), and number (percent) were used to summarize the data (percent). The Shapiro-Wilk test was used to verify that the data followed a normal distribution. Statistical analysis was performed using one-way analysis of variance, Kruskal-Wallis tests, and Pearson chi-square tests. Perinatal complication prediction performance and optimal cut-offs for the variables were established using receiver operating characteristic (ROC) analysis. Assuming a p-value less than 0.05 indicates statistical significance. The analysis was performed in IBM SPSS Statistics version 25.0. Histopathologic data were expressed as mean ± SD. Histological results were compared using Kruskal-Wallis analysis of variance.

Results

Class II-III obese patients were significantly older than class I obese and normal-weight patients (p<0.001). As expected, there was a significant difference in BMI between the normal-weight, class I obese, and class II-III obese groups (p<0.001). The groups were comparable in terms of gestational age at hospital admission (p=0.583). Additionally, there were significant differences in pre-pregnancy BMI between the groups (p<0.001). In the prenatal period, the rate of preeclampsia was significantly higher in class II-III obese pregnant women compared to the other groups (p=0.008). The rates of gestational diabetes mellitus, fetal growth restriction, and preterm birth were similar among the groups (p=0.117, p=0.913, and p=0.367, respectively). There were no significant differences in gestational age at birth or neonatal birth weight in all three groups (p=0.994 and p=0.237, respectively). Neonatal morbidity rates were comparable between groups (p=0.747), and there was no perinatal mortality in the entire study group. Maternal characteristics and perinatal and neonatal outcomes of the study population are summarized in Table 1.

The serum levels of neopterin, periostin, TnC, tissue inhibitor of metalloproteinase-1, and MMP-2 in the third trimester are summarized in Table 2. Neopterin, periostin, and TIMP-1 were significantly higher in obese class II-III patients than in class I obese and normal-weight patients (p=0.002, p<0.001, and p<0.001, respectively). There was no significant difference in MMP-2 levels between the groups (p=0.769). TnC levels were lowest in the normal-weight group and highest in class II-III obese pregnant women (p<0.001). The comparison of serum levels of neopterin, periostin, TIMP-1, TnC, and MMP-2 is shown in Figure 1.

ROC curve analysis was performed to evaluate the ability of serum biomarkers to predict pre-eclampsia. TnC showed the best performance in predicting pre-eclampsia [area under the curve (AUC)=0.82, 95% confidence interval (CI), 0.72-0.90, p<0.001]; however, periostin also proved effective (AUC=0.71, 95% CI, 0.60-0.80, p=0.007) (Table 3) (Figure 2).

In the control group, placental tissue showed a normal histological appearance. Patients in the control group had normal appearance of villi with normal vasculosyncytial membranes and syncytiotrophoblastic layer (Figure 3). Fibrin-containing fibrinoid deposits, and villous stroma disruption were observed more frequently in the placental villi of the class II-III obesity group and class I obesity group compared with the control group. Also, the fragmentation of the vasculosyncytial membranes and irregularities in the syncytiotrophoblastic layer was observed in the obese groups compared with the control group. Additionally, syncytial knots were more frequent in obese patients in class II-III obese patients than in class I obesity and control groups (Figures 4 and 5). Table 4 shows the histopathological scores of the control, class I obesity, and class II-III obesity groups.

Discussion

This study showed that serum levels of neopterin, periostin, TnC, and TIMP-1 were significantly higher in class II-III obese pregnant women than in class I obese and normal-weight women. Particularly in patients with extreme obesity, insights into the molecular factors associated with increased perinatal complications and obesity and elucidation of the pathophysiological mechanisms are needed to develop effective diagnostic and therapeutic strategies. Adequate vascular and ECM remodeling is strongly associated with the progressive and balanced increase in adipocytes in response to total calorie intake. Increasing adiposity, on the other hand, is probably linked to a condition of inflammatory processes in adipose tissue that promote ectopic lipid accumulation. The results of this study showed significantly increased serum levels of ECM proteins, which regulate inflammatory responses, in class II-III obese pregnant women. Histopathological analyses of the placentas of the obese pregnant women also showed higher histopathological scores than those of the control group. Histopathological findings of placental damage, such as fibrin deposition, villous stroma disruption, villous basement membrane thickening, syncytial knots increment, and inflammatory cell infiltration, were observed more frequently in placental samples from obese pregnant women. In line with this, recent studies have revealed that macrophages play a significant role in the inflammatory state in adipose tissue and ECM remodeling that is considered a pathological status rather than adaptive feedback[14]. Evidence also suggests

that as chronic inflammation progresses, the adipose tissue undergoes adipocyte hypertrophy, proinflammatory invasion, regulation of angiogenesis, and enhanced ECM synthesis. Adipose tissue macrophages are dominant contributors of proinflammatory mediators that sustain inflammation throughout the obesity process. Therefore, a paracrine circuit between adipocytes and macrophages may impact the progression of metabolic disorders in various organs due to obesity-related dysregulation of adipocytokine production.

Many biological processes involve pteridines like neopterin and biopterin, which are produced by the oxidation of tetrahydrobiopterin, and both tetrahydrobiopterin and its derivative products can be detected in physiological fluids and tissues. Neopterin is an effective biomarker for inflammatory diseases because it sensitively detects T helper type 1 immune response in humans[15]. In this study, we found significantly higher serum neopterin levels in women with class II-III obesity compared with the other groups. Consequently, higher neopterin levels are frequently observed in disorders associated with oxidative stress, and it has been hypothesized that neopterin may serve as a signal for oxidative stress indirectly resulting from immune system dysfunction. Sugulle et al.[16] found elevated neopterin levels in pregnant women with late-onset preeclampsia. Similarly,

Table 1. Maternal characteristics and perinatal and neonatal outcomes of groups

		Group			p-value
		Control (n=28)	Class I Obesity (n=28)	Class II-III Obesity (n=28)	
		Mean ± SD	Mean ± SD	Mean ± SD	
Age (years)		29.86a±5.32	29.11a±5.04	34.93b±4.22	**<0.001***
Prepregnancy BMI (kg/m²)		21.11a±2.05	26.48b±2.8	32.42c±3.91	**<0.001***
Weight (kg)		64.07a±5.25	82.14b±7.12	97.11c±10.39	**<0.001***
Height (cm)		163.29a±5.46	161.46a±7.14	159.75a±6.51	0.125*
BMI (kg/m²)		24.47a (18.87-24.98)	31.15b (30.08-34.14)	36.76c (35.16-46.09)	**<0.001****
		Median (Min-Max)	Median (Min-Max)	Median (Min-Max)	
Gestational age at admission		38a (37-40)	37.5a (36-40)	37.5a (36-41)	0.583**
Gravidity		2.0a (1.0-8.0)	3.0a (1.0-9.0)	3.0a (1.0-6.0)	0.309**
Parity		1.0a (0.0-4.0)	1.0a (0.0-4.0)	1.0a (0.0-4.0)	0.242**
Gestational age at birth (weeks)		38a (37-40)	38a (37-41)	38a (37-41)	0.994**
Birthweight (g)*		2945a (2545-4100)	3057.5a (2540-3700)	3180a (2530-4800)	0.237**
Cord blood pH		7.37a (7.27-7.47)	7.37a (7.17-7.6)	7.36a (7.18-7.43)	0.589**
Cord blood base excess		-3.95a (-18-6.2)	-4.4a (-10.5-0.8)	-3.7a (-8.1-1.3)	0.423**
		Count (Percent)	Count (Percent)	Count (Percent)	
Mode of delivery	Cesarean section	28a (100.00%)	27a (96.43%)	27a (96.43%)	0.599***
	Vaginal delivery	0a (0.00%)	1a (3.57%)	1a (3.57%)	
Gender	Female	17a (60.71%)	11a (39.29%)	15a (53.57%)	0.263***
	Male	11a (39.29%)	17a (60.71%)	13a (46.43%)	
Gestational DM		3a (10.7%)	3a (10.7%)	8a (28.6%)	0.117***
Preeclampsia		0a (0.00%)	2a, b (7.14%)	7b (25.00%)	**0.008*****
FGR		4a (14.29%)	5a (17.86%)	4a (14.29%)	0.913***
Preterm birth		3a (10.71%)	7a (25.00%)	6a (21.43%)	0.367***
Fetal distress		3a (10.71%)	2a (7.14%)	2a (7.14%)	0.856***
Admission to NICU		4a (14.29%)	8a (28.57%)	11a (39.29%)	0.109***
Neonatal morbidity		3a (10.71%)	4a (14.29%)	5a (17.86%)	0.747***

*: One-way ANOVA test; **: Kruskal-Wallis test; ***: Pearson chi-square; There is a statistically significant difference in the group categories that do not contain the same letter on a row basis, BMI: Body mass index, DM: Diabetes mellitus, HT: Hypertension, FGR: Fetal growth restriction, NICU: Neonatal intensive care unit, Min-Max: Minimum-Maximum, SD: Standard deviation, Bold values show p<0.05

pregnant women with gestational diabetes had greater serum neopterin levels compared with healthy controls and the inflammatory response in GDM was closely related to obesity[17]. Our current clinical finding is well supported by the literature and suggests the potential importance of neopterin in the development of pregnancy complications associated with obesity.

Table 2. Serum neopterin, periostin, Tenascin-C, Tissue inhibitor of metalloproteinase-1 and matrix metalloproteinase-2 levels of control and study groups

	Group			p-value
	Control (n=28)	Class I Obesity (n=28)	Class II-III Obesity (n=28)	
	Median (Min-Max)	Median (Min-Max)	Median (Min-Max)	
Neopterin (nmol/L)	4.07a (1.76-9.44)	4.33a (2.13-18.37)	12.39b (2.42-19.58)	**0.002****
Periostin (ng/mL)	44.57a (10.33-97.48)	63.68a (10.59-93.49)	79.39b (10.64-120.33)	**<0.001****
Tenascin C (ng/mL)	932a (557-8757)	2637b (1137-5857)	5672c (1147-12587)	**<0.001****
MMP-2 (ng/mL)	18.67a (12.03-45.56)	21.15a (13.55-80.94)	18.25a (7.5-85.14)	0.769**
TIMP-1 (ng/L)	127a (99.75-597.22)	198.48a (98.1-822.82)	795.63b (104.91-998.1)	**<0.001****

*: One-way ANOVA test; **: Kruskal-Wallis test; ***: Pearson chi-square; There is a statistically significant difference in the group categories that do not contain the same letter on a row basis, MMP: Matrix metalloproteinase, TIMP: Tissue inhibitor of metalloproteinase, Min-Max: Minimum-Maximum, bold values show p<0.05

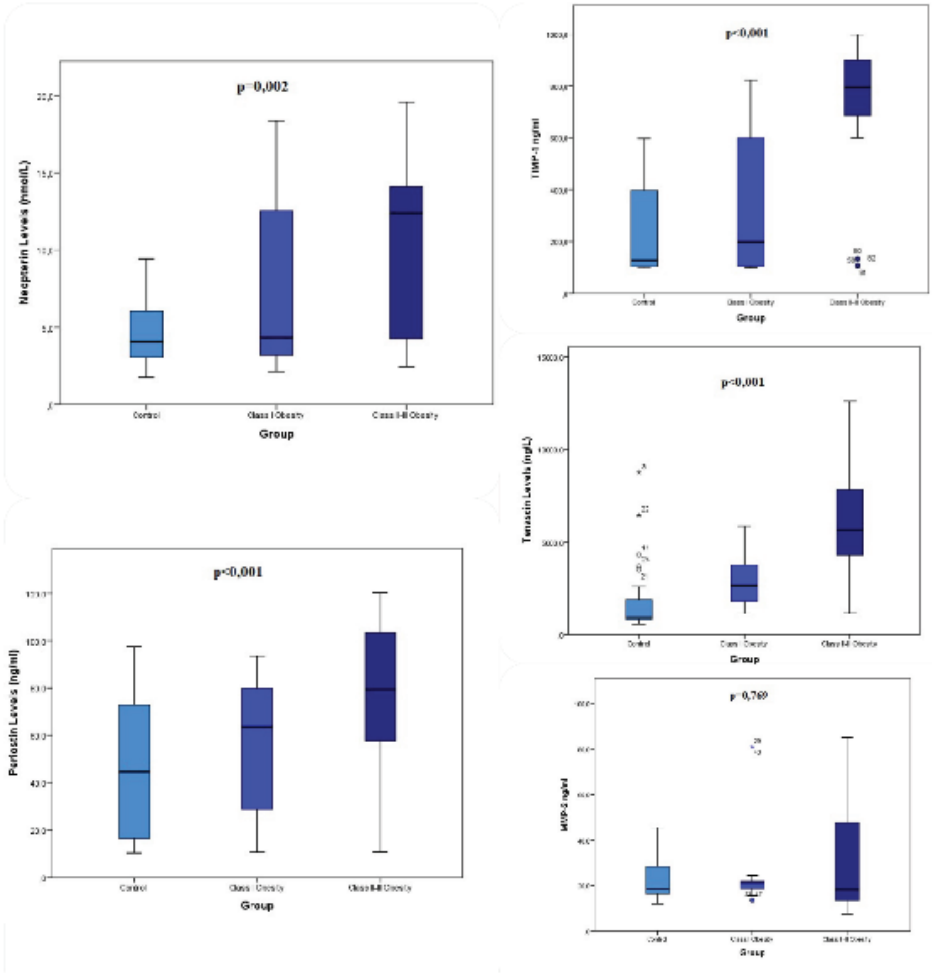

Figure 1. Comparison of neopterin, periostin, tissue inhibitor of metalloproteinase-1, Tenascin C, and matrix metalloproteinase-2 levels of the study groups compared to the control group

Maternal obesity is a major problem in today's world as more and more women of childbearing age are overweight. It has both short- and long term harmful effects on the mother and baby. The placenta of obese women exhibits altered function related to increased inflammation and oxidative stress[18]. Challier et al.[10] also observed that the chronic inflammatory state of pre-pregnancy obesity, which continues during pregnancy, increases macrophages and proinflammatory mediators in the placenta. They suggested that fetal metabolic programming of obesity and insulin resistance syndrome may occur because of this inflammatory environment. In this study, although no significant differences were found in the early neonatal outcomes of lean and obese pregnant women, the rate of preeclampsia was significantly higher in class II-III obese patients. Besides, the predictive value of ECM proteins for preeclampsia was also evaluated as new potential biomarkers, and TnC and periostin were found to be effective in predicting pre-eclampsia. Accordingly, Ribatti et al.[19] demonstrated tenascin expression in preeclamptic decidua in association with angiogenesis and showed that both angiogenesis and tenascin expression is induced by implants from preeclamptic decidua. The placenta and skin contain the greatest levels of TnC among human tissues. The placental mesenchymal villi, which are

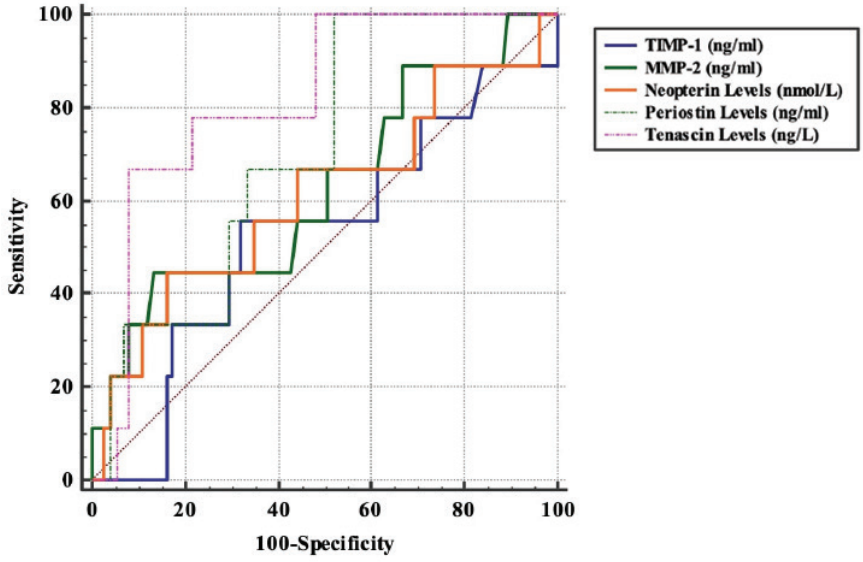

Figure 2. Receiver operating characteristic analysis showing the utility of neopterin, periostin, tissue inhibitor of metalloproteinase-1, Tenascin C, and matrix metalloproteinase-2 levels in the study cohort for predicting preeclampsia

Table 3. ROC analysis showing the predictive value of biomarkers for preeclampsia

Variables	Cut-off	Sensitivity	Specificity	LR+	LR-	PPV	NPV	AUC (95% CI)	p-value[a]
Neopterin	3.00	44.44 (13.7-78.8)	84.00 (73.7-91.4)	2.78	0.66	25.0	92.6	0.61 (0.50-0.71)	0.102
Periostin	53.4	100.0 (66.4-100.0)	48.00 (36.3-59.8)	1.92	0.0	18.8	100.0	0.71 (0.60-0.80)	**0.007**
Tenascin C	6627	66.67 (29.9-92.5)	92.00 (83.4-97.0)	8.33	0.36	50.0	95.8	0.82 (0.72-0.90)	**<0.001**
MMP-2	29.17	44.4 (13.7-78.8)	86.67 (76.8-93.4)	3.33	0.64	28.6	92.9	0.63 (0.51-0.73)	0.254
TIMP-1	599.75	55.56 (21.2-86.3)	68.00 (56.2-78.3)	1.74	0.65	17.2	92.7	0.53 (0.42-0.64)	0.806

MMP: Matrix metalloproteinase, TIMP: Tissue inhibitor of metalloproteinase, AUC: Area under the curve, LR: Likelihood ratio, PPV: Positive predictive value, NPV: Negative predictive value, ROC: Receiver operating characteristic, bold values show p<0.05

Table 4. The histopathological score of groups

Groups	Placenta histopathology score (mean ± SD)
Control group	0.51±0.07[a]
Class I obesity	1.76±0.08[b]
Class II-III obesity	2.33±0.09[c]

SD: Standard deviation, The mean differences in the values bearing different superscript letters within the same column are statistically significant (p<0.001)

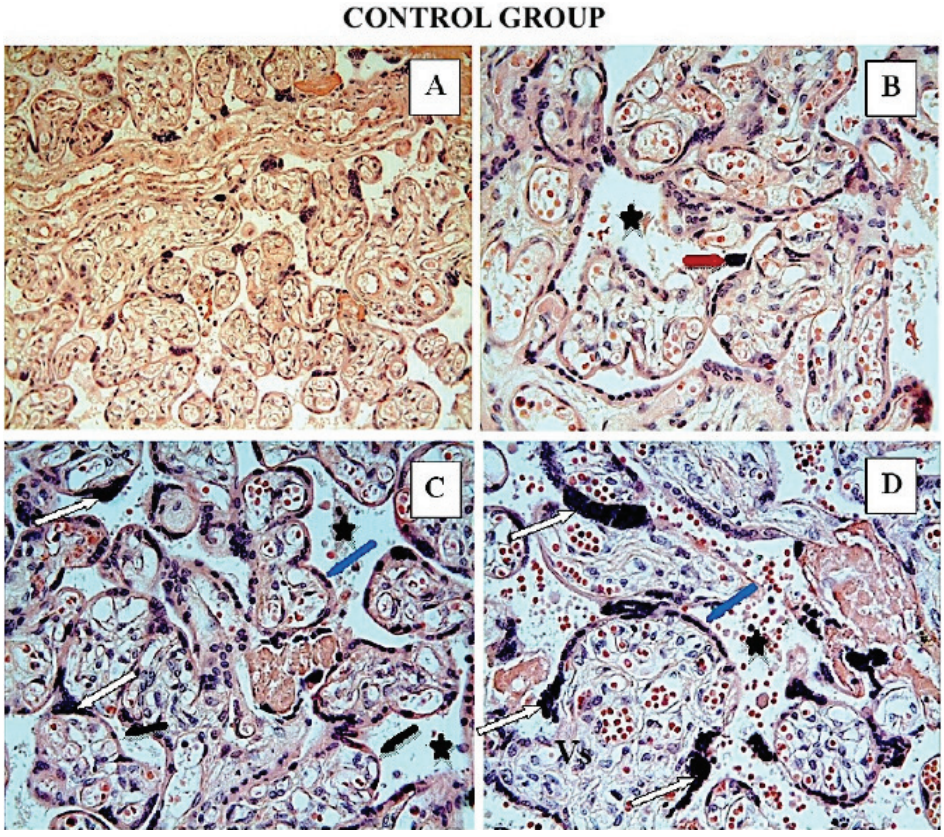

Figure 3. (Control group). Normal villi were observed in patients with body mass index between 18-24.9 kg/m^2. (A). The villi are characterized by the formation of vasculosyncytial membranes (black arrows) (C), which result from the accumulation of syncytiotrophoblasts in syncytial knots (white arrows) (C, D). The syncytiotrophoblastic layer (blue arrows) (C, D) was observed in normal appearance in this group. Maternal red blood cells (black asterisks) are present in the intervillous space (B, C, D). The villous stroma (D) contains fetal blood capillaries. The fetal blood capillaries are filled with fetal red blood cells. A: H-E; X20, B, C, D: H-E; X40

assumed to constitute the framework for the promotion and differentiation of the villous tree, exhibit high levels of tenascin. Villous expansion, cell growth, and formation of fibrinoid deposits have all been linked to TnC expression in the human placenta[20]. In agreement with our results, serum periostin concentrations were observed to be considerably higher in women diagnosed with pre-eclampsia compared with normotensive controls by Sasaki et al.[21] This study's *in situ* hybridization results showing periostin expression in placental stromal cells suggests that this molecule plays a role in adhesion. Adhesion molecules like periostin and TnC are secreted by the placenta and may regulate inflammation or disrupt cell adhesion. Therefore, inflammation, which is the most important feature of preeclampsia, may be thought to be regulated in part by adhesion molecules that modulate leukocyte and endothelial cell activation.

The ECM is critical for adipocyte development and function and therefore is central to weight regulation, obesity, and lipid metabolism[22]. Recent studies have found close associations between adipose tissue inflammation and ECM proteins, and the expression of TIMP-1 and MMPs may play a key role[23]. To the our knowledge, there are no data on serum ECM protein levels in obese pregnant women or their possible involvement in adipose tissue inflammation during pregnancy. In this study, although MMP-2 levels were similar between groups, we observed statistically significantly higher serum TIMP-1 and neopterin levels in class II-III obese pregnant women. In parallel, recent clinical studies have described changes, particularly in the circulating levels of MMP-2 and MMP-9 and endogenous mediators of these MMPs (TIMPs) in obesity and obesity-related metabolic diseases[24]. This is important because the measurement of serum levels of MMPs and TIMPs may help elucidate important mechanisms potentially involved in the pathogenesis of obesity-related pregnancy complications. One of the major roles of MMP-2 is the degradation of ECM components, particularly type IV collagen, the major component of basal membranes. It has also been suggested that MMP-2 plays a crucial role in adipose tissue development and plays a central role in inflammation and vasoconstriction. However, in this study, no statistically

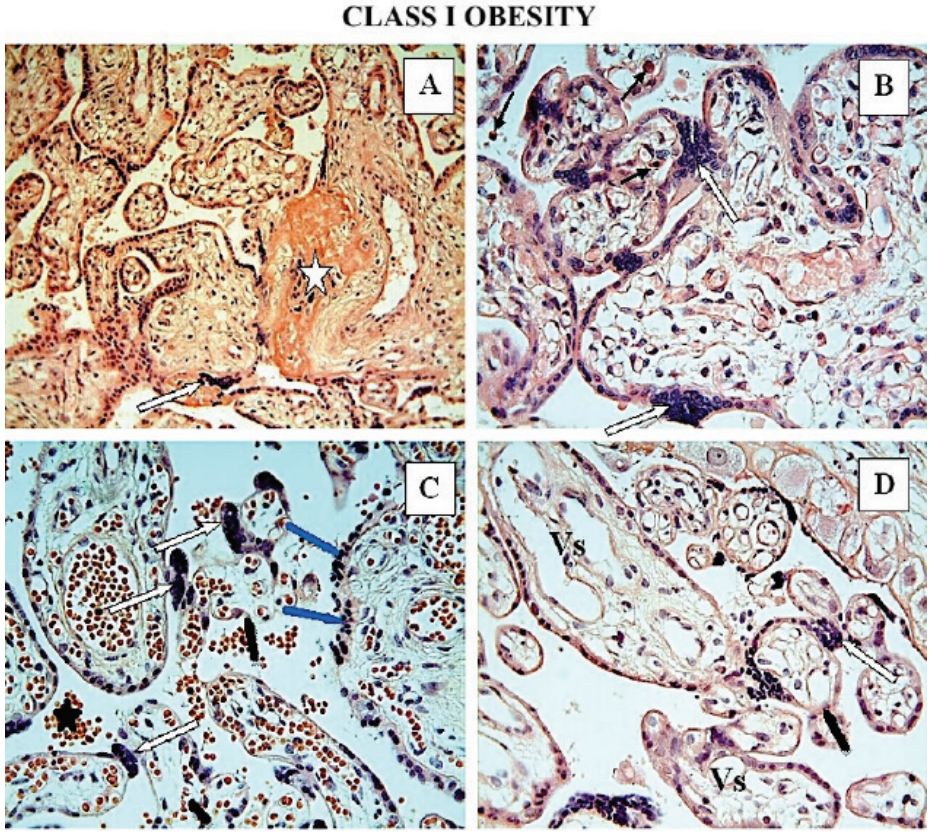

Figure 4. In the class I obesity group, white asterisks indicate some of the fibrin-containing fibrinoid deposits on the surface of the villi (A). In the class I obesity group, syncytial knots were increased. White arrows show that the syncytial knots (A, B, C) bulging into the intervillous space. Rarely fragmentation of the vasculosyncytial membranes was observed (black arrows) (C, D). Irregularities in the syncytiotrophoblastic layer (blue arrows) (C) were detected in the class I obesity group. A small amount of red blood cells were present in the intervillous space (black asterisks) (C). Disruptions of the villous stroma were also noticed. Apoptotic cells (thin black arrows) (B) were observed in the intervillous space and villi. A: H-E; X20, B, C, D: H-E; X40

significant difference was found between serum MMP-2 levels of overweight and normal-weight pregnant women. One possible explanation for these discrepant results is that the measurement of serum MMP levels rather than plasma in this study does not accurately reflect the circulating concentration of the enzyme of interest. The exact function of MMPs in the inflammatory cascade is not yet fully understood. It is assumed that they can be mediated and upregulated by inflammatory cytokines and that they also have an intrinsic influence on the inflammatory process.

Study Limitations

The small size of our sample and the fact that it was collected from a single center are two of our study's weaknesses. However, there were enough participants included in the study to reliably evaluate these protein markers. Additionally, serial serum measurements were not performed during pregnancy, even during the first and second trimesters. Moreover, we were unable to evaluate the expression of these biological markers through pathological examination of placental tissue. One of the study's strongest points is that, to the best of our knowledge, it is the first to analyze these protein biomarkers in obese pregnant women. The prospective cohort design was another strength.

Conclusion

In conclusion, this study shows that class II-III obese pregnant women had significantly higher serum levels of neopterin, periostin, TnC, and tissue inhibitor of metalloproteinase-1 in the third trimester. Thus, the fetuses of obese women are exposed to higher levels of these protein markers in utero than those of lean women. These higher serum levels may be associated with the adverse perinatal effects of obesity during pregnancy. Additionally, periostin and TnC are considered biomarkers of preeclampsia, and serum levels of these protein biomarkers could be used to support clinical decisions for predicting pre-eclampsia.

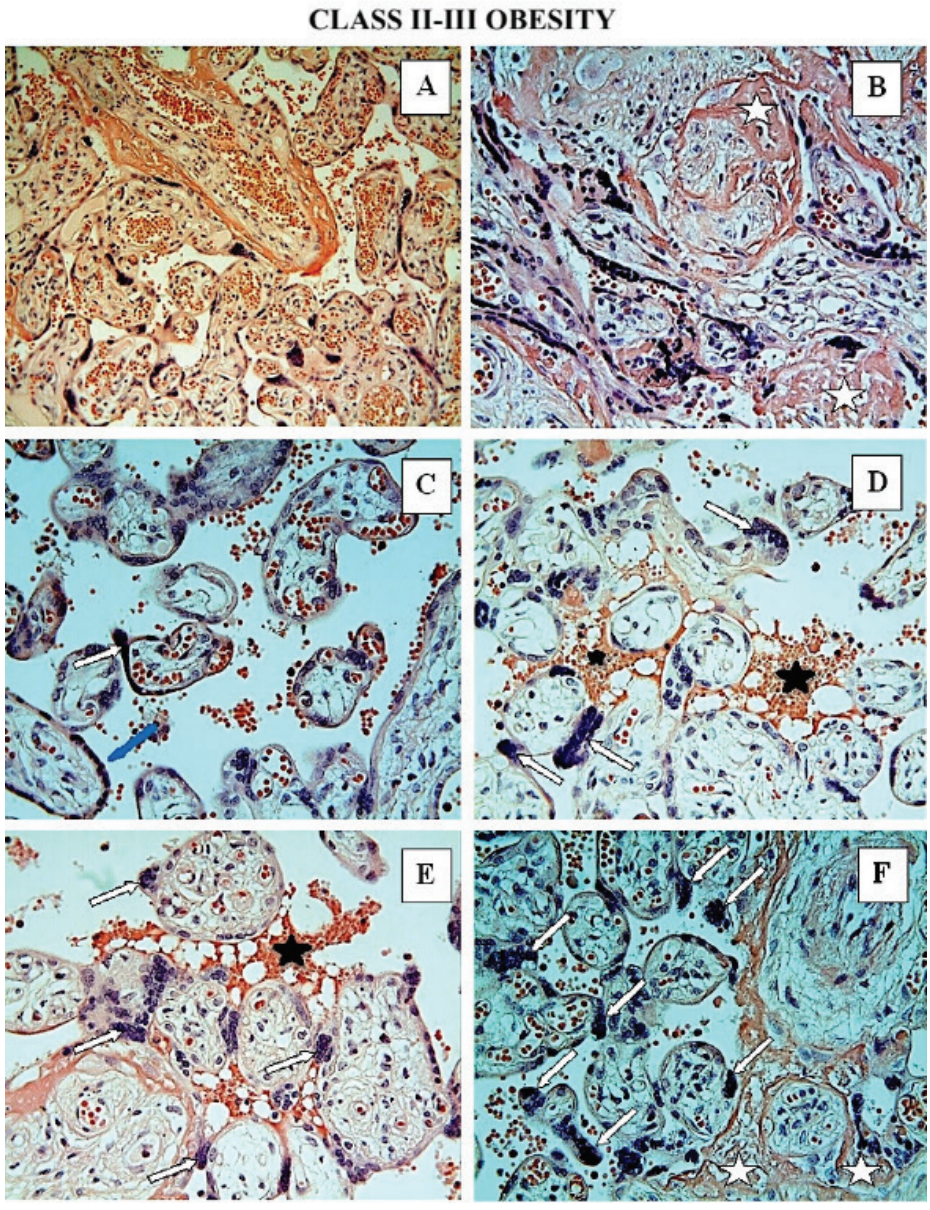

Figure 5. In the class II-III obesity group, deterioration and irregularity of villous structures were noted. White asterisks indicate some of the fibrin-containing fibrinoid deposits (B) on the surface of the villi. Syncytial knots were significantly increased in this group. White arrows indicate the syncytial knots (C, D, E, F) bulging into the intervillous space. The syncytiotrophoblatic layer (blue arrow) (C) was rarely observed in the villi. There is a large number of red blood cells (D, E) in the intervillous space (black asterisks). Disruption of villous stroma and vacuolization (thin black arrows) were observed in the class II-III obesity group. A: H-E; X20, B, C, D, E, F: H-E; X40

Ethics

Ethics Committee Approval: This research was carried out in accordance with the principles outlined in the Declaration of Helsinki, and it received ethical approval number 2021/138 from the Clinical Research Ethics Committee of Inonu University.

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

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: R.M., Concept: R.M., Design: R.M., S.Ü., Data Collection or Processing: S.Ü., N.B.T., N.Z.Ç., H.Y., Analysis or Interpretation: R.M., N.B.T., A.Ç., N.Z.Ç., Ş.Y., Literature Search: R.M., Writing: R.M., S.Ü.

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Predictive and diagnostic value of serum sVEGFR-1 level in women with preeclampsia: A prospective controlled study

Preeklampsili kadınlarda sVEGFR-1 serum düzeyinin prediktif ve tanısal değeri: Prospektif kontrollü bir çalışma

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Abstract

Objective: Pre-eclampsia (PE), a pregnancy-specific syndrome consisting of hypertension and proteinuria occurring *de novo* after the 20th week of gestation, remains the leading cause of maternal and fetal morbidity and mortality worldwide. Endothelial dysfunction is proposed to be a central feature of the pathophysiology of preeclampsia. However, the mechanism by which this endothelial dysfunction occurs remains uncertain. We investigated the predictive and diagnostic value of serum soluble vascular endothelial growth factor receptor-1 (VEGFR-1) with by comparison of its prepartum and postpartum serum levels in the management of women with PE.

Materials and Methods: This prospective case-controlled study was composed of pre-eclamptic (n=44) and normal, healthy pregnant (n=44) women. Blood samples were collected before any intervention at the first antenatal examination of the women in the control group and at the admission of the women to the hospital in the PE group, additionally, from all women in the study groups within six hours of the postpartum period, and used for the serum VEGFR-1 analyses.

Results: Within both groups, prepartum serum levels of sVEGFR-1 were higher than postpartum levels ($p<0.05$). In PE, pre-partum and postpartum serum levels of sVEGFR-1 were higher than levels in the control group ($p<0.05$). Serum sVEGFR-1 levels of preeclamptic women were positively correlated with the degree of proteinuria ($p<0.05$, $r=0.25$), systolic ($p<0.05$, $r=0.25$), and diastolic blood pressure ($p<0.05$, $r=0.31$).

Conclusion: These findings seem to point to an involvement of sVEGFR-1 in the pathophysiology of PE. Serum sVEGFR-1 has the potential to be used as a valuable biomarker in the prediction, diagnosis, and risk management of women with subtypes of PE including mild and severe PE, HELLP syndrome, and eclampsia. There is a need to study serum sVEGFR-1 as a biomarker in pregnant women with different subtypes of PE.

Keywords: Preeclampsia, eclampsia, HELLP syndrome, hypertension, pregnancy, sVEGFR-1, soluble vascular endothelial growth factor receptor

Öz

Amaç: Gebeliğin 20. haftasından sonra meydana gelen hipertansiyon ve proteinüriden oluşan gebeliğe özgü bir sendrom olan preeklampsi (PE), dünya çapında maternal ve fetal morbidite ve mortalitenin önde gelen nedeni olmaya devam etmektedir. Preeklamsi patofizyolojisinde endotel disfonksiyonunun temel bir neden olduğu bilinmektedir. Bununla birlikte, bu endotel disfonksiyonunun meydana geldiği mekanizma belirsizliğini korumaktadır. PE'li kadınların tedavisinde prepartum ve postpartum serum seviyelerinin karşılaştırılmasıyla serum çözünür, vasküler endotelyal büyüme faktörü reseptörü-1'in (VEGFR-1) öngörücü ve tanısal değerini araştırdık.

Gereç ve Yöntemler: Bu prospektif olgu kontrollü çalışma preeklamptik (n=44) ve normal sağlıklı hamile (n=44) kadınlardan oluşmaktaydı. Kan örnekleri, kontrol grubundaki kadınların ilk doğum öncesi incelemesinde ve kadınların PE grubundaki hastaneye kabul edilmesinde, ayrıca doğum gruplarındaki tüm kadınlardan, altı saat sonra serum VEGFR-1 analizleri için serum toplandı.

PRECIS: Serum sVEGFR-1 has a potential be used as a valuable biomarker in the prediction, diagnosis, and risk management of women with subtypes of preeclampsia.

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Bulgular: Her iki grupta da sVEGFR-1 prepartum serum seviyeleri postpartum seviyelerden daha yüksekti (p<0,05). PE'de, sVEGFR-1'in prepartum ve postpartum serum seviyeleri, kontrol grubundaki seviyelerden daha yüksekti (p<0,05). Preeklamptik kadınların serum sVEGFR-1 seviyeleri, proteinüri derecesi (p<0,05, r=0,25), sistolik (p<0,05, r=0,25) ve diyastolik kan basınçları (p<0,05, r=0,31) ile pozitif korelasyon göstermiştir.

Sonuç: Bu bulgular sVEGFR-1'in PE'nin patofizyolojisine etkili olduğu görülmektedir. Serum sVEGFR-1, hafif ve şiddetli PE, HELLP sendromu ve eklampsi dahil PE alt tipleri olan kadınların tahmini, tanı ve risk yönetiminde değerli bir biyobelirteç olarak kullanılabilir. PE'nin farklı alt tiplerine sahip hamile kadınlarda biyobelirteç olarak serum SVEGFR-1'i incelemeye ihtiyaç vardır.

Anahtar Kelimeler: Preeklampsi, eklampsi, HELLP sendromu, hipertansiyon, gebelik, SVEGFR-1, çözünür vasküler endotelyal büyüme faktörü reseptörü

Introduction

Pre-eclampsia (PE) is a multisystem disorder that begins after the 20th week of pregnancy, progresses by hypertension and proteinuria, and has fatal complications. The fetus is at risk because of the possibility of adverse outcomes such as intrauterine growth retardation, preterm labor, placenta abruption, and intrauterine fetal hypoxia due to hypertension and uteroplacental vascular insufficiency during pregnancy. The incidence of PE is approximately 3-8% in pregnant women[1-3]. PE usually occurs during the first pregnancy. Multiple pregnancies, history of PE, chronic hypertension, pre-pregnancy diabetes mellitus, vascular and connective tissue diseases, nephropathy, antiphospholipid antibody syndrome, obesity, dyslipidemia, high testosterone and homocysteine levels, pregnancies aged 35 and over are other risk factors[4]. In normal pregnancies, the spiral arteries are enlarging, and their walls are reshaping. These changes extend to the inner 1/3 of the myometrium, providing low resistance flow to the intervillous space and are related to the invasion of fetal trophoblasts. While the trophoblast invasion is complete before the 22nd week of normal pregnancies, in PE cases, is not complete during these weeks. The pathogenesis of PE has three components: poor placentation, placental ischemia, and endothelial cell dysfunction that causes placental vascular complications[2]. Many factors have been considered in the development of PE. Many factors, such as disruption of prostaglandin I2-thromboxane balance and nitric oxide metabolism, production of vasoconstrictor agents, increased oxidative stress, toxic compounds produced by the placenta, disruption of placental cytokine production cause endothelial dysfunction in PE. The effects of placental agents on maternal vascular structures (e.g., platelets, endothelial cells, and neutrophils), maternal risk factors (renal, metabolic, and vascular diseases), genetic factors, and immune disorders predispose for developing PE during pregnancy[5]. Secondary to the vasospasm caused by the increased sensitivity of the vessels to vasopressor agents, blood flow to all organs decreased. Perfusion impairs by the activation of the coagulation. Additionally, plasma volume decreases with fluid loss from the intravascular space[6]. The human placenta needs extensive angiogenesis to supply oxygen and nutrients to the fetus and to form its vascular network. Many angiogenic growth factors such as vascular endothelial growth factor (VEGF), placental growth factor (PIGF), and soluble VEGF receptor (sVEGFR)-1 are produced in the placenta. The biological effects of the VEGF family are regulated by the type III subgroup of receptor tyrosine kinases (RTKs), including VEGFR-1 (Flt-1), VEGFR-2 (KDR/Flk-1), and VEGFR-3 (Flt-4) that bind to VEGF family members[7].

The development of PE includes mainly three factors: Abnormal placental vasculature, endothelial dysfunction, and placental ischemia. VEGF and PIGF, having angiogenic effects, are suggested important promoters in the human placenta. Moreover, decreased concentrations of circulating free VEGF and PIGF have been reported in PE. Additionally, it has been shown that the soluble form of VEGF receptor-1 is increased in the placenta and serum in pregnant women with PE[8].

In recent studies, the disorder of circulating angiogenic and antiangiogenic factors has clarified as a significant biomolecule in the pathophysiology of PE, but there are no reliable biomarkers are available to predict and diagnose PE[9]. This condition needs the investigation of RTKs. Maynard et al.[10] have shown that sVEGFR-1 levels increase in the preeclamptic subjects. They reported that if sVEGFR-1 was removed from the preeclamptic placenta or VEGF was administered to block sVEGFR-1, the antiangiogenic situation returned to normal. Those SVEGFR-1-related data make further studies possible in clinical settings with women with or without hypertensive disorders of pregnancy. We thought that serum sVEGFR-1 may change differently in pregnant and puerperal women with or without PE. Although there are extensive research activities, it remains unclear when during the third trimester that delivery should be accomplished for maximal maternal safety and minimal fetal risk; and there is a need for clinical studies examining the potential use of possible biomarkers of PE. In this research project, we assessed the predictive and diagnostic value of serum sVEGFR-1 with the comparison of its prepartum and postpartum serum levels in the management of women with PE.

Materials and Methods

This prospective controlled study included consecutive women with or without PE in the Sivas Cumhuriyet University Department of Obstetrics and Gynecology. All participants signed a written informed consent form on enrollment. Forty-nine women diagnosed with PE, and 49 healthy pregnant women, 98 patients, were included in this study. The study protocol was performed according with the principles of the Declaration of Helsinki and was approved by the Human Ethics Committee of Sivas Cumhuriyet University (protocol ID: 05.04.2005-4/5).

The control group consisting of healthy pregnant women included pregnant women with a singleton pregnancy without health problems including hypertensive disorders of pregnancy, diabetes mellitus, and liver, kidney, or endocrine disease, pregnancy with intrauterine growth retardation (IUGR) below the 10th percentile, and multiple pregnancies, pregnant women were excluded from the control group. The PE group included women with subtypes of PE, including mild and severe PE, eclampsia, and HELLP syndrome. PE criteria in pregnant women after 20 weeks of gestation who made up the PE study group were based on blood pressure of at least 140/90 mm Hg persisting for 6 h or more and proteinuria (urine protein of 300 mg/L or more or 1+ with a urine test stick) in two random urine samples[11]. If one or more of the following criteria were present, the women were diagnosed with severe PE, if (1) systolic blood pressure of 160 mmHg or higher or diastolic blood pressure of 110 mmHg or higher twice, at least 6-hour interval; (2) proteinuria in 24-hour urine 5 g or more, or 3+ or more in two random urine samples taken at least 4-hour interval; (3) oliguria (less than 400 mL in 24 h), elevation in serum creatinine; (4) cerebral dysfunction or visual impairment; (5) pulmonary edema or cyanosis; (6) epigastric or right upper quadrant pain, nausea, vomiting; (7) impaired liver function tests; (8) thrombocytopenia (<100x10^3/mm^3); and (9) IUGR. Other patients with PE were diagnosed as mild PE. Additionally, if patients had convulsions, they were diagnosed with eclampsia. In the presence of hemolysis, high liver enzymes, and decreased platelet criteria, the women were accepted to have HELLP syndrome[11]. Preeclamptic pregnant women with a history of chronic hypertension, diabetes mellitus, renal disease, and endocrine diseases were excluded from the PE group.

Clinical parameters including the age, gravidity, body mass index (BMI), weight gain during pregnancy, smoking, gestational week, systolic and diastolic blood pressures, amount of protein in the urine, hemoglobin, platelet count, blood urea nitrogen (BUN), creatinine, alanine transaminase (ALT), aspartate transaminase (AST), lactate dehydrogenase (LDH), estimated fetal weight, and amniotic fluid index obtained by obstetric ultrasonography, mode of delivery, APGAR scores at 1 and 5 min, newborn weight, postpartum maternal complications, prepartum, and postpartum serum sVEGFR-1 levels, the presence of IUGR and placental abruption were recorded.

Before any intervention at the first antenatal examination of the women in the control group and at the admission of the women to the hospital in the PE group, additionally, from all women in the study groups with in six hours of postpartum period, blood samples were collected, and stored at -80 °C until analyses of the sVEGF-1. Collected serum samples were run on the ELISA (Triturus, Grifols Inc., California, USA) device with the sVEGFR-1 kit (BioSource International Inc., California, USA) and the results were recorded. The kit performance characteristics were sensitivity, 0.1 ng/mL, and coefficient of variation (CV%), <10.

Statistical Analysis

The conformity of the data with a normal distribution was evaluated with the Kolmogorov-Smirnov test and the variance homogeneity with the Levine test. Mann-Whitney U test was used to compare non-normally distributed variables. The medians of prepartum and postpartum serum sVEGFR-1 values in each group were compared with the Wilcoxon test. Normally distributed variables were compared with the t-test. The chi-square test was used in the comparison of the categorical variables. The relationship between clinical variables was evaluated with the Spearman and Pearson test according to the suitability of the data. A $p<0.05$ was considered statistically significant. In the power analysis with an effect size of 0.6, when the power was used as 85% and the alpha was accepted as 0.05, it was found that 41 control and 41 preeclamptic patients were required for the main variable of sVEGFR. With the addition of 20% drop-out, the study group had 49 participants.

Results

The study was completed with 44 women diagnosed with PE, and 44 healthy pregnant women, 88 patients. Eight pregnant women with PE did not participate in the study. In five pregnant women in each group, because of the work load, the study data were not collected completely and these participants were excluded from the study (Figure 1).

There were no significant differences between the pregnant women with PE and controls in terms of age, normal spontaneous vaginal delivery, and cesarean section ($p>0.05$).

In pregnant women diagnosed with PE, BMI, the arterial blood pressure (systolic and diastolic), and protein positivity in spot urine testing, the number of births with IUGR and placental abruption, was significantly higher than in healthy pregnant women ($p<0.05$).

The gestational age at delivery, birth weights were significantly lower in pregnant women diagnosed with PE compared with the control group ($p<0.05$) (Table 1).

In the preeclamptic patient group, 16 (36.4%) had mild PE, 12 (27.2%) severe PE, 3 (6.8%) eclampsia, 10 (22.8%) HELLP syndrome, 3 (6.8%) eclampsia and HELLP syndrome.

The medians of prepartum serum sVEGFR-1 values measured in the control and PE groups were higher than the postpartum serum sVEGFR-1 values ($p<0.05$). The median of prepartum serum sVEGFR-1 values of pre-eclamptic patients was

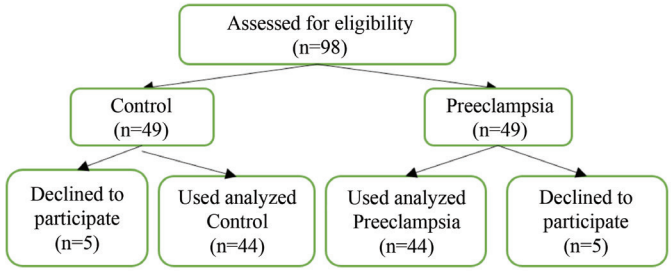

Figure 1. Flowchart of the study

significantly higher than the median of prepartum serum sVEGFR-1 values of the control group (p<0.05). The median of postpartum serum sVEGFR-1 values of pre-eclamptic patients was significantly higher than the median of postpartum serum sVEGFR-1 values of the control group (p<0.05) (Figure 2).

Platelet counts were lower and ALT, AST, and LDH levels were significantly higher in the PE group (p<0.05). There was no significant difference between the groups in terms of hemoglobin, BUN, and creatinine levels (p>0.05). In the preeclamptic patient group, 7 of the newborns had a birth weight below the 10th percentile, and 4 patients had placental abruption. There were no pregnant women with IUGR and placenta abruption in the control group. No correlation was found between IUGR and placental abruption and serum sVEGFR-1 level (p>0.05, r=0.08, and p>0.05, r=-0.08). There was a positive correlation between serum sVEGFR-1 levels and the degree of proteinuria (p<0.05, r=0.25), systolic (p<0.05, r=0.25) and diastolic blood pressure (p<0.05, r=0.31) in preeclamptic patients (Table 2).

Discussion

While regarding the age and rate of cesarean section, the control and preeclamptic groups, in terms of other baseline clinical characteristics of the study groups, there were meaningful changes supporting the adverse perinatal effects of PE. PE increased the prepartum and postpartum serum sVEGFR-1 levels. We found a meaningful association between PE with systolic blood pressure and proteinuria. PE caused some adverse results in hepatic function tests.

At currently, there is no adequate and practical screening method for PE. In the placenta, sVEGFR-1 is found in excess in the trophoblast layer. Studies have shown that sVEGFR-1 is increased in the placenta, serum, and amniotic fluid in PE[12]. It has been reported that sVEGFR-1 increases after the 12th week in patients who will develop PE, and serum levels decrease 24 h after delivery. However, VEGF level was evaluated as normal during these weeks. This indicates that sVEGFR-1 plays a more essential role in the pathogenesis of PE[13]. The increase in VEGF production in the following weeks is thought to be due to placental hypoxia resulting from insufficient invasion of spiral arteries by trophoblasts. The increase in sVEGFR-1 in PE

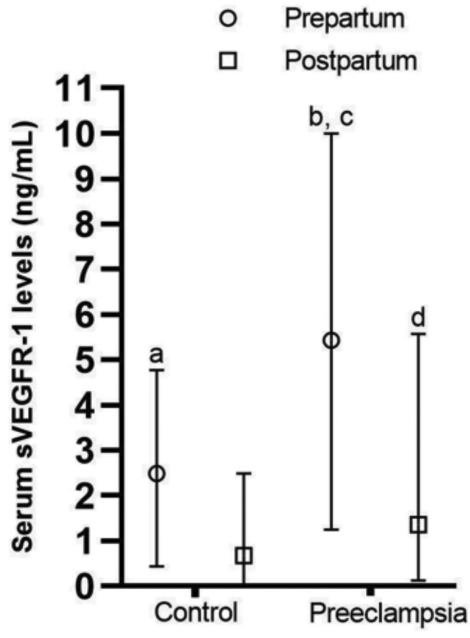

Figure 2. Prepartum and postpartum serum sVEGFR-1 levels of control and preeclampsia groups. Data are shown as median (minimum-maximum). a, p<0.05 vs postpartum serum sVEGFR-1 level of the control group. b, p<0.05 vs postpartum serum sVEGFR-1 level of preeclampsia group. c, p<0.05 vs prepartum serum sVEGFR-1 level of the control group. d, p<0.05 vs postpartum serum sVEGFR-1 level of the control group

sVEGFR-1: Soluble vascular endothelial growth factor receptor -1

Table 1. Selected clinical data of control and preeclampsia groups

	Control (n=44)	Preeclampsia (n=44)	p-value
Age (y)	27.1±5.8	29.1±6	NS
BMI (kg/m^2)	28.1±3.2	31±4.3	p<0.05
Gestational age at delivery (w), min-max (median)	29-42 (38)	26-40 (36)	p<0.05
Blood pressure (mmHg) Systolic, min-max (median) Diastolic, min-max (median)	 120 (90-135) 72.5 (60-85)	 140-200 (160) 90-140 (110)	p<0.05
Proteinuria (mg/dL), min-max (median)	0	30-500 (500)	p<0.05
Birth weight (g)	3025.9±669.8	2318±855.1	p<0.05
IUGR	0	7 (16%)	p<0.05
Placental abruption	0	4 (9.1%)	p<0.05
NSVD	18 (40.9%)	11 (25%)	NS
C/S	26 (59.1)	33 (75%)	NS

BMI: Body mass index, IUGR: Intrauterine growth restriction, NSVD: Normal spontaneous vaginal delivery, C/S: Cesarean section, NS: Non-significant

is also likely due to hypoxia or parallel to the increase in VEGF. It has been reported that VEGF stimulates the production of sVEGFR-1 in cultured endothelial cells[14].

Mateus et al.[15] found that VEGF-1 treatment in an animal study of PE reduces arterial blood pressure and prevents the onset of hypertension in late pregnancy. Their data demonstrated that VEGF administration reverses the effects of sVEGFR-1, playing an important role in PE.

Geva et al.[16] reported that pregnant women who have a baby with IUGR, had increased serum levels of VEGFR-1, VEGF-A, and sVEGFR-1, but there was no difference in the levels of free PIGF (fPIGF), VEGFR-2, neuropilin-1 (NRP-1). Bahlmann and Al Naimi[17] investigated the sFlt-1/PGF ratio and uterine artery Doppler indices for the diagnosis of PE. It studies reported that sFlt-1 levels were higher in early-onset pregnant of PE by other studies[18].

Muy-Rivera et al.[6] investigated the relationship between maternal plasma sVEGFR-1, VEGF, and PIGF levels in 206 Zimbabwean women consisting of 131 preeclamptic and 175 normal pregnancies. They found that plasma VEGF level decreased and sVEGFR-1 level increased in preeclamptic pregnant women. They did not define any relationship between PE and plasma PIGF level. The high sVEGFR-1 levels in preeclamptic pregnant women in this study are consistent with the results of our study. Maynard et al.[10] showed that low PIGF and high sVEGFR-1 levels accompanying VEGF impair endothelial functions in PE. Additionally, studies are showing that an increase in the sVEGFR-1/PIGF ratio in early gestational weeks is an important finding indicating the risk of developing PE[19].

Chaiworapongsa et al.[20] investigated that plasma levels of sVEGFR-1 are correlated with the severity of PE, the degree of proteinuria and increases in resistance of uterine artery and umbilical arteries. They have also found a negative correlation between the maternal plasma levels of sVEGFR-1 and platelet count, neonatal birth weight, as well and gestational age at delivery. Similar findings were reported for maternal plasma PlGF[20].

In a longitudinal case-controlled study, plasma sVEGFR-1 levels were measured in 44 preeclamptic and healthy pregnant women after 7-16, 16-24, 24-28, 28-32, 32-36, and 37 weeks of gestation. Mean sVEGFR-1 levels at the gestational week were higher than those in the control group. Similarly, it was reported that sVEGFR-1 levels were higher 2-5 and 6-10 weeks ago in the PE group, and sVEGFR-1 levels increased earlier in early-onset PE[21]. In our study, the median serum sVEGFR-1 level was higher in PE, and there was a positive correlation between the severity of the disease and the sVEGFR-1 level. However, no relationship was found between sVEGFR-1 level and gestational week.

sVEGFR-1 was thought to be important in assessing the severity of PE, similar to proteinuria. Also, this situation was supported by the correlations of maternal PIGF, sVEGFR-1, placental weight, and fetal weight weight[22].

Levine et al.[23] measured serum sVEGFR-1, fPIGF, and VEGF levels in 655 serum samples during pregnancy in

Table 2. The correlation between serum level of sVEGFR-1 and demographic and clinical variables

	Control		Preeclampsia	
Age (y)	r=0.19	NS	r=0.01	NS
BMI (kg/m^2)	r=-0.04	NS	r=0.01	NS
Gravida	r=-0.06	NS	r=-0.01	NS
Blood pressure (mmHg) Systolic Diastolic	 r=-0.13 r=-0.12	NS	 r=0.25 r=0.31	p<0.05
Proteinuria (mg/dL)	r=-0.15	NS	r=0.25	p<0.05
Platelet counts (10^3/mm^3)	r=-0.01	NS	r=-0.03	NS
Birth weight (g)	r=0.20	NS	r=-0.10	NS
Smoking	r=0.18	NS	r=-0.22	NS
Weight gain during pregnancy (kg)	r=0.19	NS	r=0.15	NS
AFI	r=0.17	NS	r=0.08	NS
APGAR score at 1 min	r=0.18	NS	r=0.09	NS
APGAR score at 5 min	r=0.21	NS	r=0.10	NS
Gestational age at delivery (weeks)	r=0.12	NS	r=0.01	NS
Abruptio placenta	r=-0.17	NS	r=-0.08	NS
IUGR	r=-0.19	NS	r=0.08	NS

NS: Non-significant, sVEGFR-1: Soluble vascular endothelial growth factor receptor -1, AFI: Amnion fluid index, BMI: Body mass index, IUGR: Intrauterine growth restriction

120 preeclamptic and normal pregnant women each. In normotensive pregnant women, sVEGFR-1 levels were stable in the first and second trimesters of pregnancy, and there was a linear increase starting at 33 and 36 weeks and a decrease in fPIGF levels. They reported that sVEGFR-1 started to increase in the early weeks of gestation in preeclamptic pregnant women, and serum-free PIGF and VEGF levels decreased five weeks before the onset of PE[23].

The biological mechanism of the relationship between VEGF, sVEGFR-1, and PIGF is not fully understood. Chung et al.[24] analyzed endocrine gland-derived VEGF (VEGF-ED), VEGF receptors 1 and 2 and NP-1 and NP-2 in placentas from preeclamptic and normal pregnant women by PCR method. They found that only the VEGFR-1 level of these receptors was high in the placenta of preeclamptic pregnant women. VEGF and its receptors are mainly found in syncytiotrophoblasts, villous capillaries, and endothelial cells of the great vessels. These findings suggest that VEGFR-1 regulates trophoblast function and inhibits angiogenesis and vasodilation[24].

Most of the circulating VEGF in pregnancy is found as sVEGFR-1 released from the placenta. Placental production of VEGFR-1 and sVEGFR-1 is increased in PE due to hypoxia[25]. In our study, it was observed that each serum sVEGFR-1 level decreased in the postpartum period. However, the high level of sVEGFR-1 in essential hypertension may be thought to contribute to the serum sVEGFR-1 level of some tissues other than the placenta in preeclamptic pregnant women.

Sugimoto et al.[26] demonstrated that anti-VEGF antibodies and sVEGFR-1 cause proteinuria, a single dose of intravenous administration of anti-VEGF antibodies to normal healthy mice resulted in excessive albumin excretion in the urine because of massive glomerular endothelial cell damage. These findings indicate that circulating VEGF affects glomerular endothelial cell functions, and proteinuria can be an important side effect of anti-VEGF therapy.

Study Limitations

The study design this work had some limitations because of its inclusion of all subtypes of PE. And the study population with a relatively small sample size needs to be considered during drawing a conclusion on the status of serum sVEGFR-1 in patients with several types of clinical presentation of PE. Recently, various pharmacological agents that can prevent the effects of sVEGFR-1 for treating PE have been investigated[27-29]. These agents may have a significant effect on reducing neonatal morbidity and mortality, given that they are considered safe enough to delay delivery for several weeks and alleviate end-organ findings. As a study strength supporting the value of serum sVEGFR-1 in the management of PE, the measurement of serum sVEGFR-1 may have a value in choosing their doses.

Conclusion

In our study, we obtained findings indicating the involvement of sVEGFR-1 in the pathophysiology of PE. Serum sVEGFR-1 has the potential to be used as a valuable biomarker in the prediction, diagnosis, and risk management of women with subtypes of PE including mild and severe PE, HELLP syndrome, and eclampsia. There is a need to study serum sVEGFR-1 as a biomarker in pregnant women with different subtypes of PE.

Ethics

Ethics Committee Approval: The study protocol was performed according with the principles of the Declaration of Helsinki and was approved by the Human Ethics Committee of Sivas Cumhuriyet University (protocol ID: 05.04.2005-4/5).
Informed Consent: All participants signed a written informed consent form on enrollment.
Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: S.Ş., A.Ç., Design: S.Ş., A.Ç., Data Collection or Processing: S.Ş., Analysis or Interpretation: S.Ş., N.Y., A.Ç., Literature Search: S.Ş., N.Y., Writing: S.Ş., N.Y., A.Ç.
Conflict of Interest: No conflict of interest was declared by the authors.
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What should be the strategy in case of a big follicle at the start of the cycle? Shall we start the stimulation or postpone it to the next cycle?

Siklus başlangıcında büyük folikül saptanması durumunda strateji ne olmalıdır? Stimülasyona başlanmalı mı yoksa bir sonraki siklusa mı ertelenmeli?

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Abstract

Objective: This study facilitates decision-making when an antral follicle diameter >15 mm is detected at the beginning of the menstrual cycle in poor responder (POR) patients.

Materials and Methods: Eighty-three POR patients with at least one leading follicle with a diameter of 15 to 24 mm on the 2nd-4th days of the menstrual cycle were assessed.

Results: The mean age of females was 40.1±4.8 (26-45), and the mean partners' age was 42.1±7.8 (26-65). Fifty-one (61.4%) women underwent an oocyte pick-up procedure 36 h after the first ultrasonographic examination on the 2nd-4th days of the menstrual cycle. Gonadotrophin stimulation was initiated in 32 (38.6%) patients. Among women in whom oocyte retrieval was performed, an oocyte was obtained in 49 (59.75%) patients. In 13 of 49 patients (26.5%), no mature oocytes were obtained. Fertilized 2pn embryos were obtained in 18 of 33 patients (54.5%). Among the fertilized embryos, 12 were good, six were moderate, and two were of poor quality. Following the frozen embryo transfer procedure, one of the two patients experienced a clinical pregnancy.

Conclusion: Patients with POR are still difficult to manage both clinically and therapeutically. Since every oocyte is valuable and important, patients should be carefully followed up. Our research will be directed by the need to rule out a physiological ovarian cyst when large antral follicles appear at the beginning of the cycle. The clinician should give them a chance.

Keywords: Poor responder, diminished ovarian reserve, big antral follicle

Öz

Amaç: Bu çalışmada, zayıf over yanıtlı (ZOY) kadınlarda menstrüel siklusun başlangıcında antral folikül çapı >15 mm saptanması durumunda karar vermeyi kolaylaştırmayı amaçladık.

Gereç ve Yöntemler: Menstrüel siklusun 2.-4. günlerinde, çapı 15-24 mm arasında olan en az bir önde giden folikülü olan ZOY tanısı konulan 83 kadın değerlendirildi.

Bulgular: Ortalama kadın yaşı 40,1±4,8 (26-45), ortalama partner yaşı 42,1±7,8 (26-65) idi. Elli bir (%61,4) katılımcıya siklusun 2.-4. günlerinde ilk ultrasonografik incelemeden 36 saat sonra oosit toplama işlemi yapıldı. Katılımcıların 32'sine (%38,6) gonadotropin stimülasyonu başlandı. Oosit toplama yapılan, 49 (%59,75) kadından oosit elde edildi. Kırk dokuz hastanın 13'ünde (%26,5) matür oosit elde edilemedi. ICSI yapılan 33 hastanın 18'inde

PRECIS: The emergence of big antral follicles at the beginning of the menstrual cycle should not be misdiagnosed as a physiological ovarian cyst and should be given a chance.

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(%54,5) 2pn embriyo elde edildi. Döllenen embriyolardan 12'si iyi kalitede, 6'sı orta kalitede ve 2'si kötü kalitede idi. İki hastaya dondurulmuş embriyo transferi yapıldı ve bir hastada klinik gebelikle sonuçlandı.

Sonuç: ZOY'li hastaların klinik yönetimi ve tedavisi zordur. Hastalar dikkatle takip edilmelidir; her oosit çok değerli ve önemlidir. Menstrüel siklusun 2.-4. günlerinde büyük antral foliküllerin ortaya çıkması fizyolojik over kisti ile karıştırılmamalı ve oosit elde edilebilecek folikül olabileceği göz önünde bulundurulmalıdır. Klinisyen büyük antral folikül için bir şans vermelidir.

Anahtar Kelimeler: Zayıf over yanıtı, azalmış over rezervi, büyük antral folikül

Introduction

Diminished ovarian reserve (DOR) is defined as a reduction in the quantity of the ovarian follicular reserve, which occurs in 31% of ART cycles[1]. Patients with DOR are generally considered challenging because they exhibit poor ovarian response (POR), which accounts for 9-24% of patients undergoing ovarian stimulation for in vitro fertilization (IVF) treatment[2,3]. POR leads to fewer retrieved oocytes and fewer embryos to be transferred, which lowers pregnancy and live birth rates (LBR)[4].

Patient-oriented strategies encompassing individualized oocyte number (POSEIDON) classification, which was established in 2016, is used to classify and treat patients with POR. The "low prognosis patient" is defined in this categorization and is divided into four subgroups according to (i) age, (ii) ovarian reserve markers [antral follicle count (AFC) and/or anti-mullerian hormone (AMH)], and (iii) the results of prior ovarian stimulation. Groups 1 and 2 are designated for women under the age of 35 and women over the age of 35 who have sufficient ovarian reserve parameters (AFC >5, AMH >1.2 ng/mL). Groups 3 and 4 are designated for women under the age of 35 and 35 years or older who have inadequate ovarian reserve parameters (AFC <5, AMH <1.2 ng/mL), respectively[5]. POSEIDON groups 3 and 4 are called "expected POR," constituting 10% and 55% of the IVF cycle, respectively[6].

The management of patients with POR remains a challenge for clinicians. The number of retrieved oocytes considerably influences clinical outcomes concerning cumulative LBR. The increasing number of retrieved oocytes, the higher cumulative LBR[7,8]. However, the IVF cycle for patients with POR often results in a follicular developmental arrest, premature ovulation, and cancellation of oocyte retrieval[9,10]. Additionally, these women have a high risk of not having any high-quality embryos available for transfer; they often undergo multiple ovarian stimulation cycles, which causes physical, emotional, and financial costs[6].

There is no standard treatment for POR concerning protocol and drugs. Ovarian stimulation is suggested to be started when the serum estradiol level is <50 pg/mL, the endometrial lining is <5 mm, and no dominant follicle >10 mm exists during the early follicular phase, typically on day 2nd or 3rd of the following menses[11]. When an antral follicle >15 mm exists, it can be diagnosed as a retantional ovarian follicle or physiological ovarian cyst. Controlled ovarian stimulation was postponed. Whether or not to stimulate has been a conundrum.

This study facilitates decision- making when an antral follicle >15 mm is detected at the start of the menstrual cycle.

Materials and Methods

Patient Selection

Between January 2020 and February 2021, the IVF Center at Acıbadem University Maslak Hospital in İstanbul, Turkey, conducted this retrospective cohort study. The POSEIDON groups 3 and 4 criteria were met by patients who had IVF cycles and were recruited in the study. Group 3 consisted of women <35 years of age, AFC <5, and AMH <1.2 ng/mL. Group 4 consisted of women ≥35 years of age, AFC <5, and AMH <1.2 ng/mL. Age requirements for inclusion were 25-45 years old, and at least one leading follicle must have a diameter of 15-24 mm on the 2nd-4th days of the menstrual cycle. Women with body mass indices greater than 30 kg/m^2 and partners who had severe male factor infertility were excluded (e.g., aspermia, azoospermia). Each patient's complete medical history was analyzed, including their age, the age of their partners, any prior treatments, and the duration of their infertility. The primary outcome measures were the total number of oocytes, mature oocytes, and embryos.

Controlled Ovarian Stimulation

Gonadotrophin stimulation was initiated at 300 IU recombinant follicle-stimulating hormone (FSH) (randomized Gonal F randomly; Merck, or Fostimon; IBSA) with one falcon of Gonadotropin hormone-releasing hormone (GnRH) antagonist if TV-USG revealed a follicle >15 mm on the 2nd-4th days of the menstrual cycle (0.25-mg cetrorelix; Cetrotide; Merck Serono). Serum estradiol (E2) and progesterone levels were checked at each examination. Whenever the diameter of the leading follicle at the beginning of the cycle was 18 mm, final oocyte maturation was triggered by the administration of 250 mcg recombinant human choriogonadotropin alfa (rHCG, Ovitrelle; Serono).

If TV-USG revealed a follicle with a diameter between 18 and 24 mm on the 2nd-4th days of the menstrual cycle, final oocyte maturation was triggered by the administration of 250 mcg rHCG (Ovitrelle; Serono) with one dose of GnRH antagonist (0.25-mg cetrorelix; Cetrotide; Merck Serono). After 36 h following hCG injection, oocyte pick-up (OPU) was performed under sedation anesthesia using a 35 cm 17-G double lumen needle. Four hours after retrieval, oocyte denudation and ICSI were conducted. The cell quantity and morphological quality of embryos were evaluated using the İstanbul consensus workshop criteria[12]. Good-quality embryos were frozen at the cleavage stage for embryo banking.

Statistical Analysis

SPSS software (version 22.0; SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Continuous variables were expressed using the median, mean, and standard deviation (minimum-maximum). Categorical variables were reported as numbers and percentages (%). Through using Kolmogorov-Smirnov test, the distribution of the data was examined. The two groups were compared using the Student's t-test. Statistical significance was defined as $p<0.05$.

Results

Eighty-three women were retrospectively enrolled. The mean age of the females was 40.1±4.8 (26-45), and the mean partners' age was 42.1±7.8 (26-65). Table 1 lists the baseline characteristics of the included cycles: Age, length of infertility, basal FSH levels, AMH, and the number of antral follicles. Fifty-one (61.4%) women underwent an OPU procedure 36 h after the first ultrasonographic examination on 2nd-4th days of the menstrual cycle. Gonadotrophin stimulation was initiated in 32 (38.6%) patients. Gonadotropin duration was one day in 20 (24.1%) patients, two days for 10 (11%), and three days for 2 (2.4%) patients subsequently. The mean follicular diameter of the largest follicle (mm) at the start of the cycle was 18.7±2.5 (15-24). The mean follicular diameter of the largest follicle (mm) on hCG day was 19.6±2.0 (17-24). Table 2 summarizes the clinical and IVF outcome characteristics for women. Oocyte retrieval was successfully performed in 82 women, while one woman was unable to be retrieval due to premature ovulation. At least one oocyte was found in 49 patients with oocyte retrieval (59.75%). One oocyte was obtained in 42 patients, and two oocytes were obtained in seven patients. No mature oocytes were obtained in 13 of the 49 patients (26.5%). Fertilized 2pn embryos were obtained in 18 of 33 patients (54.5%). Among the fertilized embryos, 12 were of good quality, six were moderate quality, and two were poor quality. Eighteen patients opted for embryo pooling in the cleavage stage, but unfortunately, one embryo was arrested in the 2pn stage. Two patients underwent frozen embryo transfer, and a patient experienced a clinical pregnancy as a result. When we compared the baseline characteristics of the women in whom at least one oocyte was obtained or not, the analysis revealed that the partner's age was significantly higher in at least one oocyte-obtained group (p=0.03). The peak serum E2 level was significantly higher in at least one oocyte-obtained group (p=0.05) (Table 3). The flow chart of this study is detailed in Figure 1.

Table 1. Baseline characteristics and cycle parameters of the women

	Mean ± Standard deviation (min-max)
Age (years)	40.1±4.8 (26-45)
Partner's age (years)	42.1±7.8 (26-65)
Infertility duration (months)	50.8±52.6 (5-240)
Number of previous IVF attempts (n)	2.64±3.23 (0-15)
Number of AFC (n)	1.4±1.2 (0-4)
Mean follicular diameter of the largest follicle (mm) at the start of the cycle	18.7±2.5 (15-24)
Mean follicular diameter of the largest follicle (mm) on hCG day	19.6±2.0 (17-24)
FSH (mIU/mL)	33.6±24.8 (7.3-123)
AMH (ng/mL)	0.16±0.21 (0-0.9)

IVF: In vitro fertilization, AFC: Antral follicle count, AMH: Anti-Mullerian hormone, FSH: Follicle stimulating hormone, hCG: Human chorionic gonadotrophin. Data were expressed as mean ± standard deviation

Discussion

In this study, we investigated whether the cycle should be started immediately or postponed to the next cycle in case of a large antral follicle at the start of the menstrual cycle with at least an antral follicle >15 mm in women diagnosed with POR. A total of 82 women underwent an OPU procedure. At least one oocyte was obtained in 49 (59.75%) of the 82 patients. Among them, 18/33 (54.5%) had at least fertilized cleavage stage embryos.

In the previous studies in the 1950s-1970s, the traditional theory of human folliculogenesis stated two phases[13-15]. The first two weeks of the menstrual cycle are termed the "follicular phase," that a single cohort of antral follicles grows. The last two weeks of the cycle are termed the "luteal phase," when the corpus luteum grows in the absence of a follicle. Inhibin B suppresses FSH secretion during the follicular phase, whereas by regressing the corpus luteum, inhibin A secretion decrease, and FSH inhibition escape during the luteal-follicular transition[16]. When ovarian reserve diminishes, granulosa cells show a gradual decline in inhibin B secretion, with a consequent rise in FSH levels that stimulate earlier follicular development during

Table 2. Clinical and IVF outcome parameters of women

	Mean ± Standard deviation (min-max)
Days of stimulation (in women who underwent ovarian stimulation, n=32)	1.4±0.6 (1-3)
Total gonadotrophin dose (IU) (in women who underwent controlled ovarian hyperstimulation; n=32)	512±408 (150-1800)
E2 level (pg/mL) on hCG day	177±141 (5-468)
Number of retrieved oocytes (n=49)	1.1±0.4 (1-3)
Number of mature oocytes (n=36)	0.8±0.6 (0-2)
Number of 2 pronuclei embryos (n=21)	0.6±0.6 (0-2)
Number of cleavage stage embryos (n=20)	0.58±0.6 (0-2)

hCG: Human chorionic gonadotrophin, IVF: In vitro fertilization, E2: Estradiol. Data were expressed as mean ± standard deviation

the luteal-follicular transition period. As a result, accelerated follicular growth can be pictured[17,18]. Klein et al.[19] reported that the older patients over >40-45 years of age in the control cycles demonstrated an elevated day 3 FSH and a shortened follicular phase compared with the younger patients aged 20-25 years.

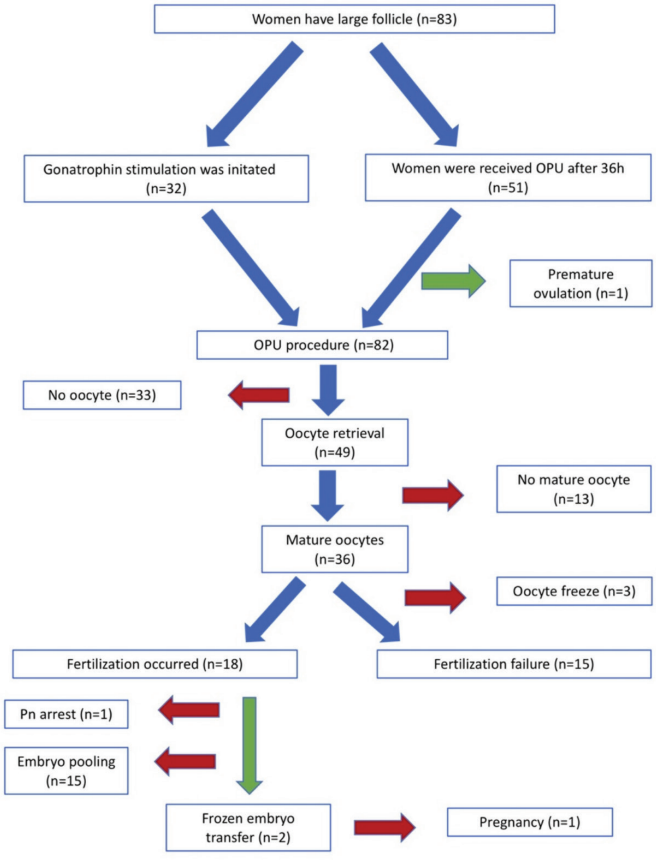

Figure 1. Flow chart of the study

OPU: Oocyte pick-up

In our study, the mean patient's age was 40.1±4.8 (26-45), the mean AFC was 1.4±1.2 (0-4), both advanced female age and DOR resulted in accelerated follicular growth. These women were also likely to have large antral follicles due to advanced follicular maturation in the very early days of the follicular phase. Turan et al.[20] reported the IVF conception of seven patients with DOR following a very short ovarian stimulation of incidentally discovered large antral follicles in the early follicular phase. Six embryos were obtained after eight oocytes from seven patients were removed, and two live births and a 50% ongoing pregnancy rate per transfer were the results[20].

In this study, oocytes were obtained in 59.75% of the patients, who was an undeniably high rate. The number of oocytes retrieved during controlled ovarian stimulation has a considerable impact on the cumulative LBR each cycle initiated[3]. The number of oocytes retrieved had a substantial relationship with LBR; the predicted LBR for one oocyte recovered at ages 18-34, 35-37, 38-39, and 40 years and older was 8%, 7%, 5%, and 1%, respectively[21]. According to the national summary report of patients with DOR in 2020, the chance of live birth with intended egg retrieval and first embryo transfer was 2.6% at more than 42 years of age, 13.6% between 38 and 40 years of age; 22.8% at <35 years[22]. In another study, Polyzos et al.[23] analyzed 14.469 patients and reported that cumulative LBRs steadily increased with the number of oocytes, which was categorized according to age (<36, 36-39, >40 years), revealed the same pattern, showing a steady increase in cumulative LBR with the number of oocytes, but cumulative LBRs decreased with increasing age for a given number of oocytes. In other

Table 3. Participant characteristics and cycle parameters of two groups

	No oocyte Mean ± Standard deviation (min-max)	At least one oocyte retrieved Mean ± Standard deviation (min-max)	p
Age (years)	39.71±5.1 (26-45)	40.61±4.3 (29-45)	0.39
Partner's age (years)	40.4±6.45 (25-57)	44.15±8.8 (29-65)	0.03
Infertility duration (months)	50.2±50.7 (5-240)	51.5±55.4 (6-240)	0.73
Number of previous IVF attempts (n)	2.62±3.4 (0-15)	2.66±2.9 (0-12)	0.96
Number of AFC (n)	1.4±1.1 (0-4)	1.4±1.1 (0-4)	0.84
Mean follicular diameter of the largest follicle (mm) at the start of the cycle	18.25±2.3 (15-23.3)	19.2±2.7 (15.3-24)	0.97
Mean follicular diameter of the largest follicle (mm) on hCG day	19.3±1.65 (17.2-23.4)	19.96±2.25 (17-24)	0.14
FSH (mIU/mL)	34.2±23 (7.3-115)	32.95±27.2 (7.8-123)	0.84
AMH (ng/mL)	0.16±0.21 (0-0.9)	0.17±0.2 (0-0.8)	0.74
E2 level (pg/mL) on hCG day	126.3±114.15	251.1±146.9	0.05

IVF: In vitro fertilization, AFC: Antral follicle count, AMH: Anti-Müllerian hormone, FSH: Follicle stimulating Hormone, E2: Estradiol, hCG: Human chorionic gonadotrophin. Data were expressed as mean ± standard deviation

words, one retrieved oocyte provides 1-8% live birth chances, so every additionally retrieved oocyte has a significant impact on the LBR. As our study proved, each follicle that develops in patients with POR is important and contributes to the LBR.

There are a limited number of studies on this subject in the literature. This study is unique, with a high number of cases. Clinicians encounter this situation frequently, but generally, oral contraceptive drugs are prescribed or postponed in stimulation protocols that may cause emotional stress and costs to the anxious infertile couple. When an antral follicle diameter more than 15 mm is detected at the start of the menstrual cycle in patients with POR, clinicians may recommend an OPU procedure. Additionally, it is a cost-effective treatment because of its short stimulation duration.

Study Limitations

The limitation of this study is that only two patients underwent frozen embryo transfer because of embryo pooling continuing. Also, thin endometrium development is not appropriate for receiving fresh embryo transfer.

Conclusion

Clinical management and treating patients with POR is still challenging. Patients should be followed cautiously because each oocyte considerably affects the LBR. Our study could be guided when the emergence of big antral follicles at the beginning of the menstrual cycle should not be misdiagnosed as a physiological ovarian cyst and should be given a chance.

Ethics

Ethics Committee Approval: This study was approved by the local ethics committee with an approval number 2021/05 (Acıbadem University Clinical Research Ethics Committee, date: 13.03.2021).

Informed Consent: Retrospective study.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Ş.Y.K., A.Y., Z.E.U.K., Concept: Ş.Y.K., Y.Ç., B.T., Design: Ş.Y.K., Y.Ç., B.T., Data Collection or Processing: Ş.Y.K., Z.E.U.K., Ö.K., Analysis or Interpretation: Ş.Y.K., A.Y., Y.Ç., Literature Search: Ş.Y.K., A.Y., Writing: Ş.Y.K., Z.E.U.K., Y.Ç.

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Copeptin: A potential marker for the prediction of poor ovarian reserve in the ınfertile women

Copeptin: İnfertil kadınlarda kötü over rezervinin ön görülmesinde potansiyel marker

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Abstract

Objective: There is actually no evidence regarding the physiological effects of copeptin in infertile women with different ovarian reserve types. This study aimed to investigate the relationship of serum copeptin level with poor ovarian reserve (POR) and to reveal the predictive value of copeptin for POR development in the infertile women.

Materials and Methods: All participant women were classified as the control group (n=77) included the women with diagnosis of unexplained infertility and the POR group (n=61) was composed of the women who met the European Society of Human Reproduction and Embryology consensus on POR [serum anti-Müllerian hormone (AMH) concentrations below 1.1 ng/mL]. The biochemical tests, including estradiol (E2), follicle stimulating hormone (FSH), luteinizing hormone, AMH and copeptin were analysed. The analyses of serum copeptin concentrations were measured by the means of competitive enzyme immunoassay.

Results: A significant increase in the serum copeptin level existed only in the POR group. There was a significant positive correlation between serum copeptin with E2 and FSH levels in the POR group. Significant negative correlations between copeptin and AMH concentrations (r=-0.310, p=0.015) and between copeptin concentration and antral follicle counts (r=-0.284, p=0.027) were detected only in the POR group. The estimated areas under receiver operating characteristic curves for serum concentration were found to be statistically significant with a cut-off value of 3.52 (95% confidence interval 0.519-0.709), sensitivity 0.90 and specificity 0.72.

Conclusion: This study confirmed that there was an elevated serum copeptin concentration in the infertile women with POR and that serum copeptin concentration may have a predictive value for POR diagnosis.

Keywords: Copeptin, infertility, poor ovarian reserve, vasopressin

Öz

Amaç: Farklı over rezerv tiplerine sahip infertil kadınlarda kopeptinin fizyolojik etkilerine dair aslında yeterli kanıt yoktur. Bu çalışmanın amacı, infertil kadınlarda serum kopeptin düzeyi ile zayıf over rezervi (POR) arasındaki ilişkiyi araştırmak ve kopeptinin POR gelişimi için prediktif değerini ortaya çıkarmaktır.

Gereç ve Yöntemler: Katılımcılar, açıklanamayan infertilite tanılı kadınları içeren kontrol grubu (n=77) ve POR grubu (n=61), olarak sınıflandırıldı. POR tanı grubu Avrupa İnsan Üremesi ve Embriyoloji Derneği kriterlerini karşılayan kadınlardan oluşturuldu [serum anti-Müllerian hormon (AMH) konsantrasyonları 1,1 ng/mL altındadır]. Estradiol (E2), folikül uyarıcı hormon (FSH), luteinize edici hormon, AMH ve kopeptin içeren biyokimyasal testler analiz edildi. Serum kopeptin konsantrasyonlarının analizleri, enzim immünoassay vasıtasıyla ölçüldü.

Bulgular: Serum kopeptin düzeyinde anlamlı artış POR grubunda mevcuttu. POR grubunda serum kopeptin ile E2 ve FSH seviyeleri arasında anlamlı pozitif korelasyon vardı. Kopeptin ve AMH konsantrasyonları (r=-0,310, p=0,015) ve kopeptin konsantrasyonu ile antral folikül sayısı (r=-0,284, p=0,027) arasında anlamlı negatif korelasyonlar POR grubunda saptandı. Serum konsantrasyonu için alıcı işletim karakteristiği eğrileri altındaki tahmini alanların, kesme değeri 3,52 (%95 güven aralığı 0,519-0,709), duyarlılık 0,90 ve özgüllük 0,72 ile istatistiksel olarak anlamlı olduğu bulundu.

PRECIS: We investigated the relationship of serum copeptin level with poor ovarian reserve (POR) and to reveal the predictive value of copeptin for POR development in the infertile women.

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Sonuç: Bu çalışma, POR grubundaki infertil kadınlarda serum kopeptin konsantrasyonunun yükseldiğini ve serum kopeptin konsantrasyonunun POR tanısı için öngörücü bir değere sahip olabileceğini doğruladı.

Anahtar Kelimeler: Copeptin, infertilite, kötü over rezervi, vazopressin

Introduction

The term "ovarian reserve" is traditionally described as a female's reproductive potential specifically in the sense of the number and content of oocytes in the ovaries[1]. The etiopathogenesis of poor ovarian reserve (POR) has not clearly documented as yet. However, many etiologies, including age-related decline in ovarian follicles, severe endometriosis, chromosomal and genetic disorders, prior pelvic surgery, metabolic, autoimmune and infectious diseases, as well as exposure to toxic agents were identified[2-4]. In the existing literature, the POR prevalence was estimated as 6-35%[5,6]. This huge discrepancy presumably arises from the absence of conclusive collectivity in the POR definition.

On the purpose of POR terminology, a European Society of Human Reproduction and Embryology Working Group reported a consensus termed as Bologna criteria[7]. Despite the Bologna criteria exhibiting unsatisfactory uniformity in the POR definition, most scientific authorities have recently accepted these criteria. Despite the considerable progress in modern assisted reproduction technology in the last 40 years, the clinical management of infertile women with POR is still a challenge in clinical practice. Consequently, this may lead to great disappointment and discouragement for the patients and clinicians.

Copeptin possesses a molecular structure of glycosylated 39-amino-acid peptide, which is a C-terminal part of pre-provasopressin (preproAVP). PreproAVP is an initial protein containing a signal peptide, arginine vasopressin (AVP), neurophysin II and copeptin[8,9]. During the transport from the hypothalamus to posterior hypophysis, copeptin and neurophysin II mainly participate as carrier proteins of AVP. All these molecules are cleavage products in their course in the pituitary gland and are concurrently secreted into the bloodstream. The main physiological actions of AVP are the homeostasis of fluid balance, the maintenance of vascular tonus and regulation of the endocrine stress response. The AVP receptors exist in many organs and tissues, including the kidney, liver, vascular smooth muscles, and brain[10]. However, the exact role of copeptin in the circulation is not clearly elucidated for this moment[11,12]. Copeptin is simultaneously synthesized with AVP and detected at equimolar concentrations[12]. Therefore, copeptin level confidentially reflects equivalent AVP concentration in the circulation.

In the present studies, copeptin emerged as a new diagnostic and prognostic marker in various diseases, including diabetes insipidus, diabetes mellitus, sepsis, pneumonia, chronic obstructive pulmonary disease, heart failure and myocardial infarction[13]. However, in the accumulated literature, no evidence regarding the physiological effects of copeptin actually exists in infertile women with POR. Hence, this study aimed to investigate the relationship between serum copeptin level and POR status and to reveal the predictive value of copeptin for POR development in infertile women.

Materials and Methods

Setting

This analysis was performed in a prospective observational (cross-sectional) manner in the Department of Reproductive Endocrinology between January 2021 and June 2021. The approval of this study approved by the Ethics Committee of the University (reference number: 386/2021) compatible with the Declaration of Helsinki. Written informed consent was collected from all participant women before the study.

Study Population

During the first visit, the detailed medical characteristics were recorded for all volunteers. Infertile women with prior pelvic surgery, endometriosis, adnexal masses, chemotherapy, radiotherapy, smoking, body mass index (BMI) ≥30 kg/m^2, systemic diseases, and medications affecting adversely fertility capacity were excluded from the study. During clinical evaluation, the total antral follicle counts (AFC) were calculated in the early follicular phase of the menstrual cycle by the means of a transvaginal 7.5 MHz probe (Toshiba Xario 100, Toshiba Medical System Co., Nasu, Japan). Eventually, the infertile women aged 20 to 40 years and who met the eligibility criteria were enrolled in this study.

Unexplained infertility is described as the lack of a definable cause to achieve a pregnancy after 12 months of attempting conception despite a thorough evaluation[1]. In the Bologna consensus on the definition of poor response, at least two of the following three criteria had to be present to establish the definition[7]:

(1) Advanced maternal age (>40 years) or any other risk factor for POR.

(2) A previous POR (≤3 oocytes with a conventional stimulation protocol).

(3) An abnormal ovarian reserve test [i.e. AFC less than 5-7 follicles or anti-Müllerian hormone (AMH) below 0.5-1.1 ng/mL].

The study groups were recruited from the infertile women who planned to receive the first in vitro fertilization therapy. All participant women (n=138) were classified as two study groups based on the ovarian reserve patterns. While the control group (n=77) included the women with a diagnosis of unexplained infertility, the POR group (n=61) was arose from the women who had serum AMH concentrations below 1.1 ng/mL as

indicated in the items of Bologna criteria for abnormal ovarian reserve test.

Biochemical Evaluations

After overnight fasting, the venous samples were drawn from the antecubital veins and collected in 5 mL seperator tubes (BD Vacutainer, Becton Dickinson, New Jersey, USA) in the early follicular phases of the menstrual cycle on day 2 to 4. The laboratory workers who studied the samples were unaware of the study groups. The blood samples for hormonal measurements were centrifuged for 20 min at 1,000 x g. The serum levels of estradiol (E2), follicle stimulating hormone (FSH), luteinizing hormone (LH) were measured using an electrochemiluminescence immunoassay (ECLIA) method by an autoanalyser (Cobas 6000, E 601 Roche Diagnostics, GmbH, Mannheim, Germany).

The sera for copeptin and AMH analyses were drained into cryo tubes to be stored at -80 °C until the day of analysis. The serum concentrations of AMH were evaluated by the ECLIA method using an autoanalyser (Cobas 6000, E 601 Roche Diagnostics, GmbH, Mannheim, Germany). The analyses of serum copeptin concentrations were measured by the means of competitive enzyme immunoassay (Human Vasopressin-neurophysin 2-copeptin ELISA kit: EIAab Wuhan EIAab Science Co. Ltd.; East Lake Hi-Tech Development Zone, Wuhan 430079, China).

Sample Size Estimation and Matching Analysis

Priori power analysis was performed for the Students' t-test when the study was designed. To specify the sample size, the Cohen effect size was estimated with aids of literature information[14]. For two-side minimum 95% power hypothesis, 0.05 margin of error, and 0.8 effect size; a minimum number of 35 participants for both the control and the study groups were, respectively, estimated. Matching analysis based on propensity score match was performed by the means of R studio software (Version 1.2.5042, R Core Team, Vienna, Austria) to eliminate the age- effect on data. To match cases and controls for the confounding variables of age, the MatchIt library 3.0.1 in the R package was used.

Statistical Analysis

All statistical the data were analyzed with SPSS (Statistical Packages for The Social Sciences) software version 21 (SPSS Inc. Chicago, IL, USA). The normality pattern of statistical distribution was evaluated by Shapiro-Wilk test. For comparisons of the study groups, the Student's t-test and the Mann-Whitney U test for normally and non-normally distributed data were, respectively, used. The descriptive statistics are given as mean (± standard deviation) and median (min-max) according to distribution patterns. The correlation analysis between the copeptin and other study parameters was assessed by Pearson's correlation test. To determine the discriminant power of the index (maximum sensitivity and selectivity) using the receiver operating characteristic (ROC) analysis method, the ROC graphs were drawn, the area under the curve with 95% confidence intervals were estimated. The Youden index was used to determine the best cut-off point in ROC analysis with sensitivity and specificity values for predicting POR development. The statistical significance level was considered $p<0.05$.

Results

We studied 138 participant women as the study population. The comparisons of clinical and biochemical characteristics are exhibited in Table 1. The means of age and BMI values were statistically comparable for the control and POR groups ($p=0.121$ and $p=0.749$, respectively). The E2, FSH, and LH concentrations were elevated in the infertile women with POR compared with the control group ($p<0.05$, for all). Unsurprisingly, the serum levels of AFC and AMH were significantly decreased in the POR group ($p<0.001$, for both). Additionally, a significant rise in the serum copeptin level existed only in the POR group ($p=0.022$).

The correlation analysis of serum copeptin level with other study parameters can be viewed in Table 2. The Pearson's analysis demonstrated that the mean ages of the participant women in the control and POR groups did not exhibit any correlation with serum copeptin level. The BMI value of the control group showed a statistically significant positive correlation ($r=0.359$, $p<0.001$). There was a significant positive correlation between

Table 1. Comparisons of clinical and biochemical characteristics of the study parameters

	Control group (n=77) Mean ± standard Median (min-max)	POR group (n=61) Mean ± standard Median (min-max)	p
Age (years)	30.2±4.8 30 (20-40)	31.4±3.7 32 (23-39)	0.121
BMI (kg/m²)	22.5±2.4 22.1 (17.1-29.1)	22.9±3.03 21.8 (17.5-29.4)	0.749
E2 (pg/mL)	77.1±11.2 77.6 (51.0-105.5)	80.3±16.6 83.9 (22.3-122.2)	0.021*
FSH (IU/L)	5.5±1.3 5.4 (2.4-9.6)	8.5±3.3 8.7 (3.4-20.4)	<0.001*
LH (IU/L)	5.5±1.4 5.7 (3.2-9.1)	7.8±2.9 7.3 (3.5-13.8)	<0.001*
AMH (ng/dL)	5.6±1.9 5.8 (1.5-9.6)	0.8±0.2 0.7 (0.5-1.0)	<0.001*
AFC	14.2±4.6 13 (4-24)	5.5±2.3 5 (1-10)	<0.001*
Copeptin (ng/mL)	4.2±0.9 4.3 (2.2-6.6)	5.1±1.7 4.4 (2.0-9.8)	0.022*

POR: Poor ovarian reserve, BMI: Body mass index, E2: Estradiol, FSH: Follicle stimulating hormone, LH: Luteinizing hormone, AMH: Anti-Müllerian hormone, AFC: Antral follicle count, min-max: Minimum-maximum, *p-values <0.05 are accepted as statistically significant

serum copeptin E2 level (r=0.434, p<0.001) and a significant positive correlation FSH level (r=0.328, p=0.01) in the POR group. However, no statistically significant correlation was observed between LH and copeptin existed in both study groups. A significant and negative correlation between AMH and copeptin (r=-0.310, p=0.015) was detected only in the POR group. Also, a significant and negative correlation between copeptin concentration and AFC existed in the POR group (r=-0.284, p=0.027).

Table 3 describes the serum copeptin concentration in predicting the POR development. The estimated areas under ROC curves for serum concentration were found to be statistically significant (p=0.022) with a cut-off value of 3.52 (95% confidence interval 0.519-0.709), sensitivity 0.90 and specificity 0.72.

Discussion

This study focused on investigating the relationship between serum copeptin level and POR status and ascertaining the predictive value of copeptin for POR development. Consequently, an increased serum copeptin level was found in the infertile women with POR diagnosis. Additionally, the serum copeptin level may predict the POR diagnosis in the study population.

Copeptin (also known as AVP-associated glycoprotein) was firstly identified in 1972 by Hanaoka and Guggino[15]. Because of the stoichiometric production with AVP, copeptin was accepted as a reflection of serum AVP concentration. The plasma AVP measurements are a great challenge because AVP is a small molecular size and shows more avidity to platelets[16]. Additionally, AVP exhibited an unstable state even when stored at -20 °C due to short- life time of AVP (about 24 min)[11,17]. All these reasons contribute to the lack of routine clinical practice of AVP. Since Morgenthaler et al.[18] defined an assay technique for copeptin, it became a preferred choice for investigators to reveal the functions of AVP.

Table 2. Correlation analysis of serum copeptin concentration with other study parameters

	Control group (n=77)		POR group (n=61)	
	r	p	r	p
Age (years)	0.106	0.359	-0.232	0.071
BMI (kg/m²)	0.359	0.001*	0.161	0.216
E2 (pg/mL)	0.026	0.823	0.434	<0.001*
FSH (IU/L)	0.001	0.995	0.328	0.01*
LH (IU/L)	-0.025	0.829	-0.235	0.068
AMH (ng/dL)	0.326	0.051	-0.31	0.015*
AFC	0.080	0.457	-0.284	0.027*

POR: Poor ovarian reserve, BMI: Body mass index, E2: Estradiol, FSH: Follicle stimulating hormone, LH: Luteinizicng hormone, AMH: Anti-Müllerian hormone, AFC: Antral follicle count, *p-values <0.05 are accepted as statistically significant

Unlike AVP, copeptin keeps stability in EDTA plasma for up to 2 weeks at room temperature[19]. In a large study by Roussell et al.[20], the authors compared the copeptin and AVP concentrations and noticed a strong correlation between the serum copeptin and AVP levels. There are four main advantages of serum copeptin measurements to AVP; (i) less sample size (ii) no extraction procedure (iii) less time to analyse and (iv) more sensitivity. Based on these facts, serum routine measurements of copeptin have become a rational reason for preference for the investigators.

The physiological role of copeptin is not elucidated at present. Prior studies reported conflicting findings regarding the physiological roles of copeptin[21,22]. In the recent studies, copeptin was professed to be a chaperone-like molecule related to the structural formation of propre AVP[23]. Despite the lack of strong evidence in the current literature, copeptin itself may have specific peripheral functions, Substantially, copeptin shows a rapid response to osmotic, hemodynamic and unspecified stress-related stimuli as occurred in AVP.

A normal range of copeptin concentration, the median plasma concentration was detected in healthy people to be 4.2 pmol/L with a wide range between 1 and 13.8 pmol/L[18,24]. Women had slightly lower values than men with a difference of only 1 pmol/L[18,24]. Copeptin concentrations did not correlate with age[18] and circadian variability[25]. Copeptin secretion appeared not to be influenced by food intake[26] and menstrual cycle in women[27]. Additionally, fasting and exercise induced an increase in copeptin concentration[28]. Based on these facts, copeptin measurements can be confidentially evaluated independently of withdrawal time, prandial state, or menstrual cycle phases.

A hallmark of stress response is the activation of the hypothamo-pituitary-adrenal axis[29]. Hormonal cascades induce the secretion of corticotropin releasing hormone (CRH) from the paraventricular nucleus of the hypothamus. AVP is another hypothalamic hormone stimulated by different stress

Table 3. Serum copeptin concentration in predicting the POR development

Copeptin (ng/mL)	
AUC (95% CI)	0.614 (0.519-0.709)
Cut-off	3.52
Sensitivity	0.90 (0.79-0.95)
Specificity	0.72 (0.61-0.81)
PPV	0.72 (0.60-0. 0.81)
NPV	0.90 (0.79-0.96)
LR+	1.23 (1.02-3.78)
p-value	0.022*

AUC: Area under the curve, PPV: Positive predictive value, NPV: Negative predictive value, LR+: Positive likehood ratio, POR: Poor ovarian reserve, CI: Confidence interval, *p-values <0.05 are accepted as statistically significant

stimuli. This seems to have a potentiating effect on CRH. Eventually, these two hormones are the main inducers of adrenocorticotropic hormone[30-33]. The duration of stressor exposure (acute vs chronic), the type of stressor (physical vs pychological), age and gender have effects on the pattern and magnititude of response to stressor. In addition to hemodynamic and osmoregulatory effects, AVP may be considered a reflection of the increased stress and inflamation[9,33]. Moreover, copeptin was demonstrated to be favorable in reflecting individual stress level due to its relatively stable serum concentration.

In this study, we demonstrated that there was a significant increase in serum copeptin concentration and significant correlations between serum copeptin level and ovarian reserve markers, including E2, FSH, AFC, and AMH, only in infertile women with POR. At this stage, it can be hypothesized that the POR status in infertile women may have received a stress stimulus by the hypothalamus. Subsequently, this stimulus may induce an increased AVP and copeptin release into the bloodstream. Therefore, serum copeptin levels may be used as a predictive marker of POR development in infertile women.

As far as we know, this study is the preliminary study revealing the relationship between serum copeptin levels with different ovarian reserve patterns. This issue can be considered the main strength of this study.

Study Limitations

The limitation of the study is that we could not investigate the stress hormone levels such as CRL, ACTH, or cortisol in the study groups.

Conclusion

In summary, this study confirmed that that there was an elevated serum copeptin concentration in the infertile women with POR and that copeptin might have a predictive value for the POR development. Future large-sized prospective studies are required to clarify the potential effects of copeptin in the POR pathogenesis.

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Ethics

Ethics Committee Approval: The approval of this study approved by the Ethics Committee of the Hitit University (reference number: 386/2021) compatible with the Declaration of Helsinki.

Informed Consent: Written informed consent was collected from all participant women before the study.

Peer-review: Externally 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., Literature Search: E.Y., Writing: Ü.G., E.Y.

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The relationship between the first trimester maternal serum PAPP-A and β-hCG values and newborn intensive care needs in low-risk pregnancies

Düşük riskli gebeliklerde birinci trimester maternal serum PAPP-A ve serbest β-hCG değerleri ile yenidoğan yoğun bakım ihtiyacı arasındaki ilişki

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Abstract

Objective: The purpose of the study was to look at the connection between newborn intensive care requirements in low-risk pregnancies and maternal blood pregnancy-associated plasma protein (PAPP-A) and free human chorionic gonadotropin (hCG) levels, which are elements of screening tests within the first trimester.

Materials and Methods: In the delivery unit of our hospital, pregnant women between the years of 18 and 35 had singleton pregnancies who delivered between 37 and 41 weeks of pregnancy between July 2021 and January 2022 were split into 2 groups. One hundred eighty two pregnant women with infants who required neonatal intensive care (NICU) were enrolled in the first group, whereas 890 pregnant women with infants who did not require NICU were enrolled in the second. These two groups' maternal blood PAPP-A and free hCG levels, which are among the first trimester screening procedures, were examined. Additionally, subgroup analysis were performed in terms of cesarean section indications and NICU admission indications. Logistic regression analysis and ROC analysis were performed with related variables for estimating NICU need.

Results: The mean serum PAPP-A value was found to be 0.91±0.34 multiples of the median (MoM) in the blood taken from the infant mothers who needed NICU, while the mean serum PAPP-A value in the blood taken from infant mothers who did not need NICU was 1.12±0.59 MoM (p<0.000). The PAPP-A MoM mean of the group with Apgar 5th minute score ≥8 (1.09±0.57) was higher than the PAPP-A mean (0.84±0.27) of the Apgar 5th minute score <7 group (p=0.013). According to the results of our study, in groups with a PAPP-A value below 0.95, the possibility of increased NICU need of newborns is higher.

Conclusion: The low serum PAPP-A level, which is used as a screening test among pregnant women, demonstrates that it is successful in predicting perinatal outcomes in the low-risk pregnancy group.

Keywords: PAPP-A, neonatal outcome, free hCG, low-risk pregnancy, neonatal intensive care

Öz

Amaç: Çalışmanın amacı düşük riskli gebeliklerde ilk trimesterde tarama testleri bileşenlerinden maternal serum gebelikle ilişkili plazma proteini (PAPP-A) ve serbest insan koryonik gonadotropin (hCG) seviyeleri ile bebeklerin yenidoğan yoğun bakım (YDYB) ihtiyacı ilişkisinin araştırılmasıdır.

Gereç ve Yöntemler: Temmuz 2021-Ocak 2022 tarihleri arasında arasında hastanemiz doğum ünitesinde 37.-41. gebelik haftası arasında doğum yapmış olan 18-35 yaşları arasındaki tekil gebeliği olan ve YDYB ihtiyacı olan 182 bebeğe sahip olan gebeler ile YDYB ihtiyacı olmayan 890 bebeğe sahip olan gebelerin ilk trimester tarama testlerinden maternal serum PAPP-A ve serbest hCG değerleri karşılaştırılmıştır. Ayrıca sezaryen endikasyonları ve YDYB kabul endikasyonları bakımından da subgrup analizler yapılmıştır. YDYB ihtiyacının tahmini için ilişkili bulunan değişkenlerle lojistik regresyon analizi ve ROC analizi yapılmıştır.

PRECIS: In this study, the relationship between first trimester maternal serum PAPP-A and free β-hCG levels and postpartum neonatal intensive care needs in low-risk pregnancies was investigated.

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Bulgular: YDYB ihtiyacı olan bebeklerin ortalama PAPP-A değeri 0,91±0,34 medyanın katları (MoM) iken, YDYB ihtiyacı olmayan bebeklerin ortalama PAPP-A değeri 1,12±0,59 MoM olarak bulunmuştur (p<0,000). Apgar 5. dk skoru ≥8 grubunun PAPP-A MoM ortalaması (1,09±0,57), Apgar 5. dk skoru <7 grubunun PAPP-A ortalamasından (0,84±0,27) daha yüksektir (p=0,013). Çalışmamız sonuçlarına göre PAPP-A değeri 0,95 MoM altında olanların YDYB ihtiyacı olması ihtimali daha yüksektir.

Sonuç: Düşük riskli gebelik grubunda, PAPP-A düzeyinin düşük olması tarama testi olarak kullanılan bu parametrenin aynı zamanda perinatal sonuçları ön görmede etkin olduğunu göstermektedir.

Anahtar Kelimeler: PAPP-A, neonatal sonuçlar, serbest hCG, düşük riskli gebelik, yenidoğan yoğun bakım

Introduction

Serum concentrations of free human chorionic gonadotropin (hCG) and pregnancy-associated plasma protein (PAPP-A) are used as indicators of chromosomal defects within the context of the first trimester screening test. However, maternal and pregnancy-related characteristics, such as maternal age, ethnicity, smoking habits, weight, and conception procedures, impact these tests. Multiples of the median (MoM) are calculated using each of these variables[1]. Recent studies have also discovered a link between poor obstetric outcomes and maternal serum-free hCG and PAPP-A levels used in chromosomal abnormality screening in the first trimester[1]. Placental dysfunction may be indicated by a low PAPP-A result. PAPP-A and free hCG levels are produced in the placenta shortly after implantation, and low levels may signify improper placentation, which explains why these hormones are associated with poor obstetric outcomes. Low PAPP-A levels have been linked to diseases including preeclampsia, stillbirth, preterm delivery, and fetal growth retardation in much more extensive investigations on PAPP-A from these two markers. However, there is ongoing debate over the findings of research using free-hCG[2-4]. Infants delivered in births with poor obstetric outcomes are becoming more in need of neonatal intensive care. As part of the first trimester screening tests, the levels of free hCG and PAPP-A were examined in this study to determine their association with low-risk pregnancy's requirement for neonatal intensive care.

Materials and Methods

The patient data were accessed using the automation system after receiving the hospital's ethical authorization. The study comprised singleton pregnant women between the ages of 18 and 35 who gave birth in our hospital's maternity ward in the 37th and 41st week of gestation between July 2021 and January 2022. Patients with placental pathologies such as ablation placenta, placenta previa, and vasa previa, pregnant women with an anomaly in their infants, and pregnant women with high-risk circumstances such as gestational diabetes mellitus, preeclampsia, multiple pregnancies, diabetes mellitus, hypertension, polyhydramnios, and oligohydramnios were excluded from the study. The study also eliminated patients whose complete follow-ups were not conducted at our institution. Body mass index (BMI), smoking status, age, method of birth, gravida, parity, and the weight, gender, Apgar score, and requirement for neonatal intensive care of the newborns were all collected from patient data. The MoMs of PAPP-A and free hCG values in the results of first-trimester screening test performed during the antenatal follow-up of infants in need of neonatal intensive care and healthy infants who did not were compared. In our clinic, during the 11th and 14th weeks of pregnancy, within the scope of the first trimester screening test, PAPP-A and hCG tests from blood samples taken from the antecubital vein were studied in our hospital laboratory (Siemens Immulte 2000 XPi) without waiting. In the first trimester screening test, crown-rump length measurement and nuchal translucency were added to serum markers as ultrasonographic measurements. The results of the tests were recorded in both ng/dL and MoM. The MoM value was determined using the prenatal risk calculation program (PRISCA software) used in the laboratory of our hospital by questioning the gestational age, maternal age and weight, smoking status, consanguineous marriage status and conception methods.

Information about the follow-up of newborns was obtained retrospectively from the data system of our hospital. Apgar scores, heights, weights, whether resuscitation done, whether there is a need for neonatal intensive care, and the postnatal laboratory parameters and daily clinical course of the infants of mothers who gave birth in our hospital are recorded on the infant screening system, which is used by pediatricians and obstetricians. All newborns were evaluated postnatally by specialist pediatrics and neonatal physicians. Information about infants in need of neonatal intensive care (NICU), vital follow-ups, indications for intensive care hospitalization and laboratory data, imaging results and physical examination findings are recorded by the specialists by filling in the NICU admission and discharge form. These forms are completed for all newborns admitted to the NICU post-birth, and the database contains all the information to including all medical conditions throughout admission, hospitalization, and repatriation. The international classification of diseases is also used to code all treatments and diagnoses (ICD-10). The admission to the NICU (NICU levels 2 and 3) within the first four weeks after delivery was referred to as a neonatal admission. The following conditions must be met for NICU admission in this study: Cardiorespiratory monitoring of neonates with transient problems, the requirement for intravenous fluid therapy, external intravenous fluid therapy, and closer monitoring of jaundiced infants, respiratory distress syndrome and neonatal septicemia, and continuous supported ventilation. One of the most frequent causes for an infant being admitted to the neonatal critical care unit is respiratory distress. One or more indicators of increased labor of breathing, such as tachypnea, cyanosis, chest retractions, stridor, nasal flaring,

stertor, or grunting, are characterized as respiratory distress in newborns. The ICD-10 code for infant distress was P22.0.

Based on measurements of the quantity of unconjugated bilirubin in the serum and the regional treatment recommendations, neonatal jaundice was characterized as a requirement for phototherapy. In the ICD-10, neonatal jaundice is coded as P59.9. A blood glucose level with less than 2.5 mmol/L, combined tube feeding, or management of hypoglycemia with parenteral glucose infusion was considered as indicators of neonatal hypoglycemia. The ICD-10 code for neonatal hypoglycemia is P70.3, P70.4, P70.8, or P70.9. Neonatal infection was categorized under the ICD-10 subheadings P36 and P39.9 and was described as a combination of clinical symptoms or cultural confirmation of infection requiring systemic antibiotic therapy. The presence of at least three of the following symptoms -inadequate body temperature, tachypnea (>70/min), eating reluctance, abdominal distention, lethargy, hepatosplenomegaly, tachycardia (HR >190 bpm), dyspnea, and bradycardia (HR 90 bpm)- was required for the diagnosis of sepsis. Asphyxia was measured using a low Apgar score, which was classified as less than 7 after 5 min. In the ICD-10, neonatal asphyxia is coded as P21.0, P21.1, and P19.9. Births with low birth weight are classified as P07.1 in the ICD-10 and is described as birth weight below 2500 g.ICD-10 code P92 is used to classify newborn feeding problems.

Statistical Analysis

The IBM SPSS (version 20.0) package application was used in a computer setting to analyze the data collected for our investigation. Cross tables and descriptive statistical data in the form of percentages and numbers were used to depict the research group's socio-demographic characteristics. For continuous variables, the standard deviation and mean values are provided. The Kolmogorov-Smirnov test was used to determine whether the data were in accordance with a normal distribution. To compare categorical variables, chi-square analysis was used. A t-test was performed to examine the normally distributed data between the two groups to compare non-normally distributed data between two groups, the Mann-Whitney U test was applied, and the Kruskal-Wallis test was used in the case of more than two groups. For investigating the linked variables, correlation analysis was performed. Order to estimate the NICU need, a logistic regression analysis was carried out with the corresponding factors. The PAPP-A value's NICU requirement was predicted using ROC analysis. The threshold for statistical significance was set at $p<0.05$.

Results

Of the 1.072 newborn babies included in the research, 17% required NICU care. Of the 1.072 babies, 496 (46.3%) were born vaginally, while 576 (53.7%) were delivered through cesarean section.

In the research, mothers of infants who required NICU had a mean age of 29.49±4.32 and mothers of infants whose did not have a mean age of 29.32±4.57. In terms of mother age, there wasn't any statistically meaningful difference between the two groups (p=0.656). The gravida variable and the requirement for NICU have a statistically significant connection (p=0.001). The parity variable and the requirement for NICU have a statistically significant connection (p=0.002). Multigravid women make up 63.7% of moms of infants who require NICU care and 74.2% of mothers of newborns who do not (p=0.004). Newborns of multigravid mothers had a lower requirement for NICU care. Additionally, there was a statistically significant link (p=0.036) between multiparity and the requirement for a NICU. Infants born to multiparous pregnant mothers had lower NICU needs. Between these two groups, there wasn't a statistically significant difference in the mean maternal BMI (p=0.181). The average gestational week for mothers of infants who require NICU is 38.19±1.17 weeks at the time of delivery, compared to 38.36±1.11 weeks for mothers of infants who do not. When it came to the mothers' gestational week at birth, there wasn't any statistically meaningful difference between the two groups (p=0.056). Whether or whether the infant requires a NICU is significantly correlated with smoking status (p=0.027). Infants of smokers require more NICU care (8.8% vs. 4.7%). 56.6% of mothers of infants who required NICU care underwent a cesarean section, compared to 53.61% of mothers of infants whose did not (p=0.395). The gender of the newborns and the requirement for NICU have a statistically significant link (p=0.007). Newborn boys require more NICU care than infant females. Newborns that require NICU have an average birth weight of 3187.66±661.76 gr, whereas healthy infants have an average birth weight of 3357,12430.47 gr. However, there wasn't any statistically significant link between the requirement for a NICU and fetal macrosomia (p=0.457). Infants who required NICU care had an average PAPP-A value of 0.91±0.34 MoM. Infants who do not require NICU care have an average PAPP-A value of 1.12±0.59 MoM. In terms of PAPP-A value, there is a statistically significant disparity between the requirement for NICU (p=0.000). The subgroup that does not require NICU has a PAPP-A value that is, on average, greater than the subgroup that does. However, the difference between the requirement for NICU and the free hCG value was not statistically significant (p=0.134) (Table 1).

Cesarean section grounds were studied in our study as a subgroup, and they are displayed in Table 2. The necessity for an NICU is statistically different from the criteria for a cesarean section (χ^2=21.723; p<0.001). Infants who had cesarean delivery with fetal distress indication require NICU care the most. Cesarean grounds and PAPP-A (MoM) values did not differ in a way that was statistically significant (F=1.407; p=0.230). Cesarean grounds and hCG (MoM) value did not change in a way that was statistically significant (F=1.517; p=0.196).

The between infant hospitalization causes and hCG (MoM) levels, there wasn't any statistically significant difference

(F=0.852; p=0.545). There wasn't any statistically significant relationship between the PAPP-A (MoM) value and neonatal hospitalization indications (F=1.878; p=0.087) (Table 3).

A statistically significant distinction existed between the PAPP-A (MoM) mean of the Apgar 5th minute score <7 group and the PAPP-A (MoM) mean of the Apgar 5th minute score greater than or equal to 8 group (p=0.013). A statistically significant distinction existed between the mean of the hCG (MoM) of the Apgar 5th minute score <7 group and the hCG (MoM) mean of the Apgar 5th minute score greater than or equal to 8 group (p=0.019) (Table 4).

Table 5 lists correlation analysis results. PAPP-A value has a strongly negative statistical connection (r=-0.102, p=0.001) with age, gravida (r=-0.147, p=0.001), and parity factors (r=-0.125, p=0.001). PAPP-A value has a positive and extremely weak statistical correlation with BMI (r=0.073, p=0.017), gestational week at delivery (r=0.067, p=0.029), and birth weight (r=0.099, p=0.001) variables. PAPP-A value and free hCG have a positive and statistically weakly significant connection (r=0.246, p=0.001), as do PAPP-A value and Apgar 5th minute score (r=0.159, p=0.001).

Our regression model (χ^2=15.038; p<0.000) was significant due to the forward (wald) approach logistic regression analysis. According to Nagelkerke, our binary logistic regression model, which was created to forecast the requirement for newborn intensive care, has a 26.9% explanation rate. Smoking and the need for a NICU had a significant positive connection, using the established model (B=2.069; p=0.001). When we look at the odds ratios (to the quantity) of this link, we find that infants of smoker mothers are more likely to need an NICU than those of non-smokers to be 7.055 times more likely. PAPP-A value and NICU necessity have a compelling negative connection in our constructed model (B=-1.227; p=0.000). In this connection, NICU requirement increases by 0.370 times for every unit reduction in PAPP-A score. Low birth weight and the requirement for NICU were positively and significantly correlated (B=3.198; p=0.000). Low birth weight infants cause NICU use to increase by 24.473 times. The need for a NICU was significantly negatively correlated with the need for a cesarean section (B=-0.943; p=0.001). Comparing individuals who have had prior uterine surgery to those who have fetal distress owing to cesarean indication, the requirement for NICU is increased by 0.390 times (Table 6).

The area under curve =0.593 was used to calculate the proportion of PAPP-A variables that predicted the requirement for NICU care. This determined PAPP-A value allows an accurate estimation of the NICU need at a rate of 59.3%. The test's cut-off point was 0.955, its specificity was 45.2%, and it

Table 1. Comparison of demographic and clinical characteristics of the groups

	NICU (+) (n=182)	NICU (-) (n=890)	p
Age**	29.49±4.32	29.32±4.57	0.656
Gravida***	2 (1-11)	2 (1-8)	<0.001
Parity***	1 (0-3)	1 (0-5)	0.002
Multigravida***	116 (63.7)	660 (74.2)	0.004
Multiparity***	47 (25.8)	301 (33.8)	0.036
BMI (kg/m^2)*	29.60±3.57	29.22±3.48	0.181
Obesity***	87 (47.8)	387 (43.5)	0.285
Gestational age (week)**	38.19±1.17	38.36±1.11	0.056
Smoking***	16 (8.8)	42 (4.7)	0.027
Cesarean delivery***	103 (56.6)	473 (53.1)	0.395
Infant gender*** Female Male	 80 (44.0) 102 (56.0)	 488 (54.8) 402 (45.2)	0.007
Birth weight (gr)**	3187.66±661.76	3357.12±430.47	<0.001
LBW***	40 (22.0)	6 (0.7)	<0.001
Macrosomia***	18 (9.9)	73 (8.2)	0.457
PAPP-A (MoM)**	0.91±0.34	1.12±0.59	<0.001
Free hCG (MoM)**	1.05±0.46	1.11±0.49	0.134

NICU: Neonatal intensive care unit, BMI: Body mass index, LBW: Low birth weight, PAPP-A: Pregnancy-associated plasma protein A, MoM: Multiples of the median, hCG: Human chorionic gonadotropin, *Continuous variable with normal distribution; t-test, **Continuous variables not normally distributed; Mann-Whitney U test, ***Categorical variable; chi-square test, p<0.05 was considered statistically significant

Table 2. Evaluation of the relationship between cesarean indications and the need for neonatal intensive care

	NICU (+) (n=182)	NICU (-) (n=890)	Total
Fetal distress	45 (26.2)	127 (73.8)	172 (100.0)
Malpresentation	2 (7.7)	24 (92.3)	26 (100.0)
Previous uterine surgery	40 (12.3)	284 (87.7)	324 (100.0)
CPD	12 (30.0)	28 (70.0)	40 (100.0)
Macrosomic fetus	4 (28.6)	10 (71.4)	14 (100.0)
Total	103 (17.9)	473 (82.1)	576 (100.0)
χ^2=21.723 p<0.001*			

NICU: Neonatal intensive care unit, CPD: Cephalopelvic disproportion, *chi-square test was used. P<0.05 was considered statistically significant

Table 3. Evaluation of the relationships between indications for hospitalization of newborns and PAPP-A and hCG values

	N	PAPP-A (MoM) Mean ± standard deviation	hCG (MoM) Mean ± standard deviation
Respiratory distress	53	0.82±0.32	0.93±0.33
Indirect hyperbilirubinemia	63	0.92±0.36	1.04±0.46
Low birth weight	40	0.94±0.25	1.17±0.57
Neonatal infection	8	0.98±0.43	1.22±0.53
Neonatal hypoglycemia	6	1.15±0.33	1.22±0.40
Asphyxia	4	0.89±0.19	1.11±0.58
Nutritional intolerance	8	1.20±0.58	0.85±0.16
		F=1.878 p=0.087*	F=1.463 p=0.194*

PAPP-A: Pregnancy-associated plasma protein A, hCG: Human chorionic gonadotropin, MoM: Multiples of the median, *Kruskal-Wallis multiple comparison test. P<0.05 was considered statistically significant

Table 4. Evaluation of the relationships between the fifth-minute Apgar score and PAPP-A values

	n	PAPP-A (MoM) Mean ± standard deviation	hCG (MoM) Mean ± standard deviation
Apgar score 5th min <7	32	0.84±0.27	0.89±0.23
Apgar score 5th min ≥8	1.040	1.09±0.57	1.10±0.49
		p=0.013*	p=0.019*

PAPP-A: Pregnancy-associated plasma protein A, MoM: Multiples of the median, hCG: Human chorionic gonadotropin. *Continuous variable not normally distributed; Mann-Whitney U test. P<0.05 was considered statistically significant

had a 52.7% sensitivity rate (Figure 1). Our study's findings indicate that individuals with PAPP-A values below 0.95 are more likely to have an increase in their demand for NICU care.

Discussion

PAPP-A and hCG, two of the first trimester blood screening tests, are linked to pregnancy problems in addition to being predictive of fetal defects[5]. In our investigation, blood samples from mothers of infants who required NICU care and had an Apgar score at the five-minute mark below seven had a substantially lower PAPP-A value. Additionally, despite a correlation between the hCG value and the Apgar score in the fifth minute, it was believed that this circumstance was caused by a discrepancy in the distribution of the data because it did not result in a greater requirement for NICU care. There are a few research looking at the connection between PAPP-A and various poor neonatal and perinatal results in the literature. According to these studies, low PAPP-A levels in the first trimester of pregnancy are linked to an increased risk of miscarriage, preeclampsia, pregnancy-induced hypertension, small-for-gestational-age delivery, stillbirth, premature membrane rupture, and placental abruption[3,6]. Although it is possible that the need for NICU for newborns might be connected with low PAPP-A levels, this is unlikely given that the study group in our study was made up of a low-risk group and there were no concomitant events that would enhance the need for NICU in infants throughout this group. This relationship can be explained by low placental size and decreased placental perfusion, which is probably associated with decreased PAPP-A production[7,8].

People who are most prone to stillbirth, IUGR, and early labor have smaller placentas and higher levels of alpha-fetoprotein in the absence of damaged uterine artery Doppler velocimetry in those with low PAPP-A levels[9]. PAPP-A levels are expected to be associated with a disturbance of placental circulation, even if the AFP quantity and Doppler measurements were excluded from our analysis. The neonatal outcomes of 9.450

Table 5. Evaluation of the relationships between PAPP-A and other parameters

	r	p
Age	-0.102	0.001
Gravida*	-0.147	<0.001
Parity*	-0.125	<0.001
BMI (kg/m^2)	0.073	0.017
Pregnancy week	0.067	0.029
Birth weight (gr)	0.099	0.001
Free hCG (MoM)	0.246	<0.001
Apgar 5th min score	0.159	<0.001

r: Corelation coefficient, MoM: Multiples of the median, BMI: Body mass index, hCG: Human chorionic gonadotropin, *Spearman correlation analysis was used, p<0.05 was considered statistically significant

singleton pregnant women who participated in the prenatal screening program were also studied by Kirkegaard et al.[10]. They asserted a high correlation between low PAPP-A levels and neonatal diseases, such as the requirement for NICU admission and hypoglycemia. PAPP-A and hCG values were examined with newborn critical care hospitalization indications in our investigation, but no significant correlation between the variables was discovered. Examining the studies on this topic shows that the connection between pregnancy issues such preterm birth, SGA, preeclampsia, and IUGR as well as first trimester screening tests are studied, and negative newborn outcomes are disclosed based on these results[6-9]. Fox and Chasen[11] looked at the connection between PAPP-A levels, the second trimester growth restriction, associated problems, and NICU admissions. Although there are not enough patients with lower PAPP-A values, it has been demonstrated a need for NICU rises in these infants. But we found that there wasn't a parameter we identified as a risk factor, like smoking. Although the results are similar to our findings, this study is not entirely appropriate for comparison because our study only looked at low-risk pregnant women[11]. However, an association between low birth weight and low PAPP-A values was noticed in a study with a huge number in which the findings of 12,592 pregnant women across Britain were published, comparable to our study[12]. A few studies, in particular, have suggested that elements like premature delivery and SGA impact negative newborn outcomes[13-16]. Since the low-risk pregnancy population was the focus of our study, there shouldn't be a significant NICU demand. The pregnant women in the NICU-required group, however, should be noted since they smoked, and smoking was linked to low birth weight and poor PAPP-A readings. All these findings clearly imply that placental perfusion dysfunction may be the etiology of the disease. The requirement for NICU has increased because of multigravida and multiparity rates, which is another finding of our study.

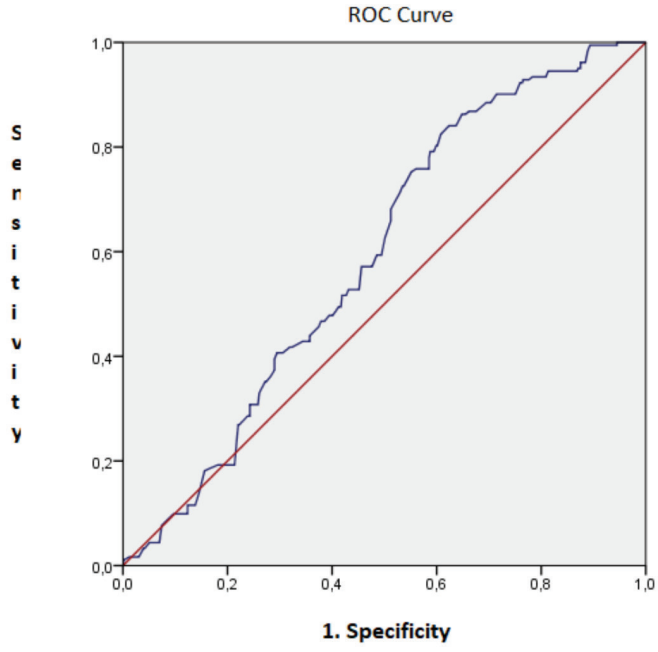

Figure 1. ROC analysis

ROC: Receiver operating characteristic

Although the requirement for NICU grew exponentially as the parity ratio increased, a statistically significant difference was also undiscovered, according to Madan et al.[17]. Similar to our investigation, the same study concluded that smoking enhanced the requirement for NICU care. Additionally, it was found in our study that male infants required more NICU care. Although it has been noted in the literature that male newborns are more likely to experience negative outcomes and require NICU care[18], we believe that the results of our study are a result of the quantitative and distributional properties of the data. The need for NICU was greater in infants whose cesarean sections were performed for fetal distress, even though cesarean section rates were similar between infants who required NICU regardless of the mode of delivery, as predicted by the subgroup analysis carried out in terms of cesarean delivery indications. However, there was no association between PAPP-A and hCG levels and cesarean section indications. According to a study looking at first trimester PAPP-A levels and the chance of developing

Table 6. Results of logistic regression analysis of variables associated with NICU

Variables	B	St. error	p	β	95% confidence interval for β	
					Bottom	Top
Still	-3.365	0.994	0.001*	0.035	-	-
Smoking (1)	2.069	0.420	0.000*	7.914	3.475	18.025
PAPP-A value	-1.227	0.336	0.000*	0.293	0.152	0.566
LBW (1)	3.198	0.557	0.000*	24.473	8.208	72.970
Cesarean indication (3)	-0.943	0.274	0.001*	0.390	0.228	0.667
Nagelkerke R2=0.269 χ^2=15.038 p=0.000*						

Reference variable: NICU (-) Cesarean indication reference variable: Fetal distress; (3): Previous uterine surgery; *: Statistical significance. PAPP-A: Pregnancy-associated plasma protein A, LBW: low birth weight. P<0.05 was considered statistically significant

intrapartum fetal distress, low PAPP-A concentration increases the likelihood of experiencing intrapartum fetal distress and, consequently, the probability of cesarean birth[19]. One thousand thirty seven pregnant women with low PAPP-A levels were included in the study by Uccella et al.[20] The umbilical artery pH was considerably lower and the incidence of emergency cesarean sections was greater in the low PAPP-A group after correcting for potential confounding factors such hypertension, small for gestational age, preterm birth, and labor induction. It was discovered the existence of a statistically significant distinction was observed between fetal weight, 5th minute Apgar scores, and gestational age was observed in a study that examined PAPP-A values in pregnancies complicated by preterm delivery, preeclampsia, and fetal growth restriction. They concluded that variations in PAPP-A levels should be considered to remember that more attentive and cautious antepartum observation may be necessary to prevent negative perinatal outcomes in a particular patient group[21]. In our study, a relationship between PAPP-A levels, gestational week, 5th minute Apgar score, and birth weight was found. With the help of this information, we think that low PAPP-A in the group of low-risk pregnancies may be able to predict low birth weight, which in this instance is evident in the Apgar score and increases the requirement for NICU. The significantly low level of PAPP-A suggests that this parameter, which is employed as a screening test, is helpful at predicting perinatal outcomes, particularly in low-risk pregnancies, even though all cases were chosen from term and low-risk pregnancies. We believe that a low PAPP-A level can be used as an indicator, although the sensitivity and specificity values are not particularly strong.

Study Limitations

The inclusion of a carefully chosen community of low-risk pregnant is the study's strength, even though the sample size and retrospective nature of the study are two of its most obvious weaknesses.

Conclusion

The ideal postpartum scenario for the family and doctor in the low-risk pregnancy group is for the mother to be able to stay with her healthy newborns. NICU care is not preferred in low-risk pregnancies, although it is more acceptable for infants in the dangerous pregnancy group. However, because the newborns of this group of expectant women may also require NICU care, a marker-like PAPP-A, which we routinely scan, can be used to foretell this potential need to inform the patient and prepare them for potential risks. On the other hand, avoiding this scenario altogether will be advantageous for both the economy and public health. As a result, necessary precautions should be taken and preparations should be made accordingly, as infants may need NICU in pregnant with low PAPP-A value, one of the first trimester screening tests.

Ethics

Ethics Committee Approval: Ethics committee approval was obtained from the University of Health Sciences Turkey, Ankara City Hospital (approval no: 7, date: 25.01.2022).
Informed Consent: Retrospective study.
Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: B.E., B.L.K., D.T.E., U.Z., E.A., G.Y., E.Ü.Ö., N.H., Concept: Ö.M.T., Design: B.E., N.H., Data Collection or Processing: U.Z., E.A., Analysis or Interpretation: B.L.K., D.T.E., G.Y., Literature Search: N.H., Writing: B.E., E.Ü.Ö.
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Short interdelivery interval in modern obstetrics: Maternal and neonatal outcomes

Modern obstetride doğumlar arası kısa interval: Maternal ve neontal sonuçlar

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Abstract

Objective: To investigate the maternal, neonatal outcomes of the patients with short interdelivery interval (IDI) considering initial pregnancy outcomes.

Materials and Methods: Women with two consecutive deliveries between 2016 and 2020 were included in the study. The maternal and neonatal outcomes of both pregnancies were reviewed. The time interval between consecutive deliveries was calculated. The patients were divided into two groups in terms of IDI either less or more than 24 months.

Results: The number of patients with short IDI (≤24 months), and normal IDI was 1.915 and 1.370, respectively. About 15% of the women in both groups had at least one obstetric morbidity. The rates of uterine rupture, placenta previa, and peripartum hysterectomy were higher in women with short IDI. The number of patients with low birth weight, very low birth weight, and stillbirth was higher in the short IDI group.

Conclusion: Patients with short interpregnancy intervals should be considered high-risk pregnancy. Adequate contraceptive methods should be used to prevent unintended pregnancies.

Keywords: Interdelivery interval, birth spacing, inter-pregnancy interval, stillbirth, neonatal morbidity

Öz

Amaç: Bu çalışmada doğumlar arası intervali kısa olan gebeliklerin maternal ve neonatal sonuçlarının incelenmesi amaçlanmıştır.

Gereç ve Yöntemler: 2016 ve 2020 yılları arasında iki kez doğum yapmış kadınlar çalışmaya dahil edildi. Doğumlar arasındaki süre hesaplandı. Katılımcılar, doğumlar arası interval 24 aydan az veya fazla olmak üzere iki grupta incelendiler.

Bulgular: Doğumlar arası interval 24 aydan az olan hasta sayısı 1,915 ve 24 aydan fazla olanların sayısı 1,370 olarak belirlendi. Her iki grupta en az bir obstetrik morbiditesi olan hasta oranı %15 olarak bulundu. Uterus rüptürü, plasenta previa, peripartum histerektomi oranları doğumlar arası intervali kısa olanlar grubunda daha yüksekti. Düşük doğum ağırlığı, çok düşük doğum ağırlığı ve ölü doğum oranları doğumlar arası intervali kısa olanlar grubunda daha yüksekti.

Sonuç: Doğumlar arası interval 24 aydan az olan gebeler yüksek riskli gebelik olarak kabul edilmelidir. İstenmeyen gebelikleri önlemek açısından doğum kontrol yöntemlerinin doğru uygulanması çok önemlidir.

Anahtar Kelimeler: Doğumlar arası aralık, doğum aralığı, gebelikler arası aralık, ölü doğum, neonatal morbidite

PRECIS: Pregnancies with short interdelivery interval should be considered as high-risk. Two years seems as an appropriate interval between consecutive pregnancies. Contraception should be provided to individuals to avoid unintended pregnancies.

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Introduction

Childbearing potential during the reproductive lifespan has utmost importance for women order to plan their families in the way that they desire. Women inquire about the adequate time for the subsequent pregnancy after the delivery. The obstetricians may struggle to respond to this request because there is not even a consensus on the explanation of that period. Researchers have suggested various definitions to determine the appropriate timing for a subsequent pregnancy, such as inter-pregnancy interval (IPI) (time between live birth and subsequent conception), interdelivery interval (IDI) (the period between consecutive live births), and inter-outcome intervals (timing between two pregnancies). The World Health Organization recommends a two-year interval between subsequent pregnancies due to increased perinatal adverse outcomes and a short IPI is referred to a period of less than 2 years[1]. However, there are controversies in the definition of short IPI with various durations ranging from 3 to 24 months in different articles[2-4]. Several studies have revealed the association of various maternal and neonatal adverse outcomes with short IPI[2-6]. The hypotheses were put forth to explain the adverse outcomes of short IPI based on maternal nutrition depletion, or maternal folate depletion[7,8]. Besides these hypotheses; antenatal care, socioeconomic status, lifestyle behaviors might play a role in adverse outcomes; nonetheless, it has been emphasized that these concomitant issues had a small effect, and mainly short IPI played an independent role in adverse maternal and perinatal outcomes[9]. Patients who suffered a missed abortion, stillbirth, or early neonatal death may not be willing to comply with obstetricians' recommendations[10,11]. Furthermore, unintended pregnancies are common in the first 2 years after a delivery. A recent report has shown that about 30% of American women had an IPI at less than 18 months[12]. Considering such a high population in a developed country; the maternal and neonatal outcomes of the patients with a short IDI between subsequent pregnancies in developing countries is worthy of investigating. The definition of IPI was calculated in almost all studies on mothers' recall. Thus, IDI ensures an accurate duration between two deliveries. This study aimed to investigate the maternal and neonatal outcomes of women who gave consecutive births within a short IDI.

Materials and Methods

Pregnant women who were delivered between April 2016 and April 2020 at a tertiary-care center in Bursa, Turkey was reviewed. Among these, patients with two consecutive births who received antenatal care at this institution were included in the study. Ethical approval was obtained from the Institutional Review Board at University of Health Sciences Turkey, Bursa Yüksek İhtisas Training and Research Hospital. The data for the study were obtained from the birth certificates and from the hospital records. IDI was calculated as the number of months between deliveries. The cut-off for IDI were selected as 12 and 24 months. Therefore, patients were grouped based on their IDI being less or more than 12 and 24 months separately. The adverse outcomes were defined as preterm delivery, primary cesarean section (indications were as fetal malpresentation, fetal distress, prolonged or arrest of labor, cephalopelvic disproportion, suspected fetal macrosomia, umbilical cord prolapse etc.), pre-eclampsia, fetal anomaly (detected by ultrasound before birth), placental abruption, gestational diabetes mellitus, complicated vaginal delivery (operative delivery, serious perineal trauma that defined as 3rd or 4th degree perineal laceration or need of anesthesia for repairing), complicated cesarean section (Placenta previa, uterine atony, bladder or bowel injury, uterine rupture defined as disruption of uterine muscle and visceral peritoneum, need of B-Lynch suture, Bakri balloon, hypogastric artery ligation or hysterectomy). To evaluate the number of patients affected by any of the aforementioned complications, an individual parameter was created postulated as obstetric morbidity. Patients with more than one adverse outcome were included in each relevant obstetric morbidity group as one patient.

Postpartum complications were recorded as febrile morbidity, retained placenta, endometritis, abdominal hemorrhage or abscess in the postoperative period, need for transfusion, wound site infection or dehiscence, thromboembolic events, and maternal death.

Neonatal outcomes included; birth weight, 1st and 5th minute APGAR scores, admission to the neonatal intensive care unit (NICU), stillbirth, low birth weight [(LBW)-less than 2.500 gr], and very low birth weight [(VLBW)-less than 1.500 gr].

Statistical Analysis

Statistical analyses were performed using the SPSS software version 24 (Armonk, NY, USA). The Kolmogorov-Smirnov test was used to determine the normal distribution of the variables. Primarily, patients with short IDI (<24 months) were compared with the normal IDI period (>24 months). Secondarily, evaluation of these patients' first deliveries and the characteristics were achieved. Paired student's t-test and chi-square test were used appropriately. A p-value of less than 0.05 was considered as statistically significant.

Results

A total number of 50,938 births were identified on the review of records during the 4-year period. The number of patients who had two subsequent deliveries during the study period was 3.285. Of these, the number of patients with an IDI ≤24 months (short IDI), and above 24 months (normal IDI) was 1.915 and 1.370, respectively.

The characteristics, maternal and neonatal outcomes of first and second pregnancies for the short IDI and normal IDI are shown in Table 1. Women with short IDI were younger and had a lower mean birth weight during their second pregnancy. Neonatal outcomes revealed that rates of stillbirth, LBW, VLBW and admission to NICU were 1.8%, 7%, 0.7%, and

8.7% respectively, in the short IDI group. Maternal outcomes of interest, which were referred to as delivery method (p=0.085), primary cesarean indications, preeclampsia (p=0.740), preterm delivery (p=0.102), GDM (p=0.082), postpartum complications (p=0.566) did not differ significantly in women with short IDI compared with the normal IDI group. Women with both short and normal IDI had approximately 15% obstetric morbidity, which was postulated as an aggregate of the complications.

The overall complications in cesarean section deliveries in all groups are demonstrated in Table 2. Although there was no statistically significant difference between short and normal IDI patients, rates of complications such as cesarean hysterectomy, uterine rupture, and uterine atony was higher in the short IDI group (p=0.078). Despite the small number of patients who underwent two cesarean sections within two years, these patients experienced a 5% rate of surgery-related complications. Individual numbers of complications are depicted on the table.

Table 1. Evaluation of the patients' maternal and neonatal outcomes for the first and the second deliveries, and comparison of patients with the short and normal interdelivery interval

	Outcomes of 1st pregnancies in patients with IDI <24 months (n=1.915)	Outcomes of 2nd pregnancies in patients with IDI <24 months (short IDI) (n=1.915)	Outcomes of 1st pregnancies in patients with IDI >24 months (n=1.370)	Outcomes of 2nd pregnancies in patients with IDI >24 months (normal IDI) (n=1370)	Comparison of short IDI and normal IDI p-value
Age	23.6±5.3	25.1±5.4	23.8±4.9	26±4.9	<0.001
Delivery interval	-	17.9±4.1	-	31±5.5	-
Birth weight	3.111±540	3.157±555	3.207±481	3.251±498	<0.001
Hospital stay (median, std)	2±1	2±1	2±1	2±1.1	0.853
APGAR 1st min	8.8±0.8	8.8±0.6	8.8±0.6	8.9±0.4	0.027
APGAR 5th min	9.7±0.7	9.8±0.6	9.7±0.6	9.9±0.3	0.001
Newborn gender					
Male	979	986	636	726	0.395
Female	936	929	734	644	
Stillbirth	44 (2.3%)	35 (1.8%)	7 (0.5%)	10 (0.7%)	0.008
Delivery type					
Vaginal	69.7%	62.5%	70.9%	59.5%	0.085
Cesarean	30.3%	37.5%	29.1%	40.5%	
Primary cesarean section	20%	9.1%	22%	10%	0.378
Macrosomia	68 (3.6%)	81 (4.2%)	54 (3.9%)	84 (6.1%)	0.014
Complicated vaginal delivery	1 (0.1%)	0%	3 (0.2%)	0.1%	0.417
Complicated cesarean section	29 (5%)	36 (5%)	18 (4.5%)	27 (4.8%)	0.851
Obstetric morbidity	297 (15.5%)	288 (15%)	162 (11.8%)	208 (15.2%)	0.910
Preeclampsia	34 (1.8%)	28 (1.5%)	9 (0.7%)	22 (1.6%)	0.740
Preterm delivery	76 (4%)	60 (3.1%)	36 (2.6%)	30 (2.2%)	0.102
Fetal anomaly	8 (0.4%)	13 (0.7%)	5 (0.4)	3 (0.2%)	0.062
Abruptio placenta	10 (0.5%)	13 (0.7%)	2 (0.1%)	3 (0.2%)	0.062
GDM	18 (0.9%)	20 (1%)	11 (0.8%)	24 (1.8%)	0.082
Postpartum complication	36 (1.9%)	43 (2.2%)	30 (2.2%)	35 (2.6%)	0.566
NICU admission	132 (6.9%)	163 (8.7%)	52 (3.8%)	106 (7.8%)	0.368
Birth weight <1.500	29 (1.6%)	13 (0.7%)	8 (0.6)	6 (0.4%)	0.356
Birth weight <2.500	173 (9.3%)	131 (7%)	88 (6.5%)	61 (4.5%)	0.003

IDI: Interdelivery interval, NICU: Neonatal intensive care unit, Std: Standard deviation

Characteristics of the women with stillbirth are demonstrated in Table 3. Ninety-six cases of stillbirth occurred in this study with an incidence of 1.4%. The incidence of stillbirth in the short IDI group was 1.8% and in the normal IDI group was 0.7% and this difference was statistically significant (p=0.048). In the short IDI group, the mean weight weight was lower (p=0.045). However, rates of preterm delivery (p=0.036), placental abruption (p=0.044), and fetal anomaly (p=0.023) were higher than the normal IDI group.

Post partum complications are demonstrated in Table 4. The number of patients who experienced post partum complications was 43 and 35 and this difference was not statistically significant (p=0.088). Only one maternal mortality occurred due to amniotic fluid embolism.

Discussion

The effect of the short IDI on maternal and neonatal outcomes was reviewed. It has been reported that consecutive cesarean deliveries within a short IDI are associated with increased uterine rupture[13,14]. The healing process was the main determinant as depicted that the lower segment of the uterus regenerates gradually and could need at least 6 months to heal completely[15]; a recent study demonstrated that there was no relationship between short IDI and uterine rupture[16]. In our

Table 2. Demonstration of the events that complicated cesarean operations in each group individually

	Outcomes of 1st pregnancies in patients with IDI <24 months	Outcomes of 2nd pregnancies in patients with IDI <24 months (short IDI)	Outcomes of 1st pregnancies in patients with IDI >24 months	Outcomes of 2nd pregnancies in patients with IDI >24 months (normal IDI)	Comparison of outcomes of 2nd pregnancies in short IDI and normal IDI p-value
Frequency	29 (5%)	36 (5%)	18 (4.5%)	27 (4.8%)	0.078
Age	29±5.5	26±4.9	23.8±4.4	26.7±4.4	0.102
Birth weight	2.783±893	2.851±806	3.019±467	2.894±790	0.115
Stillbirth	1	4	0	1	
Abruptio placenta	1	2	0	1	
Placenta previa	8	7	6	5	
Atony	21	18	12	16	
Blood transfusion	3	5	4	4	
Hysterectomy	0	5	0	1	
Uterine rupture	0	6	0	1	
Bladder injury	0	2	0	2	
DIC	0	2	0	0	
B-Lynch suture, bacri balloon	0	4	0	2	

IDI: Interdelivery interval

Table 3. Outcomes of patients who experienced a stillbirth

	1st pregnancy outcomes of patients IDI <24 months	IDI <24 months (short IDI)	1st pregnancy outcomes of patients IDI >24 months	IDI >24 months (normal IDI)	Comparison of short IDI and normal IDI p-value
Frequency	44 (2.3%)	35 (1.8%)	7 (0.5%)	10 (0.7%)	0.048
Age	24.4±5	27.1±7	22.4±6	25.7±4	0.069
Birth weight	1.677±1.167	1.812±984	1.826±1.025	2.177±993	0.045
Preterm delivery (n-%)	28 (65%)	22 (65%)	4 (57%)	4 (40%)	0.036
Abruptio placenta	2 (5%)	6 (17%)	2 (28%)	1 (10%)	0.044
Fetal anomaly	1 (3%)	6 (17%)	0	1 (10%)	0.023
Delivery interval	-	17±3.6	-	31±5	-

IDI: Interdelivery interval

study, six patients in the short IDI group experienced uterine rupture, whereas only one uterine rupture occurred in the normal IDI group. Evaluation of these rupture cases revealed that all cases that were incomplete rupture were detected during the operation, and none of the patients underwent hysterectomy. Thus, it is difficult to associate the uterine rupture with pregnancy interval[16].

To evaluate further, a subgroup was formed to include complicated cesarean section deliveries. There was no statistical difference between the short and normal IDI groups. However, the need for uterus conserving interventions (such as B-Lynch suture, Bakri balloon placement, or hypogastric artery ligation), number of uterine ruptures, and hysterectomy procedures were higher in the short IDI group. These could be clinically important despite the statistically insignificant results.

The relationship between long IPI and primary cesarean delivery rate has been revealed[17,18]. However, limited data exists about short IDI and cesarean frequency. This study shows that there is no association between the primary cesarean delivery frequency and IDI intervals. Short IDI might be suggested to complicate the vaginal delivery such as dystocia, need for operative delivery, or perineal trauma; yet, none of the patients experienced such complications in this study. Post-partum complications were also evaluated and no significant difference was found between the groups.

In this study, a unique group was composed to determine each patient affected by any complications. The results showed that no significant difference occurred between the patients with short and normal IDI. Approximately 15% of women experienced at least one complication. Statistical analysis did not reveal any significant difference between the groups in terms of preeclampsia, preterm delivery, fetal anomaly, abruptio placenta, and GDM. Patients with short IDI might have adverse perinatal outcomes and fetal anomalies due to the folate depletion hypothesis. Despite there being no statistically significant difference within the groups, more women in the short IDI group had newborns with fetal anomaly and most of the anomalies consisted of neural tube defects, which might be related to the folate depletion hypothesis[8]. Hanley et al.[19] depicted that short IDI could be a risk factor for GDM. They stated that obesity before conception might be associated with increased GDM rates, which was contrary to the hypothesis of maternal nutrition depletion[7]. The most important point to emphasize is that maternal nutrition and obesity are the circumstances which can be managed during the period between consecutive pregnancies to avoid adverse outcomes.

The IDI was not detected as a risk factor for GDM in this study, by the way, women with normal IDI had a higher mean birth weight and more women had newborns with macrosomia. The frequency of preeclampsia, which was one of the major reasons for maternal morbidity, was not affected by short IDI in this study. The current literature has conflicting data on this issue, there are studies stating that either the short IDI[20,21], or the long IDI are associated with preeclampsia[19,22,23]. Preeclampsia is a multifactorial disease which may not be directly linked to interpregnancy interval. However, long IDI might be associated with an increased risk of preeclampsia due to advancing age. Abnormal healing processes in the endometrial cavity, suboptimal vascular regeneration, or defective implantation might be the reason for placental abruption or placenta previa. Contemporary studies focused on the effect of short IDI and these placental pathologies and stated that short IDI increases the risk factors of placenta abruption and placenta previa[9,21,24]. More repeat cesarean deliveries within a short time interval would inevitably increase the rate of placenta previa and placental invasion anomalies[25]. Placenta previa and placenta

Table 4. Evaluation of the post-partum complications of the patients in each group

	1st pregnancy outcomes of patients IDI <24 months	IDI <24 months (short IDI)	1st pregnancy outcomes of patients IDI >24 months	IDI >24 months (normal IDI)	Comparison of outcomes of 2nd pregnancies in short IDI and normal IDI p-value
Frequency	36 (1.9%)	43 (2.2%)	30 (2.2%)	35 (2.6%)	0.088
Wound site infection	6	5	9	4	
Blood component transfusion	24	28	18	26	
Endometritis	1	2	0	1	
Retained placenta	3	2	3	1	
Post-operative intraabdominal abscess or hematoma	2	2	1	1	
Disseminated intravascular coagulation	0	4	1	1	
Febrile morbidity	1	1	0	1	
Maternal mortality	0	0	0	1	

IDI: Interdelivery interval

accreta spectrum, which resulted in hysterectomy, were higher in patients with short IDI. Placental abruption was detected in 0.7% of the short IDI group whereas in the normal IDI group the rate was 0.2%. Although the results did not significantly differ, they were clinically important because each of these complications severely impacts maternal and fetal morbidity and mortality.

Even although most of the maternal outcomes did not differ statistically significantly between patients with short and normal IDI, there was a remarkable difference in neonatal results. Mean birth weight, APGAR 1st, and 5th minute scores differed significantly, although the results were supposed to be clinically insignificant. The number of newborns with low birth weights and very low birth weights were higher in the short IDI group. Additionally, the results worsened in patients with 12 months delivery interval. These outcomes were compatible with recent studies in the literature[2,4,6,19,26]. The most crucial data of this study was the higher incidence of stillbirth. Women with short IDI experienced stillbirth at a rate of 18.2 per 1.000 births. This increase was statistically significant compared to the normal IDI patients. A recent study declared that the stillbirth rate was 11.2 per 1.000 births in the same hospital[27]. The delivery interval might not be the only explanation for the increased rate of stillbirth. This was one of the most important findings of this study. Evaluating of patients who had a stillbirth revealed that the main reason was the preterm deliveries, placental abruption, and fetal anomaly. Contemporary studies revealed that women with an IPI of 6 months, which was approximately 12 months of IDI, were at risk of stillbirth[28,29]. However, contrary to these studies, Stephansson et al.[30] stated that short IPI was not associated with stillbirth after adjusting the maternal characteristics and previous pregnancy outcomes. According to the findings of this study, patients with short IDI experienced more stillbirths than women with normal IDI. Thus that should be kept in mind that patients should be informed about stillbirth as a possible adverse outcome of short IDI.

Study Limitations

The retrospective nature of this study stands as a major limitation.

Conclusion

Despite the difficulty in defining a universally accepted period for birth spacing after a delivery, patients with short interdelivery intervals should be considered high-risk pregnancies. The interval of two years seems as an appropriate period and is recommended by many studies. However, approximately 30% of the women conceive in that period. Appropriate contraceptive methods should be used to prevent unintended pregnancies. Women with consecutive deliveries less than 2 years apart might be at risk of stillbirth, preterm delivery, intrauterine growth restriction, and low birth weight. It is important to emphasize that pregnancies with short interdelivery intervals should never be understated and should be managed appropriately.

Ethics

Ethics Committee Approval: Ethical approval was obtained from the Institutional Review Board at University of Health Sciences Turkey, Bursa Yüksek İhtisas Training and Research Hospital (approval number: 2011-KAEK-25 2020/06-23, date: 10.06.2020).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Techinal Assistance: A.D., Data Collection or Processing: S.Ü., O.İ., Analysis or Interpretation: M.İ., G.Ö., Editing: A.G.İ., B.D., Writing: M.İ., D.Ş.

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Fetal arrhythmias: Ten years' experience and review of the literature

Fetal aritmiler: On yıllık deneyim ve literatür taraması

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Abstract

Objective: Fetal arrhythmias complicate 1-2% of all pregnancies. Ultrasound evaluation and Doppler technology are indispensable in both diagnosis and management. Digoxin, sotalol, flecainide and amiodarone are widely accepted antiarrhythmic agents that are frequently. We reviewed the maternal and fetal outcomes in cases with fetal arrhythmia in a tertiary care center in the last decade.

Materials and Methods: Fetal arrhythmias were classified under three main groups: Irregular rhythms, tachyarrhythmia and bradyarrhythmia. Detailed anatomical evaluation and fetal echocardiography were performed in all cases to determine whether a structural cardiac and extracardiac anomaly accompanied fetal arrhythmia and the type of fetal arrhythmia. Digoxin was started primarily as first-line therapy in patients with persistent fetal tachyarrhythmia. In cases, not responding to digoxin, other antiarrhythmic agents (sotalol, flecainide) were combined with treatment without discontinuing digoxin.

Results: Fetal arrhythmia was detected in 36 cases during the study period. 50% (n=18/36) of the cases had supraventricular tachycardia, whereas 28% (n=10/36) of them were fetal bradyarrhythmia and 22% (n=8/36) of them were with various irregular rhythms. Transplacental therapy was initiated in 13 patients with persistent supraventricular tachycardia and atrial flutter regardless of the presence of hydrops. The success rate in transplacental therapy was 77% (n=10/13).

Conclusion: Successful transplacental therapy was achieved in approximately 80% of cases and delivery could be postponed to advanced gestational weeks, confirming the crucial role of this treatment for the management of tachyarrhythmia.

Keywords: Fetal arrhythmia, fetal tachyarrhythmia, fetal bradyarrhythmia, transplacental therapy, hydrops fetalis

Öz

Amaç: Fetal aritmiler tüm gebeliklerin %1-2'sini komplike etmektedir. Ultrason değerlendirmesi ve Doppler teknolojisi hem tanı hem de yönetimde vazgeçilmezdir. Digoksin, sotalol, flekainid ve amiadaron transplasental tedavide sıklıkla kullanılan, yaygın olarak kabul görmüş ajanlardır. Son 10 yılda, üçüncü basamak bir sağlık merkezinde fetal aritmili olgularda maternal ve fetal sonuçları gözden geçirmeyi amaçladık.

Gereç ve Yöntemler: Fetal aritmiler üç ana gruba ayrıldı: Düzensiz ritimler, taşiaritmiler ve bradiaritmiler. Tüm olgularda fetal aritmiye kardiyak yapısal ve ekstrakardiyak anomalinin eşlik edip etmediğini ve fetal aritminin tipini belirlemek için detaylı anatomik değerlendirme ve fetal ekokardiyografi yapıldı. Persistan fetal taşiaritmili olgularda ilk basamak tedavide digoksin başlandı. Digoksine yanıt vermeyen olgularda digoksin kesilmeden diğer antiaritmik ajanlar (sotalol, flekainid) tedaviye kombine edildi.

Bulgular: Çalışma süresi boyunca 36 olguda fetal aritmi tespit edildi. Olguların %50'sinde (n=18/36) supraventriküler taşikardi, %28'inde (n=10/36) fetal bradiaritmi ve %22'sinde (n=8/36) çeşitli düzensiz ritimler vardı. Persistan supraventriküler taşikardisi ve atriyal flatteri olan 13 hastaya hidrops varlığına bakılmaksızın transplasental tedavi başlandı. Transplasental tedavide başarı oranı %77 (n=10/13) idi.

Sonuç: Transplasental tedavinin olguların yaklaşık %80'inde başarılı olduğunu, doğumun ileri gebelik haftalarına ertelenebileceğini ve bu tedavinin taşiaritmi yönetimi için çok önemli rolünün doğruladığını belirtmek önemlidir.

Anahtar Kelimeler: Fetal aritmi, fetal taşiaritmi, fetal bradiaritmi, transplasental tedavi, hidrops fetalis

PRECIS: Especially in fetuses with persistent SVT and AF, successful transplacental treatment is achievable in approximately 80% of cases and delivery can be postponed to advanced gestational weeks in these cases.

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Introduction

The fetal cardiac conduction system is functionally developed at 16 weeks of gestation[1]. Any deviation from normal rhythm and speed is defined as a fetal arrhythmia[2]. Fetal arrhythmias complicate 1-2% of all pregnancies[3]. The vast majority of these arrhythmias are benign. However, arrhythmias such as supraventricular tachycardia (SVT), ventricular tachycardia, atrial flutter (AFL), atrial fibrillation (AF), and atrioventricular block (AVB) might be associated with low cardiac output, heart failure, hydrops, and subsequent fetal loss[4].

Diagnostic modalities such as fetal electrocardiography and magnetocardiography have been developed for the diagnosis of arrhythmias in the past few decades. However, pulse wave Doppler and M-Mode Doppler are more frequently used in clinical practice[5-7]. Ultrasonography is the primarily preferred method for diagnosis and management of arrhythmias because of its ability to enable a detailed analysis of fetal cardiac anatomy. Transplacental treatment is the mainstay of perinatal management in earlier gestational weeks, especially in cases of tachyarrhythmia, unless any maternal contraindication is present[8]. Digoxin, sotalol, flecainide and amiodarone are the most commonly used antiarrhythmic agents for transplacental therapy[9,10]. No standard protocol regarding drug selection, loading, and maintenance dose has yet been established. Additionally, transplacental therapy is almost always individualized based on the dynamically changing maternal and fetal status.

In our study, we retrospectively evaluated the outcomes of pregnancies complicated by fetal arrhythmias at a single tertiary care hospital.

Materials and Methods

Pregnant women with fetal arrhythmias who received prenatal care in the Department of Obstetrics and Gynecology, Ege University Faculty of Medicine Hospital between January 2011 and January 2021 were retrospectively analyzed. Cases which were delivered at a different hospital and without complete records were excluded from the study.

Approval from the Human Ethics Committee of Ege University was obtained and the World Medical Association Declaration of Helsinki was compiled regarding the ethical conduct of this study (approval ID: 20-6.1T/55). Fetal arrhythmias were classified under three main groups: Irregular rhythms, tachyarrhythmia and bradyarrhythmia. Fetal ventricular beats of more than 160 beats/minute (bpm) are defined as fetal tachycardia. Fetal tachycardia is defined as persistent or intermittent depending on whether the tachycardia lasts longer than 50% of the duration of echocardiographic evaluation or less. Sinus tachycardia (ST), SVT and AFL are the main types of fetal tachycardia. ST presents with an atrial rate of 160-200 bpm and 1:1 atrioventricular (AV) conduction rate. SVT is manifested with a heart rate of 220-260 bpm (Rarely as high as 300 bpm) and an AV conduction rate of 1:1 as well. However, AFL presents with an atrial rate of 400-500 bpm and a ventricular rate of 200-220 bpm due to the variety of AV block rates (2:1, 3:1, 4:1).

Fetal bradycardia is defined as an intermittent or persistent heart rate slower than 110 bpm. Sinus bradycardia, persistent block ectopic beats and AV heart blocks are the main causes of fetal bradycardia. Second degree AV block presents with a regular atrial beat at a constant 2:1 AV conduction rate (Mobitz type 2). Third-degree AV block is characterized by a complete lack of interaction between the atria and the ventricles and presence of independent activities of both structures (normal atrial beat, slower ventricular beat). Any deviation in the rhythm with a normal heart rate (110-160 bpm) is defined as an irregular rhythm (premature atrial contraction, premature ventricular contraction, premature junction contraction).

Fetal echocardiography was performed using a Voluson-E8 Expert Scanner and a 4-9 MHz transducer ultrasound device (General Electric Healthcare, Wauwatosa, WI, USA). The ultrasound device was featured with M-Mode, pulse Doppler, color Doppler, and power Doppler functions.

Hydrops fetalis is defined as the presence of abnormal fluid collections in the fetus at least in two different potential spaces, including fluid in serous cavities (e.g., ascites, pleural effusions, pericardial effusions) and generalized skin edema. Cardiovascular profile scoring was performed due to the high risk of heart failure, hydrops and fetal loss in patients with continuous fetal tachyarrhythmia and fetal bradyarrhythmia[11,12].

Digoxin was initiated as the first-line therapy in patients with persistent fetal tachyarrhythmia regardless of the presence of hydrops. The oral digoxin loading dose was between 1 and 2 mg and was administered in three equal doses. Following the loading dose, digoxin level was checked (target values: 1-2 ng/mL) and the maintenance therapy (in the form of 0.5-0.75 mg divided doses) was initiated following a normal result. The fetal response to initial therapy was evaluated in 48-72 hours after the first dose. Second-line therapy was initiated only if no improvement was identified despite adequate digoxin levels (1-2 ng/mL). Second-line agents (sotalol, flecainide) were added to the regimen without discontinuation of digoxin. Flecainide treatment was initiated at 100 mg every eight hours, up to a daily maximum dose of 400 mg. Sotalol (80 mg) was administered every eight hours as well. All patients were referred to cardiology for initial workup before initiation of antiarrhythmic therapy. Maternal cardiac activity was assessed daily with EKG, particularly to monitor QRS and QT lengths.

In patients with maternal antibody positivity (anti-Ro/SSA or anti-LA/SSB), the PR length was evaluated with pulse Doppler starting at 18-week gestation, and 4 mg of dexamethasone treatment was initiated for patients with a PR length greater than 150 msec.

Statistical Analysis

Descriptive statistics are presented. The numerical variables are given as mean, standard deviation, or median (minimum-maximum). The categorical variables are given in numbers and percentages. Because of the sample size and lack of significant results, statistical analysis was not performed and multivariate analysis was not performed.

Results

A review of records revealed 36 fetal arrhythmia cases between January 2011 and January 2021. 50% (n=18/36) of the cases had SVT, whereas 28% (n=10/36) of them were fetal bradyarrhythmia and 22% (n=8/36) of them were with various irregular rhythms.

Most tachyarrhythmia cases consisted of SVT. At the time of diagnosis, hydrops was observed in 39% of all fetal tachyarrhythmia cases. Structural cardiac anomalies were found in only two cases, which were rhabdomyomas. Expectant management was sufficient in this case, and transplacental treatment was not needed. The clinical features of cases with fetal tachyarrhythmia are given in Table 1.

Expectant management was sufficient in 27.8% of fetal tachyarrhythmia cases (ST: 1 and SVT-I: 4) and spontaneous return to sinus rhythm was observed within days (range 1-7 days). First-line therapy (oral digoxin) was initiated in the remaining fetal tachyarrhythmia cases, regardless of the presence of hydrops in the fetus. The success rate in digoxin mono-therapy (first-line therapy) was 38% (n=5/13). In cases with no response to first-line therapy, a second antiarrhythmic agent (sotalol, flecainide) was added to the treatment without discontinuing digoxin. Normal sinus rhythm could not be achieved despite second-line therapy in two patients (1 SVT-P, 1 AFL). These patients delivered because of deteriorating fetal status. Hydrops fetalis was observed in these refractory cases.

Intrauterine fetal demise was identified in one case. This patient was diagnosed with AFL at 27 weeks of gestation (atrial rate 550 bpm, ventricular rate 225 bpm, 2: 1 AV block). Oral digoxin was started initially, followed by digoxin-sotalol combined treatment due to the lack of improvement. The fetal demise occurred on the 6th day of transplacental therapy. The success rate in transplacental therapy was 77%. The follow-up and treatment scheme of fetal tachyarrhythmias are shown in Figure 1 in detail.

In the study population, AV heart blocks were the main cause of fetal bradyarrhythmia. Hydrops fetalis was noted in 40% of the cases with bradyarrhythmia. Structural cardiac anomalies were diagnosed in four cases and maternal antibody positivity (anti-Ro/SSA) was observed in two cases. Eight cases resulted in live birth. Intrauterine fetal demise (AV septal defect, 34 gestational weeks) occurred in two cases. Pregnancy was terminated at 23 weeks of gestation in another case which was complicated by a double outlet right ventricle.

Patients with maternal antibody (anti-Ro/SSA) positivity was followed by PR interval starting at the 18th week, and two cases with a PR interval of 150 ms received corticosteroid therapy. However, complete AV block was identified in both patients.

Table 1. Clinical features of patients with tachyarrhythmia

Parameter	Results
Fetal tachyarrhythmia	
SVT	14/18 (78%)
AFL	3/18 (17%)
ST	1/18 (5%)
Maternal age	28.6±4.1 (22-36)
Hydrops	7/18 (39%)
IUFD	1/18 (5%)
Additional echocardiography findings	2/18 (11%)
GA at referral weeks	30±3.99 (25-36)
Average HR (bpm)	228.94±22.6 (190-283)
CVPS	8.72±1.17 (7-10)
Mean GA at delivery	36.2±2.75 (28-40)
≥37 week	11/18 (61%)
<37 week	7/18 (39%)
Mode of delivery	
CS	14/18 (78%)
VD	4/18 (22%)

Data are given as mean ± standard deviation. Percentage or range is given in parentheses, SVT: Supraventricular tachycardia, AFL: Atrial flutter, ST: Sinus tachycardia, IUFD: Intrauterine fetal demise, GA: Gestational age, HR: Heart rate, bpm: Beats per minute, CVPS: Cardiovascular profile score, CS: Cesarean section, VD: Vaginal delivery

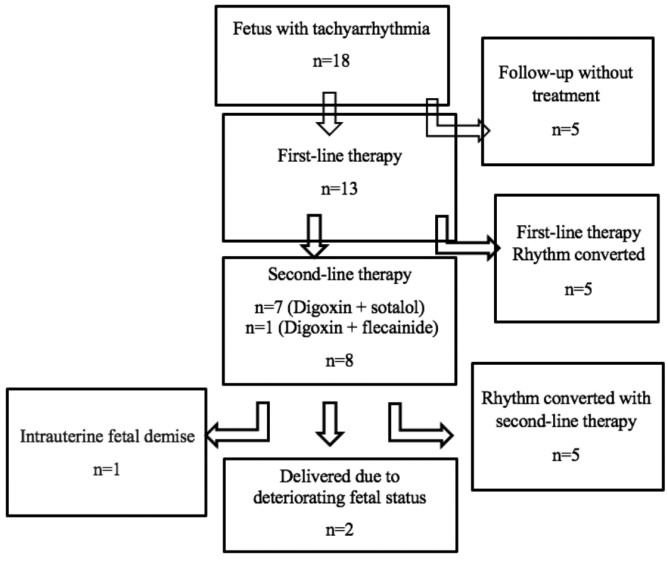

Figure 1. Flow chart of treatment

Detailed information on cases with fetal bradyarrhythmia is given in Table 2.

Irregular rhythms are well tolerated and rarely cause symptoms or progress to a serious disease. Therefore, follow-up visits were planned on a weekly basis following a fetal EKG. No structural cardiac anomaly was observed in any case, and no serious arrhythmia was seen during the follow-up.

Discussion

In our study, the most common cause of fetal arrhythmias was fetal tachyarrhythmias, followed by fetal bradyarrhythmia and irregular rhythms, respectively. In the literature, most fetal arrhythmias are benign and irregular rhythms that do not affect fetal hemodynamics and often spontaneously regress without any further treatment[4]. This relatively low prevalence of irregular rhythms in our study can be explained by inadequate diagnosis or by the fact that obstetricians do not refer these cases to a tertiary care center because of the favorable prognosis of this condition. None of the fetuses with irregular rhythm progressed to SVT in our study. The delivery time and method were determined based on obstetric indications and no premature delivery occurred.

Table 2. Clinical features of patients with bradyarrhythmia

Parameter	Results
Fetal bradyarrhythmia	10
AVB	8/10 (80%)
SB	1/10 (10%)
BABPB	1/10 (10%)
Maternal age	28±5.6 (22-35)
Additional echocardiography findings	4/10 (40%)
Hydrops	4/10 (40%)
CVPS	8.2±1.54 (6-10)
IUFD	1/10 (10%)
Maternal antibody positivity	2/10 (20%)
GA at referral weeks	28±5.6 (22-35)
The average HR (bpm)	70.2±17.75 (46-106)
Termination of pregnancy	1/10 (10%)
Mean GA at delivery	36.8±2.8 (24-39)
Mode of delivery	
CS	7/10 (70%)
VD	3/10 (30%)

Data are given as mean ± standard deviation. Percentage or range is given in parentheses. AVB: Atrioventricular clock, SB: Sinus bradycardia, BABPB: Blocked atrial bigeminy presenting bradycardia, CVPS: Cardiovascular profile score, IUFD: Intrauterine fetal demise, GA: Gestational age, HR: Heart rate, bpm: Beats per minute, CS: Cesarean section, VD: Vaginal delivery

11% of the patients with fetal tachyarrhythmia have structural cardiac anomaly in our study, and this result is consistent with the literature[4]. Hydrops is a strong indicator of arrhythmia that impacts the cardiovascular system by reducing ventricular filling and cardiac output. It also seems to be the most important factor affecting the success of transplacental therapy. Hydrops was observed in 39% of the fetal tachyarrhythmia cases at the time of diagnosis, and this rate was reported to 21% in the study by van der Heijden et al.[11].

Although the knowledge on transplacental treatment of fetal tachyarrhythmia has increased over the past few decades, there is no standard protocol for antiarrhythmic drug selection, loading, or maintenance doses. To date, there is no randomized study has clearly documented the superiority of an antiarrhythmic drug over another. Therefore, the choice of medication to start the treatment should depend on the condition of the mother and fetus as well as the provider preference. In our study, the conversion to sinus rhythm was achieved in 38% of fetuses with digoxin monotherapy. Digoxin monotherapy has been used in first-line therapy in many centers because of its safety and ease of monitoring serum levels. It was found to be effective in approximately 50% of fetal SVT and AFL cases[9,13].

A second antiarrhythmic agent (sotalol-flecainide) was added in cases that failed to improve despite adequate digoxin levels in 48-72 hours. The rate of failure in transplacental therapy was 23% in patients on second-line agents. This rate was reported as 5% in the study of Krapp et al.[14] with digoxin + flecainide combination, and 17% in the study of Oudijk et al.[15] with digoxin + sotalol combination. In our study, digoxin + sotalol (88%, n=7/8) combination was used as the second-line therapy.

Invasive treatment options (fetal intramuscular injection, umbilical vein injection) were not preferred in cases of fetal tachyarrhythmia due to the low efficacy of the treatment, need for multiple interventions and high risk of fetal loss. In fetal tachyarrhythmia cases, the major delivery route was cesarean section (78%), and the mean delivery week was 36.2. The high rate of the cesarean section can be explained by the difficulty of fetal assessment during labor, especially in patients with persistent SVT and AFL.

Ten cases of fetal bradyarrhythmia were observed in our study. Structural cardiac anomalies were diagnosed in 40% of cases and maternal antibody positivity (anti-Ro/SSA positivity) was observed in 20% of cases. In the literature, it has been reported that fetal bradyarrhythmia is accompanied by structural cardiac anomalies in approximately half of the cases, and complete AVB develops in 2-3% of maternal anti-Ro/SSA and anti-La/SSB positivity[16,17]. While two patients with prolonged PR interval received corticosteroid treatment, intrauterine treatment options such as beta-adrenergic agents, immunoglobulin, plasmapheresis were not preferred in other cases due to unclear efficacy and possible maternal risks[18,19].

In our study, approximately one-fourth of the fetal tachyarrhythmia cases did not respond to intrauterine treatment despite all treatment combinations. A new treatment modality or a new antiarrhythmic agent is needed in refractory cases. In cases with fetal bradyarrhythmia, detailed fetal cardiac examination and maternal blood sampling for anti-Ro/SSA and anti-La/SSB antibodies might be helpful to determine the etiology. In cases of fetal bradyarrhythmia of unknown etiology, novel treatment options with low maternal risks may improve fetal outcomes.

Study Limitations

The strength of our study can be stated as the inclusion of all three types of fetal arrhythmia and the contribution to the management. The retrospective nature of this study stands as a limitation. Incomplete data on the postnatal course of the newborns are another limitation of our study.

Conclusion

Our study clearly indicated that successful transplacental treatment was achieved in approximately 80% of cases and delivery could be postponed to later gestational weeks. This is applicable especially in fetuses with persistent SVT and AF. Patients with irregular rhythm have a benign course, and in non-immune bradyarrhythmia, the effectiveness of intrauterine fetal therapy has not been proven with possible maternal risks. Therefore, expectant management with regular follow-up visits should be sufficient in these two sub-groups of patients. Further studies are required to elaborate the mechanisms of fetal arrhythmias and to evaluate treatment options to reduce fetal mortality and morbidity.

Ethics

Ethics Committee Approval: This study was approved by the Local Ethics Committee of the Faculty of Medicine at Ege University (approval ID: 20-6.1T/55).
Informed Consent: Informed consent was obtained from all individual participants included in the study.
Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: H.E., F.Ö., Design: H.E., F.Ö., A.M.E., Supervision: F.Ö., A.M.E., Data Collection or Processing: M.İ., A.G.İ., Analysis or Interpretation: H.E., M.İ., Literature Search: A.G.İ., Writing: H.E., F.Ö.
Conflict of Interest: No conflict of interest was declared by the authors.
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Evaluation of vaginal brachytherapy for treating early-stage endometrial cancer according to the European Society of Medical Oncology 2020 risk stratification

Avrupa Tıbbi Onkoloji Derneği 2020 kılavuzu risk sınıflamasına göre erken evre endometrium kanseri tedavisinde vajinal brakiterapinin etkinliğinin incelenmesi

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Abstract

Objective: The aim was to evaluate vaginal brachytherapy (VB) after surgery in early-stage endometrial cancer.

Materials and Methods: The patients with Stage I-II endometrial adeno-cancer operated between 1998 and 2018 and whose adjuvant therapies had been arranged were evaluated retrospectively.

Results: A total of 618 patients were enrolled. In 409 patients in the low-risk group, the vaginal, pelvic recurrence, and distant metastasis rates were found to be higher in the VB group. When the results of 112 patients in the intermediate-risk group were evaluated, there was no statistically significant difference between the vaginal, pelvic recurrence, and distance metastasis rates. In 89 patients in the intermediate-high risk group, vaginal recurrence rates were 0%, 4.8%, 0%, and 25% for VB, external beam radiotherapy, combination radiotherapy, and the follow-up groups, respectively (p=0.010), and pelvic recurrence rates were found to be 18.2%, 0%, 1.9% and 0% (p=0.036). Distant metastasis rates were 0%, 0%, 9.6% and 0% (p=0.229). When the overall survival in all groups was examined, no significant difference was found between the groups.

Conclusion: In conclusion, no adjuvant treatment is a proper approach for low-risk patients. Brachytherapy can be considered a suitable option for the intermediate risk group. Combined treatments instead of VB in the high-intermediate risk group would be preferred in terms of local control.

Keywords: Brachytherapy, adjuvant radiotherapy, vaginal administration, endometrial cancer

Öz

Amaç: Çalışmada amacımız erken evre endometrium kanserinde vajinal brakiterapinin (VB) etkinliğini araştırmaktır.

Gereç ve Yöntemler: 1998-2018 yılları arasında ESMO kliniğimizde opere edilen ve adjuvan tedavileri düzenlenen Evre I-II endometrioid adeno karsinom tanılı hastalar retrospektif olarak değerlendirilmiştir.

Bulgular: Çalışmaya 618 hasta dahil edildi. Düşük riskli hasta grubunda olan 409 hasta incelendiğinde vajinal, pelvik rekürrens ve uzak metastaz oranları VB grubunda daha yüksek bulundu. Orta risk grubunda olan 112 hastanın sonuçları değerlendirildiğinde vajinal, pelvik rekürrens ve uzak metastaz oranlarında istatistiksel olarak anlamlı bir sonuç bulunmadı. Yüksek-orta risk grubunda olan toplam 89 hastanın VB, dış ışın radyoterapi, kombine radyoterapi ve takip grupları için sırası ile vajinal rekürrens oranları %0, %4,8, %0, %25 (p=0,010), pelvik rekürrens oranları %18,2, %0, %1,9, %0

PRECIS: In this study, using our 20-years data we examined the efficacy of vaginal brachytherapy in early-stage endometrial cancer.

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(p=0,036) olarak bulundu. Uzak metastaz oranları %0, %0, %9,6, %0 idi (p=0,299). Genel sağkalımlar karşılaştırıldığında sırası ile %100, %95,2, %92,3, %75 olarak bulundu (p=0,534).

Sonuç: Beklenenin aksine düşük risk grubunda VB uygulanan hastaların lokal yineleme oranları daha yüksek bulundu. Bu grubun sağkalımları karşılaştırıldığında VB uygulanan hastaların sağkalımlarının istatistiksel anlamlı olmamak ile birlikte daha düşük olduğu görüldü. Orta risk grubundaki hastalarda tedavi grupları arasında rekürrens, uzak metastaz ve genel sağkalım açısından fark saptanmamıştır. Beklenildiği gibi yüksek-orta risk grubunda ise takip ve brakiterapi uygulanan hastalarda istatistiksel anlamlı olarak yüksek lokal yineleme gözlenmiştir. Bu grup hastalarda dış ışın radyoterapi ya da kombine tedavi seçeneği tercih edilmelidir.

Anahtar Kelimeler: Brakiterapi, adjuvan radyoterapi, vajinal uygulama, endometrium kanser

Introduction

The adjuvant therapy approach has changed over time in early-stage endometrial cancer. This approach began with combination therapies like external beam radiotherapy (EBRT)+vaginal brachytherapy (VB), which has left its place to no adjuvant or single modality therapies. The indication for adjuvant therapy is based on the evaluation of clinicopathological prognostic factors, including age, grade, stage of the disease, myometrial invasion, and lymphovascular space invasion (LVSI). These factors guide predicting the likelihood of disease recurrence after surgery. In patients with low-risk endometrial cancer (stage 1 endometrioid, grade 1-2, <50% myometrial invasion, LVSI negative), adjuvant treatment is not recommended[1]. A randomized trial of 645 patients with low-risk endometrial cancer treated with brachytherapy also showed no advantage for the use of adjuvant VB[2]. Large randomized trials with patients are considered intermediate risk, and a meta-analysis by Kong et al.[3] found that EBRT reduced pelvic recurrence but had no benefit on overall survival[4,5]. These trials identified a subgroup of patients who benefited the most from adjuvant EBRT, named as the high-intermediate risk group. Only in this subgroup was the risk of relapse was observed to be high enough to consider adjuvant radiotherapy (RT)[4]. In patients with intermediate risk endometrial cancer (stage1 endometrioid, grade 1-2, >50% myometrial invasion, LVSI negative) adjuvant brachytherapy is recommended[1]. However, not performing routine adjuvant RT is also an alternative approach[6]. A recent pool data analysis from the PORTEC-1 and PORTEC-2 trials showed that both LVSI and grade 3 are risk factors for distant metastasis and/or or regional nodal recurrence[7]. In patients who have high-intermediate risk endometrial cancer, adjuvant VB is recommended to decrease vaginal recurrence and adjuvant EBRT is recommended for LVSI unequivocally positive to reduce pelvic recurrence[1].

Materials and Methods

In our study, patients diagnosed with Stage I and Stage II endometrioid adenocarcinoma underwent surgery in our clinic between 1998 and 2018, and adjuvant therapy was decided in the multi-disciplinary tumor council and was retrospectively evaluated. Ethics committee approval was obtained from the Health Sciences University İzmir Tepecik Training and Research Hospital Ethics Committee (decision no: 2020/12-44, date: 12.10.2020). Our study was conducted following the ethical standards described in the 1975 Declaration of Helsinki, as revised in 2000.

The inclusion criteria were being over 18 years of age, Stage I-II cancer with endometrioid histology, and not having received adjuvant chemotherapy. The exclusion criteria were history of concomitant or past malignancy, non-endometrioid histology, advanced-stage disease, patients who received adjuvant chemotherapy, and those without follow-up data that could not be accessed. Risk classification was based on the 2020 European Society for Medical Oncology (ESMO)/European Society of Gynaecological Oncology/European Society for Radiotherapy & Oncology guideline[6]. Table 1 presents the summary of the risk classification created based on the guideline.

After endometrial biopsy and radiological staging, all patients underwent routine hysterectomy and bilateral salpingo-oophorectomy. Lymph node assessment was made according to pre-operative imaging and intraoperative frozen section results. The surgical stage was performed according to the International Federation of Gynecology and Obstetrics 2009 classification[8].

Table 1. Risk classification according to the 2020 ESMO/ESGO/ESTRO guideline

Low risk	-Patients in Stage 1A endometrioid, low grade and not having lymphovascular invasion (or local lymphovascular invasion)
İntermediate risk	-Patients in Stage 1B endometrioid, low grade and not having lymphovascular invasion (or local lymphovascular invasion) -Patients in Stage 1A endometrioid, high grade and not having lymphovascular invasion (or local lymphovascular invasion) -Stage 1A non-endometrioid tumor, no myometrial invasion
High-intermediate risk	-Patients with Stage 1 endometrioid lymphovascular invasion (irrespective of grade and myometrial invasion) -Patients with Stage 1B endometrioid, high grade (irrespective of lymphovascular invasion) -Patients in Stage 2

ESMO: European Society for Medical Oncology, ESGO: European Society of Gynaecological Oncology, ESTRO: European Society for Radiotherapy & Oncology

Adjuvant treatment was determined at the multi-disciplinary gynecological oncology tumor council. According to the final pathology report, patients have suggested no adjuvant treatment, VBT, EBRT, or combination therapy (EBRT+VB).

Patients who had received radiotherapy before 2004 were treated with 2-dimensional radiotherapy, and those who had received radiotherapy after 2004 were treated with 3-dimensional conformal RT and intensity-adjusted RT technique. Pelvic external RT was administered as a total of 45-50.4 Gy/1.8 Gy daily. Brachytherapy was applied at a dose of 6-7 Gy daily in 1-3 fractions. The patients were followed up once every three months during the first two years, every six months during the following five years, and once yearly for up to 10 years. When required, the patients underwent vaginal examination, ultrasound examination, and recurrences were evaluated with positron emission tomography-computed tomography, magnetic resonance imaging, and biopsy. In our study, the mean postoperative follow-up period of the patients was 130.29±14.41 months.

The study's primary endpoint was to determine vaginal, pelvic recurrence, or distant metastases in patients undergoing brachytherapy compared with other adjuvant therapy options. The secondary endpoint was to evaluate survival according to the risk groups.

Statistical Analysis

Continuous data are given as mean ± standard deviation. Categorical data are given as a percentage (%). Shapiro-Wilk test was used to investigate the suitability of the data for normal distribution. In the comparison of normally distributed groups, independent sample t-test analysis was used for cases with two groups, and One-Way ANOVA for cases with three or more groups. The Mann-Whitney U test was used for cases with two groups and the Kruskal-Wallis H test for cases with three or more groups in the comparison of groups that did not conform to the normal distribution. Pearson chi-square and Pearson Exact chi-square analyzes were used in the analysis of the created cross tables. Survival Analyzes were used to calculate and compare lifespans. IBM SPSS Statistics 21.0 (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.) program was used in the analysis. A value of $p<0.05$ was accepted as a criterion for statistical significance.

Table 2. Demographic information

	n	%
Stage		
IA	439	71.0
IB	145	23.5
II	34	5.5
Lymphovascular invasion		
Negative	559	90.5
Poisitive	59	9.5
Age		
<60	399	64.6
≥60	219	35.4
Grade		
1	339	54.9
2	247	40
3	32	5.1

Results

The data of 618 patients who met the inclusion criteria were analyzed. The median duration of follow-up was 72 months (36-132), and the median age was 56 (50-62). Hysterectomy was performed in all patients. Of the cases, 523 underwent abdominal, 84 underwent laparoscopically, 8 underwent robotically and 2 underwent vaginal hysterectomy. Laparotomy was completed in 6 of the 84 patients who had undergone laparoscopy. Five patients underwent ovarian sparing surgery. Median 4500 cGy (4500-5040) adjuvant external RT and 1800 cGy (1300-2100) brachytherapy was administered.

The distribution of demographic information and the prognostic characteristics according to the adjuvant treatment options is presented in Table 2 and Table 3.

In our study, 618 early-stage patients were compared according to the risk stratification and treatment groups.

A comparison of low-risk patients according to treatment groups is given in Table 4.

When the treatment results were compared in the low-risk group, vaginal recurrence, pelvic recurrence, distant metastasis, and total recurrence rates were significantly higher in the VB group (p=0.007 for vaginal recurrence, p=0.027 for pelvic recurrence, p=0.034 for distant metastasis). The overall survival rates were found to be 94.9%, 97.8%, 100% and 98.3% (p=0.567).

The evaluation of patients in the intermediate-risk group by treatment groups is presented in Table 5.

When the recurrence rates of patients were compared according to the treatment groups between intermediate-risk patients, no pelvic recurrence was observed in this group, and no statistically significant difference was observed. The overall survival rates were 100%, 97.8%, 93.9%, 80%, respectively (p=0.223). The follow-up group had the shortest survival.

The treatment groups' evaluation of the high-intermediate risk patients is been presented in Table 6.

The vaginal recurrence rate was 4.8% and 25% in the EBRT and follow-up groups, respectively (p=0.01), and the pelvic recurrence rate was 18.2% in the VB group, in the high intermediate risk group the difference was statistically significant (p=0.036). When the overall survival rates were compared, they were 100%, 95.2%, 92.3%, and 75% for VB, EBRT,

combination therapy, and the follow-up groups, respectively (p=0.534). In the intermediate-high risk group, significantly higher recurrence rates were observed in the follow-up and VB groups.

When the recurrence rates were evaluated according to age, as a cut of 60 years, there was no statistical significance in terms of vaginal and pelvic recurrence (p=0.702 and p=0.671 for vaginal and pelvic recurrence, respectively). However, the distant metastasis rate was significantly higher in patients greater than or equal to 60 years (1.3% vs. 5%, p=0.01).

As the prognostic factors such as age, grade, stage, presence of LVSI for overall survival and disease-free survival were analyzed, the existence of lymphovascular invasion was the most influential variable on overall survival (hazard ratio: 5.855, 95% confidence interval, p<0.001). When the same data were analyzed for disease-free survival, stage and tumor grade were the most influential variables.

Discussion

According to our results, adjuvant therapy did not provide additional benefits in low-risk patients, similar to the literature. In a prospective study by Sorbe et al.[2] comparing VB and no adjuvant treatment groups in low-risk patients, no significant difference was observed in terms of recurrence and overall survival. In our study, no adjuvant treatment decision was taken in 72% (n=294), and VB was applied to 14.5% (n=59) of low-risk group patients, and vaginal, pelvic recurrence, and distant metastasis rates in the VB group were found to be significantly higher. That is because of unbalanced randomization between the groups due to the retrospective nature of the data. This is a limitation of our study. While 84.7% of the patients in the VB group had grade 2 disease, this rate was 16% in the no adjuvant treatment group. This may be a reason for high recurrence rates in the brachytherapy arm. Additionally, the biological equivalent dose of VB used in the study of Sorbe seems higher than that in our research. This was evaluated as one of the influencing factors even though it was insufficient to explain the difference.

The PORTEC-1 and the GOG-99 studies investigated the effectiveness of adjuvant radiotherapy for intermediate early-stage endometrial cancer[4,5]. These studies were designed to evaluate the effectiveness of pelvic radiotherapy according to recurrence. According to the PORTEC-1 research, while the local recurrence rates were observed to decrease significantly in patients who had received pelvic radiotherapy (14% vs. 4%), no significant difference was found in the overall survival (81% in the radiotherapy group, 85% in the control group). The treatment-related toxic effects were observed at a rate of 25% in the radiotherapy group; this rate was 6% in the control group[4]. The results of the GOG-99 study also supported these data. Twenty-four-month cumulative outcomes were compared while the recurrence rate was 3% in the radiotherapy group, 12% recurrence was observed in the group not receiving radiotherapy, and the difference was statistically significant

Table 3. Prognostic characteristics

	VBT (n=97)	EBRT (n=115)	VBT + EBRT (n=94)	No adjuvant treatment (n=303)	p
Stage					
IA	64 (66%)	62 (54%)	12 (13%)	296 (98%)	<0.001
IB	33 (34%)	51 (44%)	52 (55%)	6 (2%)	
IC	0 (0%)	2 (2%)	30 (32%)	1 (0%)	
Grade					
1	16 (16%)	40 (35%)	28 (30%)	251 (83%)	<0.001
2	78 (80%)	61 (53%)	54 (57%)	51 (17%)	
3	3 (3%)	14 (12%)	12 (13%)	1 (0%)	
LVI					
Positive	11 (11%)	18 (16%)	25 (27%)	3 (1%)	<0.001
Negative	86 (89%)	97 (84%)	69 (73%)	300 (99%)	
Risk classification					
High-intermediate	11 (11%)	21 (18%)	52 (55%)	4 (1%)	<0.001
Intermediate	27 (28%)	47 (41%)	33 (35%)	5 (2%)	
Low risk	59 (61%)	47 (41%)	9 (10%)	294 (97%)	
Age	61.0 (54.0-64.0)	57.0 (52.0-62.5)	57.0 (53.0-63.0)	54.0 (48.0-59.0)	<0.001

EBRT: External beam radiotherapy, VB: Vaginal brachytherapy

Table 4. Distribution of low-risk patients

n=409	VB (n=59)	EBRT (n=47)	VB+EBRT (n=9)	No adjuvant treatment (n=294)	p
Age (median)	52.00 (50-62)	60.00 (54-66)	64.00 (52-64)	57.00 (52-63)	0.149
Grade					
1	9 (15.3%)	9 (19.1%)	2 (22.2%)	247 (84.0%)	<0.001
2	50 (84.7%)	38 (80.9%)	7 (77.8%)	47 (16.0%)	
3	(0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Lymp node dissection					
Without	8 (13.6%)	10 (21.3%)	2 (22.2%)	61 (20.7%)	0.634
With	51 (86.4%)	37 (78.7%)	7 (77.8%)	233 (79.3%)	
Vaginal recurrence	3 (5.1%)	0 (0.0%)	0 (0.0%)	1 (0.3%)	0.007
Pelvic recurrence	2 (3.4%)	1 (2.1%)	0 (0.0%)	0 (0.0%)	0.027
Distant metastasis	3 (5.1%)	0 (0.0%)	0 (0.0%)	2 (0.7%)	0.034
Overall recurrence	8 (13.6%)	1 (2.1%)	0 (0.0%)	3 (1.0%)	0.001
5-years survival	58 (98%)	47 (100%)	9 (100%)	294 (99%)	0.536
Overall survival	56 (94.9%)	46 (97.8%)	9 (100%)	289 (98.3%)	0.567

EBRT: External beam radiotherapy, VB: Vaginal brachytherapy

Table 5. Distribution of intermediate-risk patients

(n=112)	VBT (n=27)	EBRT (n=47)	VBT+EBRT (n=33)	Noadjuvant treatment (n=5)	p
Age (median)	58.00 (53.00-60.00)	53.00 (50.50-55.50)	56.50 (50.25-61.25)	51.00 (34.00-51.00)	0.107
Grade					
1	6 (22.2%)	29 (61.7%)	11 (33.3%)	2 (40.0%)	<0.001
2	19 (70.4%)	11 (23.4%)	22 (66.7%)	3 (60.0%)	
3	2 (7.4%)	7 (14.9%)	0 (0.0%)	0 (0.0%)	
Lymp node dissection					
Without	6 (22.2%)	8 (17.0%)	4 (12.1%)	2 (40.0%)	0.426
With	21 (77.8%)	39 (83.0%)	29 (87.9%)	3 (60.0%)	
Vaginal recurrence	1 (3.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.365
Pelvic recurrence	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	-
Distant metastasis	0 (0.0%)	1 (2.1%)	3 (9.1%)	0 (0.0%)	0.222
Overall recurrence	1 (3.7%)	1 (2.1%)	3 (9.1%)	0 (0.0%)	0.470
5-years survival	25 (100%)	47 (100%)	32 (97%)	4 (80%)	0.110
Overall survival	27 (100%)	46 (97.8%)	31 (93.9%)	4 (80%)	0.223

EBRT: External beam radiotherapy, VB: Vaginal brachytherapy

(p<0.01). The forty-eight-month overall survival rates were reported to be 92% in the RT group and 86% in the non-RT-receiving group (p=0.55)[5]. The number of patients followed in the intermediate-risk group was small in our study. Although this was a limitation of the study, not finding a difference between the treatment groups was consistent with the literature. After the GOG-99 and PORTEC-1 trials, for the intermediate risk group, radiation therapy was evaluated as an effective treatment. The effectiveness of VB and pelvic radiation therapy in the high- intermediate-risk group was compared in the

Table 6. Distribution of high-intermediate risk group patients

(n=89)	VBT (n=11)	EBRT (n=21)	VBT+EBRT (n=52)	No adjuvant treatment (n=4)	p
Age	61.50 (55.00-61.50)	52.00 (49.00-63.50)	58.00 (51.00-63.00)	50.00±6.16 -	0.559
Grade					
1	1 (9.1%)	2 (9.5%)	15 (28.8%)	2 (50.0%)	0.169
2	9 (81.8%)	12 (57.1%)	25 (48.1%)	1 (25.0%)	
3	1 (9.1%)	7 (33.3%)	12 (23.1%)	1 (25.0%)	
Lymp node dissection					
Without	2 (18.2%)	3 (14.3%)	11 (21.2%)	1 (25.0%)	0.909
With	9 (81.8%)	18 (85.7%)	41 (78.8%)	3 (75.0%)	
Vaginal Recurrence	0 (0.0%)	1 (4.8%)	0 (0.0%)	1 (25.0%)	0.010
Pelvic Recurrence	2 (18.2%)	0 (0.0%)	1 (1.9%)	0 (0.0%)	0.036
Distant metastasis	0 (0.0%)	0 (0.0%)	5 (9.6%)	0 (0.0%)	0.299
Overall recurrence	2 (18.2%)	1 (4.8%)	6 (11.5%)	1 (25.0%)	0.541
5-years survival	11 (100%)	20 (95%)	47 (94%)	3 (75%)	0.332
Overall survival	11 (100%)	20 (95%)	46 (92.3%)	3 (75%)	0.534

PORTEC-2 study[9]. In the 5-year results of this study, vaginal and pelvic recurrence rates were not statistically significant between the groups. No difference was observed in the distant metastasis rates and survival. Wortman et al.[10] reported the 10-year outcomes of the study, and no differences were observed between the groups in local recurrence, distant metastasis, and overall survival. When the results of our study were analyzed, no significant difference was observed in the disease-free survival and the overall survival between VB, EBRT, combination therapy, and the follow-up groups in high-intermediate-risk patients.

When we examined the results of high-intermediate-risk patients, the presence of vaginal recurrence in the EBRT group and pelvic recurrence in the VB group brought the question of whether we should choose combination therapy for these patients. No significant difference was observed in distant metastasis or overall survival. However, the highest distant metastasis rate found in the combination therapy group was striking. The high rate of grade 3 disease in this group may have increased the distant metastasis risk.

In a retrospective study by Jin et al.[11], VB and combination therapy were compared in the high-intermediate and the high-risk groups of patients according to the 2016 ESMO guideline, and no significant difference was not observed between the groups in overall survival. The study did not conduct a subgroup analysis between the two risk groups. In our study, the intermediate and high-intermediate risk groups were evaluated individually. This may explain the higher recurrence rates in the high-intermediate risk group. Additionally, the inclusion of Stage II patients according to the new classification system[12] who received combination therapy may be the other reason to explain the high recurrence rate.

The most critical step in deciding on adjuvant therapy is being able to determine the recurrence risk. The multi-variable analysis performed age, grade, LVSI, and stage. While stage and grade were the most important variables for disease-free survival, lymphovascular space involvement was the most influential variable for overall survival. In the PORTEC-1 study, lymphovascular space was not evaluated, and age was the most important risk factor. In the PORTEC-2 study, which included patients above 60 years, lymphovascular invasion was reported to be the most critical factor for local recurrences. In the variation analysis by Jin et al.[11], tumor grade was found to be the most important variable for disease-free and overall survival.

In the study by Cisek et al.[13], the age above 70 years was the most important variable for overall survival; and stage was the most important variable for disease-free survival. Our study determined a statistically significant difference for only distant metastasis in the group above 60.

Study Limitation

This article is a retrospective article based on 20 years of data. In this period, diagnostic methods used and possible method and device differences in radiotherapy may adversely affect the reliability of the data. We revised these data to current clinicopathological parameters but could not use molecular parameters. Additionally, in this period, diagnostic methods

used and possible method and device differences in radiotherapy may adversely affect the reliability of the data.

Conclusion

In conclusion, no adjuvant treatment is an appropriate approach for low-risk patients. VB did not provide an additional benefit for these patients.

In the intermediate-risk group, VB seems to be a preferable option as EBRT and combination radiation therapy exhibited similar results.

In the high-intermediate risk group, VB alone was insufficient compared with treatment modalities. In these patients, selecting combination therapies may be an appropriate approach for local control.

We believe that there will be changes in the decision of adjuvant treatment of endometrial cancer with further studies evaluating age, clinicopathological data, genetic characteristics and immunohistochemical data together.

Ethics

Ethics Committee Approval: Ethics committee approval was obtained from the Health Sciences University İzmir Tepecik Training and Research Hospital Ethics Committee (decision no: 2020/12-44, date: 12.10.2020).

Informed Consent: Retrospective study.

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Authorship Contributions

Surgical and Medical Practices: D.C.Ö., Z.G., M.Ş., Concept: D.C.Ö., Z.G., M.Ş., Design: D.C.Ö., Z.G., M.Ş., Data Collection or Processing: D.C.Ö., Z.G., M.Ş., Analysis or Interpretation: D.C.Ö., Z.G., M.Ş., Literature Search: D.C.Ö., Z.G., M.Ş., Writing: D.C.Ö., Z.G., M.Ş.

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Systematic review and meta-analysis of the efficacy of acupuncture as an adjunct to IVF cycles in China and the world

Akupunkturun Çin ve dünyada IVF döngülerine yardımcı olarak etkinliğinin sistematik incelemesi ve meta-analizi

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Abstract

Acupuncture has been introduced as an adjuvant therapy to in vitro fertilization (IVF) cycles in many randomized controlled trials (RCTs). However, there has been a debate among trials regarding the effectiveness and safety of the procedure. To determine how effective and safe acupuncture is as an adjunct to IVF cycles for primary and secondary female infertility. We conducted a literature search for relevant RCTs and ultimately included nine studies. The main selected outcomes included the rates of clinical pregnancy, ongoing pregnancy, miscarriage, live birth, and side effects. Patients receiving acupuncture were grouped together regardless of the acupuncture points used or the protocol for the insertion of needles. We performed a subgroup analysis according to whether studies originated inside and outside China to investigate the results of the different RCTs. We pooled outcomes as a risk ratio (RR) with 95% confidence interval (CI). The analysis revealed that in China, acupuncture led to lower clinical [RR=0.80, 95% CI (0.66, 0.97), p=0.02] and ongoing [RR=0.78, 95% CI (0.63, 0.97), p=0.03] pregnancy rates than placebo. Outside China, acupuncture increased clinical pregnancy rates [RR=1.38, 95% CI (1.11, 1.71), p=0.003] and ongoing [RR=1.73, 95% CI (1.29, 2.31), p<0.001] pregnancy rates. Rates of live birth and miscarriage did not significantly differ between the arms. Regarding side effects, acupuncture groups had a significantly higher rate of puncture site itching compared to control groups [RR=1.51, 95% CI (1.12, 2.04), p=0.007]. Overall analysis does not show a statistically significant increase in clinical pregnancy rates worldwide when using acupuncture as an adjunct therapy to IVF. There were no issues regarding patient safety from any included study. Subgroup results indicated that better rates for clinical pregnancy seem to be occurring more often in RCTs performed outside China than within.

Keywords: Acupuncture, Chinese medicine, in vitro fertilization, traditional medicine, traditional Chinese medicine, alternative medicine, integrative medicine

Öz

Akupunktur, birçok randomize kontrollü çalışmada (RKÇ) in vitro fertilizasyon (IVF) döngülerine adjuvan bir tedavi olarak sunulmuştur. Ancak, prosedürün etkinliği ve güvenliği ile ilgili çalışmaların tartışmalı sonuçları mevcuttur. Biz bu sistematik inceleme ve meta-analizde, primer ve sekonder kadın kısırlığında IVF döngülerine ek olarak akupunkturun ne kadar etkili ve güvenli olduğunu belirlemek istedik. İlgili RKÇ'ler için bir literatür taraması yaptık ve sonuçta 9 çalışmayı dahil ettik. Seçilen ana sonlanımlar, klinik gebelik, devam eden gebelik, düşük, canlı doğum ve yan etki oranlarını içermekteydi. Akupunktur alan hastalar, kullanılan akupunktur noktalarından veya iğnelerin yerleştirilmesi protokolünden bağımsız olarak gruplandırıldı. Farklı RKÇ'lerin sonuçlarını araştırmak için çalışmaların Çin içinden mi yoksa Çin dışından mı kaynaklandığına göre bir alt grup analizi yaptık. Sonuçları %95 güven aralığı (GA) ve bir risk oranı (RR) şeklinde havuzladık. Analiz, Çin'de akupunkturun plasebo ile kıyaslandığında daha düşük oranda klinik gebeliğe [RR=0,80, %95 GA (0,66, 0,97), p=0,02] ve devam eden gebeliğe [RR=0,78, %95 GA (0,63, 0,97), p=0,03] yol açtığını ortaya koydu. Çin dışında akupunktur klinik gebelik oranlarını [RR=1,38, %95 GA (1,11, 1,71), p=0,003] ve devam eden gebelik oranlarını [RR=1,73, %95 GA (1,29, 2,31), p<0,001] artırdı. Canlı doğum ve düşük oranları kollar arasında anlamlı farklılık göstermedi. Yan etkilerle ilgili olarak, akupunktur gruplarında, kontrol gruplarına kıyasla anlamlı olarak daha fazla

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oranda ponksiyon yerinde kaşıntı vardı [RR=1,51, %95 GA (1,12, 2,04), p=0,007]. Genel analiz, IVF'ye ek tedavi olarak akupunktur kullanıldığında dünya çapında klinik gebelik oranlarında istatistiksel olarak anlamlı bir artış olmadığını göstermektedir. Dahil edilen hiçbir çalışmada hasta güvenliği ile ilgili herhangi bir sorun yoktu. Alt grup sonuçları, klinik gebelik için daha iyi oranların Çin dışında gerçekleştirilen RKÇ'lerde daha sık ortaya çıktığını gösterdi.

Anahtar Kelimeler: Akupunktur, Çin tıbbı, tüp bebek, geleneksel tıp, geleneksel Çin tıbbı, alternatif tıp, bütünleştirici tıp

Introduction

Infertility is defined as the failure of a couple to achieve pregnancy after 12 months of attempting. A recent analysis showed that 48.5 million couples are suffering from infertility worldwide, with a global incidence of 9-18%[1,2]. The causes for infertility vary widely, with polycystic ovarian syndrome, and advanced maternal age being some of the more common causes, and secondary infertility being more common than primary[3-5]. Even with extensive workup, greater than 15% of couples suffering from secondary infertility will not find a cause[6].

As treatment, the rate of couples seeking assisted reproductive technology (ART), particularly in vitro fertilization (IVF), has been increasing since the development of these technologies[7]. Although the cost of these procedures has been decreasing, they are still unaffordable for many couples in many countries[8].

Due to the high cost of IVF in developed countries, low-cost complimentary procedures that may increase the efficacy of IVF cycles are widely sought after[9,10].

In Chinese medicine, acupuncture is a well-known therapeutic approach that depends on the insertion of fine special needles at certain points of pressure through the human body. Several studies have proven the benefits of acupuncture in limited circumstances, including improvement of digestive and emotional health, therefore the risk to infertile couples seem minimal[11]. Complications with acupuncture are exceedingly rare, with sporadic reports in the literature of pneumothorax and nerve injury[11]. As for the benefits related to reproductive organs, increased blood flow to the female reproductive organs is seen through the application of acupuncture in the lower limbs and parts of the abdomen; therefore, increasing and enriching the lining of the uterus[11]. Additionally, as inferred indirectly through laboratory analysis, several authors have postulated that acupuncture can increase ovarian follicle reserve[11,12]. As a result, acupuncture has become one of the most popular complementary therapies to IVF, used widely by couples hoping to increase their chance of success[13].

Several trials have attempted to study the effect of acupuncture on IVF cycles[14,15]. In 2020, Coyle et al.[16] conducted a systematic review and meta-analysis on the role of acupuncture versus placebo acupuncture in IVF. Building on the work of previous researchers, we endeavored to create the broadest systematic review to date, by including many new randomized controlled trials (RCTs), and including an analysis of adverse events that has not previously been studied.

Materials and Methods

To perform this systematic review and meta-analysis, the authors followed the preferred reporting items for systematic reviews and meta-analyses (PRISMA) statement[17]. All the steps were performed in strict accordance with the Cochrane Handbook of systematic reviews of interventions[18].

Eligibility Criteria

Retrieved studies were marked as included if they met the following inclusion criteria: 1) studies that are RCTs, 2) population: Women with primary or secondary infertility, 3) intervention: Acupuncture as an adjuvant to IVF, 4) comparator: Placebo acupuncture, sham acupuncture, or no intervention, and 5) outcome: Clinical pregnancy, ongoing pregnancy, live birth, rate of miscarriage, and side effects. The following studies were excluded: 1) non-RCTs, 2) women receiving acupressure, laser acupuncture, or other types of acupuncture that excluded needle penetration of the skin, 3) other comparator arms than placebo acupuncture, sham acupuncture, or laying quietly, and 4) studies with no accessible data, conference abstracts, and animal studies.

Literature Search

We searched the following databases for published articles from inception to February 2020: EBSCO, PubMed, Embase, COCHRANE, MEDLINE, the Menstrual Disorders and Subfertility Group (MDSG, Specialized Register), and Clinical Trial registers (CENTRAL, clinicaltrial.gov, and the WHO International Clinical Trials Registry Platform). We also searched opengrey.eu for gray literature and Google Scholar for additional sources. No language restrictions were applied.

We used a combination of medical subject headings and text words to include a) acupuncture studies: "acupuncture", "moxibustion", "TCM", "traditional Chinese medicine", "electroacupuncture", "electro-acupuncture"; and b) the intervention: "IVF", "in vitro fertilization", "in vitro fertilization", "assisted reproduction technologies", "ART", "embryo transfer", "ET". Supplemental Figure S1 shows the PRISMA flowchart for our literature search. We developed the following search strategy for all databases: (acupuncture OR moxibustion OR "traditional Chinese medicine" OR electroacupuncture OR "electro-acupuncture") AND (IVF OR "in vitro fertilization" OR "assisted reproduction technologies" OR "embryo transfer").

Data Collection and Analysis

Screening of Results

Following the literature search of selected databases, eligible studies and relevant controlled trials were exported and

screened in two steps. The first step involved title and abstract screening to exclude other study designs and animal trials. The second step involved full-text screening to ensure that the controlled trials met the inclusion criteria. After screening and reaching the full-text of the included papers, we performed an additional step through searching the references of the studies for possible missed trials.

Data Extraction

After a thorough reading of the included trials, we used Microsoft Excel for extracting data. Extracted data included three main groups: 1) baseline characteristics of participants, 2) data for outcomes to be incorporated in the analysis, 3) data for the assessment of the risk of bias among trials. Demographic data included patients' age, infertility duration, sample size, country, number of retrieved oocytes, percentage of patients with primary infertility, and body mass index. Outcome analysis included clinical pregnancy as the primary outcome, in addition to other secondary outcomes such as ongoing pregnancy, live birth, rate of miscarriage, and side effects.

Quality Assessment

Only RCTs were included to ensure high-quality evidence. We used Cochrane's risk of bias tool[19] for the assessment of the risk of bias. The Cochrane risk of bias tools works by assessing the risk of bias over seven stated domains. These domains include the proper randomization of patients (Domain #1), the blinding of the allocation of patients into the study's treatment arms (also called allocation concealment) (Domain #2), whether the intentional blinding of patients only (also termed single blinding) or the intentional blinding of both personnel and participants (also termed double-blinding) was used (Domain #3), Attrition bias (Domain #4), whether the outcomes used in the protocol are all reported (also termed selection bias) (Domain #5), blinding of outcome assessors to prevent underestimation or overestimation of outcome values (Domain #6), and other bias (Domain #7). Each domain was judged as a low, high, or unclear risk of bias.

Data Synthesis

Dichotomous data were pooled as a risk ratio (RR) and 95% confidence intervals (CI). We used Review Manager Software (Version 5.3) to perform the data analysis[20]. We used the method of inverse variance for the analysis of continuous outcomes and the Mantel-Haenszel method for the analysis of dichotomous outcomes. We used two main tests to measure inconsistency among the studies[21]. These tests included the I-square test (I^2) and the p-value of the chi-square test. Values of $I^2>50\%$ and $p<0.1$ were considered significant identifiers of heterogeneity, as found within the Cochrane Handbook[18]. We analyzed homogeneous data under a fixed-effects model. As recommended by the Cochrane Handbook, a random-effects model was employed when necessary to solve for heterogeneous data. We performed a subgroup analysis according to the country in which each RCT was conducted and included two main groups, China group, and the outside China group.

Results

Summary of Included Studies

This review presents the analysis of 3.020 patients (1.515 and 1.505 were allocated to the acupuncture and control group, respectively). The mean age of patients in the acupuncture group was 34.3 years, and 34.5 in the control groups. A total of 990 patients (489 in the intervention arm, and 501 in the control group) had not experienced any prior IVF cycles, and an average of 10.3 oocytes was retrieved from the patients. All studies included the previously described acupuncture procedures and acupoints, and were performed by appropriately licensed or trained personnel. There was a variation in chosen acupoints and temporal relationship to the planned IVF cycle.

Acupoints Used in the Included Trials

Regarding the included trials, Paulus et al.[22] were the first to conduct a randomized controlled trial comparing acupuncture with placebo in women receiving IVF cycles. The study included 160 patients and found acupuncture to be effective in increasing pregnancy rates ($p<0.01$). The following points were chosen for needle insertions before embryo transfer: Cx6 (Neiguan), Sp8 (Diji), Liv3 (Taichong), Gv20 (Baihui), and S29 (Guilai). After embryo transfer, the following points were selected: S36 (Zusanli), Sp6 (Sanyinjiao), Sp10 (Xuehai), Li4 (Hegu), ear point 55 (Shenmen), ear point 58 (Zhigong), ear point 22 (Neifenmi), and ear point 34 (Naodian). Dieterle et al.[23] included 116 patients in the acupuncture group, and 109 in the control group, and found that acupuncture increased clinical pregnancy rates ($p<0.01$). The needles were inserted at the following points: Guanyuan [ren (RN)4], Qihai (RN6), Guilai [stomach (ST)29], Neiguan [pericardium (PC)6], Xuehai [spleen (SP)10], and Diji (SP8). During the same year, two additional trials were initiated. Smith et al.[24] performed a similar trial in Australia with the same points as Paulus et al.[22], except for liver 4 and governing vessel 20, which were excluded. The study found no significant differences between the groups ($p=0.08$). Westergaard et al.[25] conducted a trial in Denmark and found that acupuncture leads to more pregnancy rates ($p<0.01$) using the same acupuncture points as Paulus et al.[22]. Later studies initiated by Domar et al.[26] and Andersen et al.[27] found no significant effect of acupuncture ($p=0.69$ and $p>0.05$ respectively) depending on the same acupuncture points as Paulus et al.[22]. Contrary to the previous results, two controlled trials were initiated in China by So et al.[28,29], the trials found that acupuncture has no effect on increasing clinical pregnancy rates [95% CI (0.898, 1.561)] and $p=0.06$ respectively. The points of acupuncture were similar to the previous trials, including PC6 (Neiguan), SP8 (Diji), LR3 (Taichong), GV20 (Baihui) and ST29 (Guilai) before embryo transfer. After embryo transfer,

the needles were inserted at ST36 (Zusanli), SP6 (Sanyinjiao), SP10 (Xuehai) and LI4 (Hegu).

Results of the Risk of Bias Assessment

The risk of bias assessment revealed an overall low risk of bias. All included trials reported adequate randomization of patients and allocation concealment. Regarding the blinding of participants and personnel, two studies[24,26] were single-blind, therefore they were classified as high risk of bias. Four studies[22,23,25,30] did not report enough evidence to ensure double-blinding, and therefore were put at "unclear" risk. The remaining three studies[27-29] were double-blind studies. All studies reported proper blinding of outcome assessment, except three studies[22,23,25], which did not report enough evidence. All studies were at low risk of bias regarding other domains, and no other bias was found as well. Supplemental Figure S2 shows a risk of bias graph and a summary of the risk of bias assessment among included studies.

Analysis of Efficacy Endpoints

Clinical Pregnancy

The clinical pregnancy outcome was reported in the nine studies. The overall RR did not reveal any significant difference between the groups [RR=1.14, 95% CI (0.93, 1.40), p=0.21]. Pooled results were heterogeneous (I^2=70%, p<0.001). Therefore, subgroup analysis according to the country was conducted.

In China, the results significantly favored the control group over the acupuncture arm [RR=0.80, 95% CI (0.66, 0.97), p=0.02]. Pooled data were homogeneous (I^2=0%, p=0.86). Conversely, the combined RR conducted outside China revealed that there was no significant difference between both groups [RR=1.28, 95% CI (1.02, 1.61), p=0.03]. Pooled results were heterogeneous (I^2=62%, p=0.02) (Figure 1A). According to Cochrane's leave-one-out method, inconsistency was best solved by excluding the Andersen et al.[27] study and the homogeneous results favored the acupuncture group significantly [RR=1.38, 95% CI (1.11, 1.71), p=0.003] (Figure 1B).

Ongoing Pregnancy

The ongoing pregnancy outcome was reported in seven studies[23-25,27-30]. The combined effect estimate showed no significant difference between both arms [RR=1.12, 95% CI (0.81, 1.82), p=0.34]. Pooled results were heterogeneous (I^2=79%, p<0.001). Subgroup analysis showed that in China, the control group was significantly associated with more clinical pregnancies than the acupuncture group [RR=0.78, 95% CI (0.63, 0.97), p=0.03]. Data were homogeneous (I^2=0%, p=0.94). While outside China, ongoing pregnancies were not different between the groups [RR=1.41, 95% CI (0.89, 2.23), p=0.14]. Pooled results were heterogeneous (I^2=79%, p=0.003), (Figure 2A). Heterogeneity was best solved after excluding the Andersen et al.[27] study (citation) and homogeneous results favored the acupuncture group [RR=1.73, 95% CI (1.29, 2.31), p<0.001] (Figure 2B).

Live Birth

Four studies[27-30] reported the outcome of a live birth. The overall RR showed no significant difference between both groups [RR=0.87, 95% CI (0.75, 1.01), p=0.06]. Pooled results were homogenous (I^2=0%, p=0.58), (Figure 3).

Miscarriage

Miscarriage was reported in five studies[24,27-30]. No statistically significant difference was found between both groups [RR=1.23, 95% CI (0.89, 1.70), p=0.21]. Pooled results were homogenous (I^2=0%, p=0.37) (Figure 4).

Analysis of Side Effects

Three studies[28-30] reported the intervention-related side effects (Figure 5). The results showed that the acupuncture group was associated with a significantly increased incidence rate of puncture site itching [RR=1.51, 95% CI (1.12, 2.04), p=0.007]. There were no statistically significant differences between the groups regarding nausea (p=0.21), dizziness (p=0.34), fainting (p=0.07), tiredness (p=0.75), drowsiness (p=0.55), headache (p=0.08), and chest pain (p=0.26).

Discussion

The results of this research suggest that outside China, acupuncture is an effective adjuvant approach that leads to increased rates of clinical and ongoing pregnancies, while contrary results are revealed for studies conducted within China, in which acupuncture actually leads to lower clinical and ongoing pregnancy rates. Other secondary outcomes, such as live birth were significantly lower in the acupuncture groups as well. Regarding side effects, acupuncture increases the incidence of headache and puncture site itching, while there was no difference between the two groups regarding the incidence of miscarriage, nausea, dizziness, fainting, tiredness, drowsiness, and chest pain.

The results of the subgroup analyses are consistent with the findings of other studies in the literature. A clinical trial[28] conducted in China found that the placebo acupuncture group was associated with higher overall pregnancy rates than the real acupuncture group. Another trial conducted a year later found that patients who received placebo acupuncture intervention had more ongoing pregnancy and implantation rates than those who discontinued the trial[29]. Another recent meta-analysis, Quan et al.[31] in 2022 agreed with our findings of a benefit to acupuncture in their analysis of 27 studies. Their analysis included studies from worldwide without a specific subgroup analysis. Differing from our findings, Tyler et al.[32] in 2022 found no significant difference in patients undergoing acupuncture versus those who did not, although this analysis included fewer studies than ours secondary to their goal of analyzing many factors outside acupuncture with IVF.

The exact explanation of why acupuncture decreases pregnancy rates among Chinese patients while increasing them among

other populations remains unclear. However, increasing evidence supports the effect of placebo acupuncture. A study by Birch[33] has shown that placebo acupuncture may induce a clinical response and is not an inert control. Vincent and Lewith[34] obtained the same results. Lund et al.[35] found that placebo acupuncture is not an inactive control. A large controlled trial[36] studied the effect of acupuncture in reducing chronic back pain. The study included three arms: Real acupuncture,

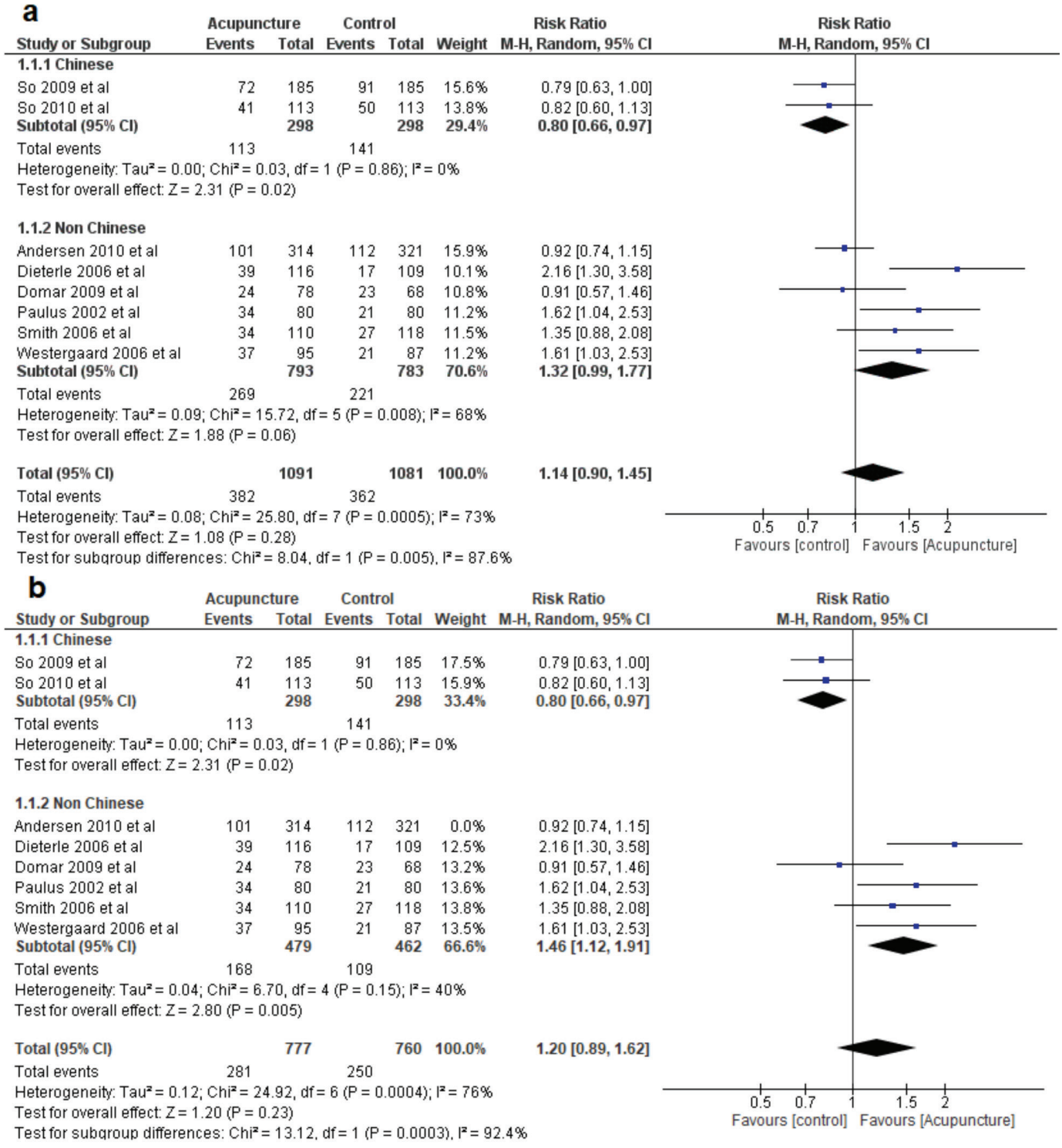

Figure 1. Shows a forest plot for the analysis of the clinical pregnancy outcomes, **a)** results before leave-one-out analysis, **b)** after leave-one-out analysis

CI: Confidence interval

a placebo acupuncture group, and a control group receiving conventional care. The study found that both real and placebo acupuncture had the same efficacy, which was superior to conventional care methods.

Contrary results were found in controlled trials performed outside China. Three RCTs[22,23,25] reported that acupuncture led to increased clinical and ongoing pregnancy rates, while another three RCTs found no significant difference between both groups[24,26,27]. A previous meta-analysis found that acupuncture increased pregnancy rates in patients undergoing IVF[37]. Another meta-analysis[38] found higher live birth and pregnancy rates in the real acupuncture group. Although the exact pathogenesis of how acupuncture increases pregnancy rates remains unknown, these studies relied on the evidence that acupuncture has been suggested to stimulate the release of several neurotransmitters (including serotonin and

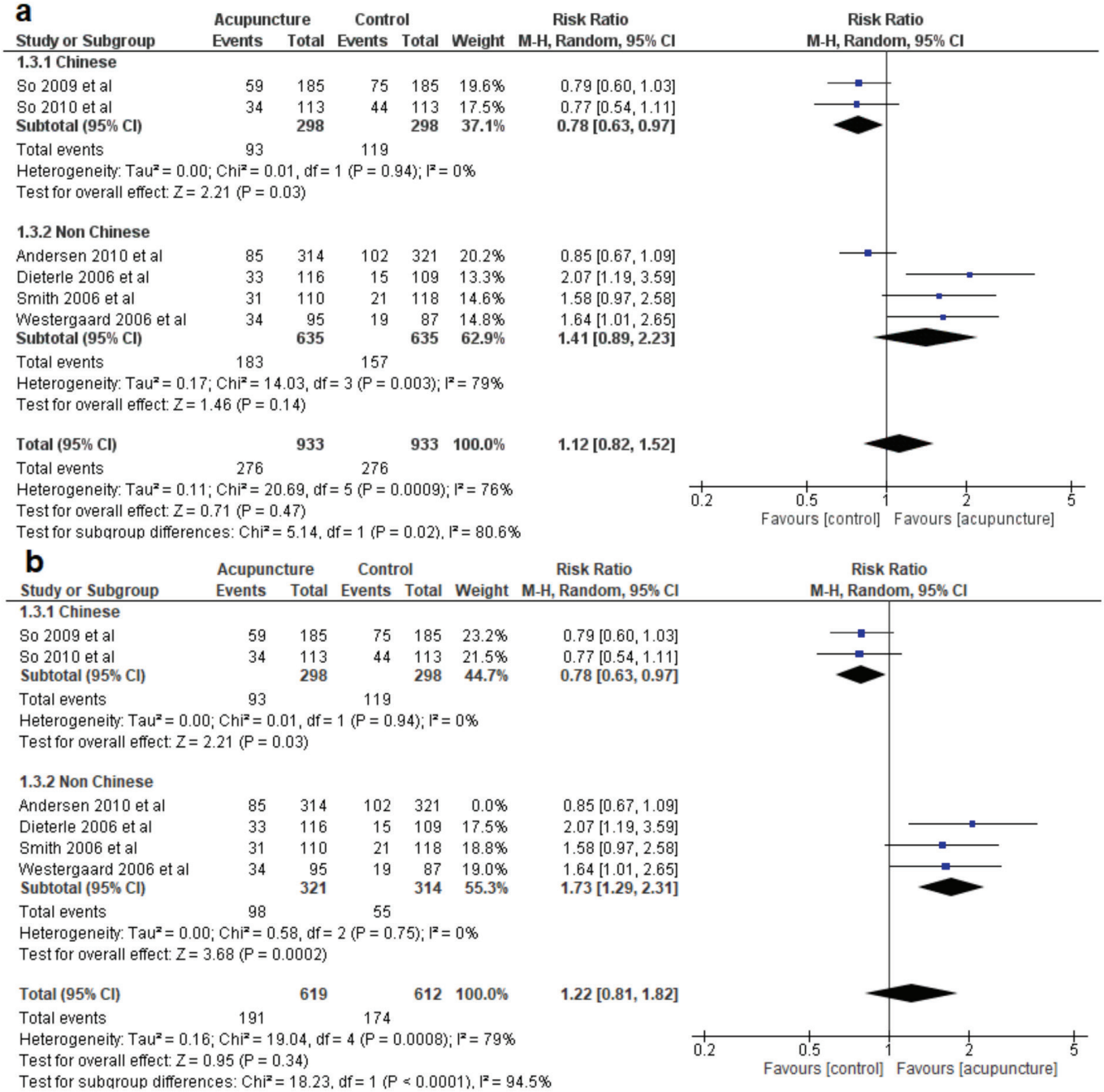

Figure 2. Shows a forest plot for the analysis of ongoing pregnancy outcome, **a)** before leave-one-out analysis, and **b)** after leave-one-out analysis

CI: Confidence interval

endorphins). These neurotransmitters enhance the production of gonadotropin-releasing hormones from the hypothalamus, which in turn increase the production of follicular-stimulating hormone that leads to stimulating and improving female ovulation[39-41]. It is also possible that these same processes lead to improved egg quality and ovarian follicle reserve, which would produce the same results[41].

There have been no reported serious adverse events with acupuncture. However, RCTs have shown that many patients may experience mild-to-moderate side effects of nausea and headache[28,29]. Other RCTs have reported that acupuncture leads to positive side effects such as the feeling of relaxation, calm and peace[24]. Therefore, acupuncture is considered a safe therapeutic approach.

In the present systematic review and meta-analysis, only RCTs were included. This gave the review a significant strength. The risk of bias assessment revealed an overall low risk of bias, which further supports the results of this study. However, several limitations need to be considered. The results of the analysis, though it is correct, may not represent the "true effect" of acupuncture. This may be primarily due to the small number of included studies in the China group (two studies). More controlled trials are needed with a larger sample size to provide solid evidence to solve this debate. Moreover, double-blinding may play a critical role in affecting the results. This is supported by the fact that sham acupuncture has been effective in increasing pregnancy rates in double-blind studies only[28,29]. Other trials included in this analysis were either single-blind[24,26] or did not report data about blinding[22,23,25]. Heterogeneity is the most important limitation, nevertheless, pooled homogeneous analysis was obtained. One final limitation is the lack of data required to conduct a true subgroup analysis of the different acupuncture sites to draw a connection between each individual point and the likely benefit or adverse effect associated with that point. Massive numbers of RCTs would be necessary to begin obtaining those answers on even a preliminary level of evidence.

Conclusion

As a conclusion, there is no clear evidence at this time to support the role of acupuncture. Results indicate that acupuncture reduces clinical and ongoing pregnancy rates among Chinese patients, while increasing them among patients outside China. The overall analysis of live birth and miscarriage outcomes showed no significant difference between the groups. As for the side effects, no side effects were associated with the procedure, except for puncture site itching.

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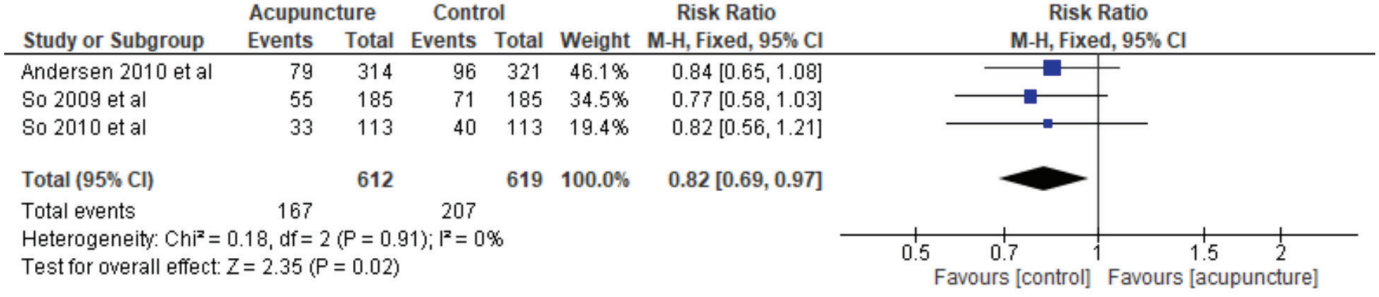

Figure 3. Shows a forest plot for the analysis of the live birth outcome

CI: Confidence interval

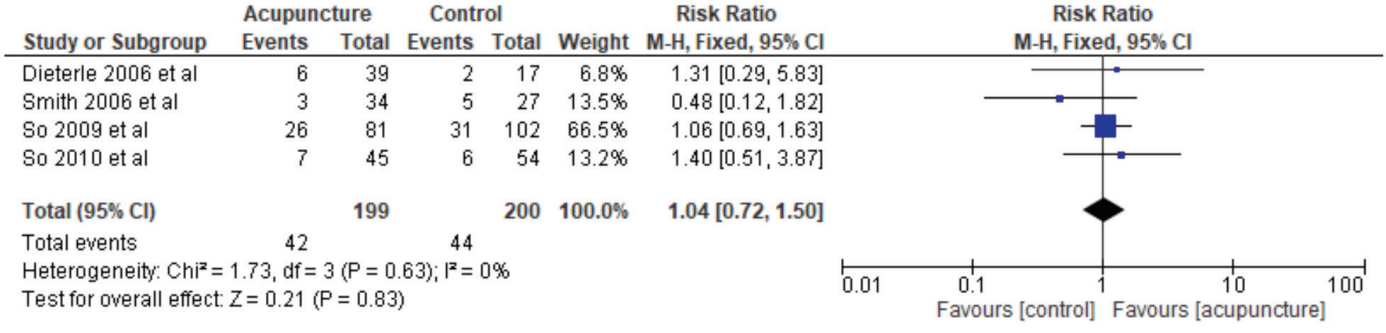

Figure 4. Shows a forest plot for the analysis of the miscarriage outcome

CI: Confidence interval

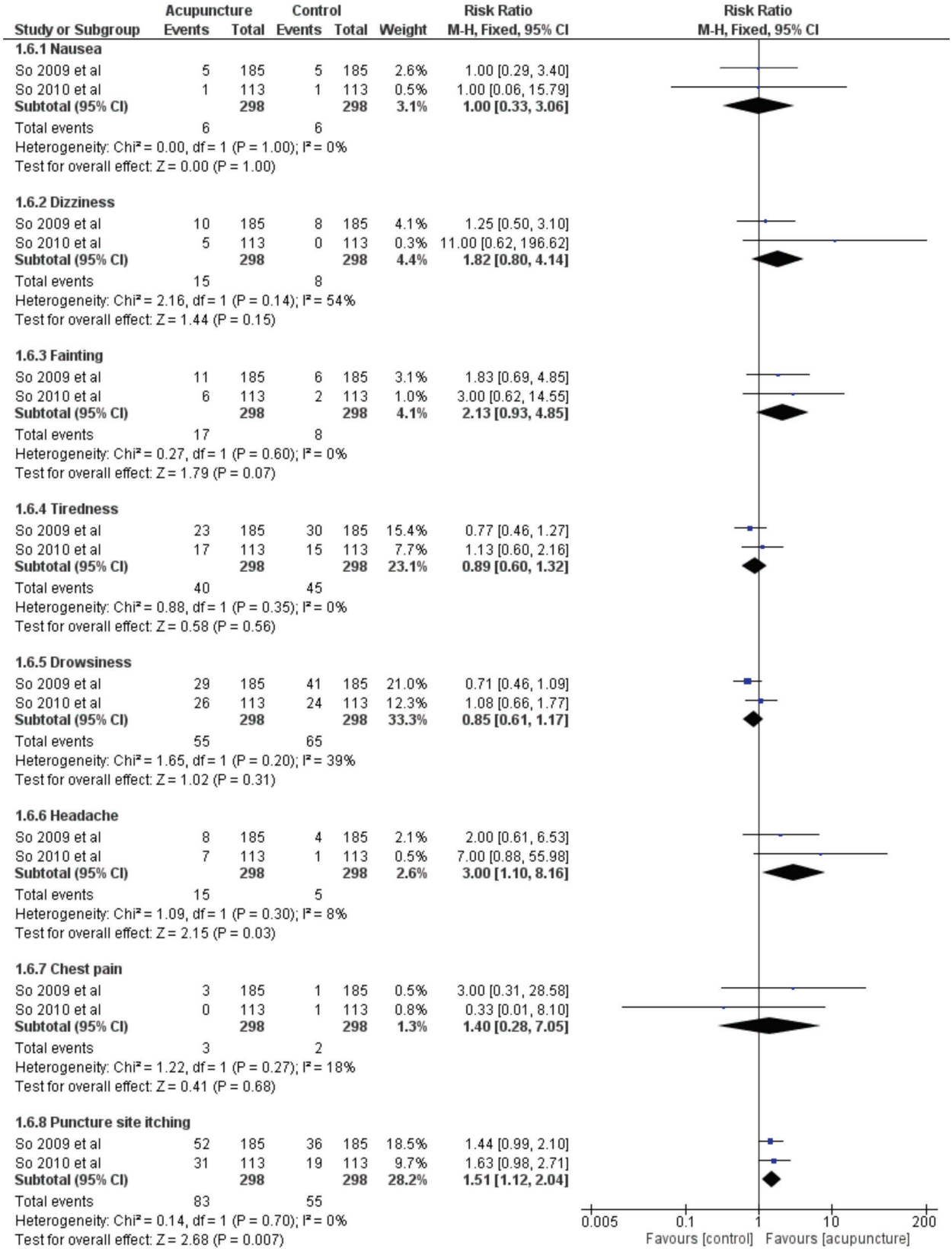

Figure 5. Shows a forest plot for the analysis of intervention-related side effects including nausea, dizziness, fainting, tiredness, drowsiness, headache, chest pain, and puncture site itching

CI: Confidence interval

Ethics

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: G.M., A.M., A.A.Z., F.E., Data Collection or Processing: G.M., R.L., M.J., Analysis or Interpretation: F.E., A.A.Z., M.J., Initial Draft: G.M., R.L., B.L., A.M., M.J., Final Draft: G.M., A.M., B.L., R.L., Writing: G.M., A.M., B.L., R.L.

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

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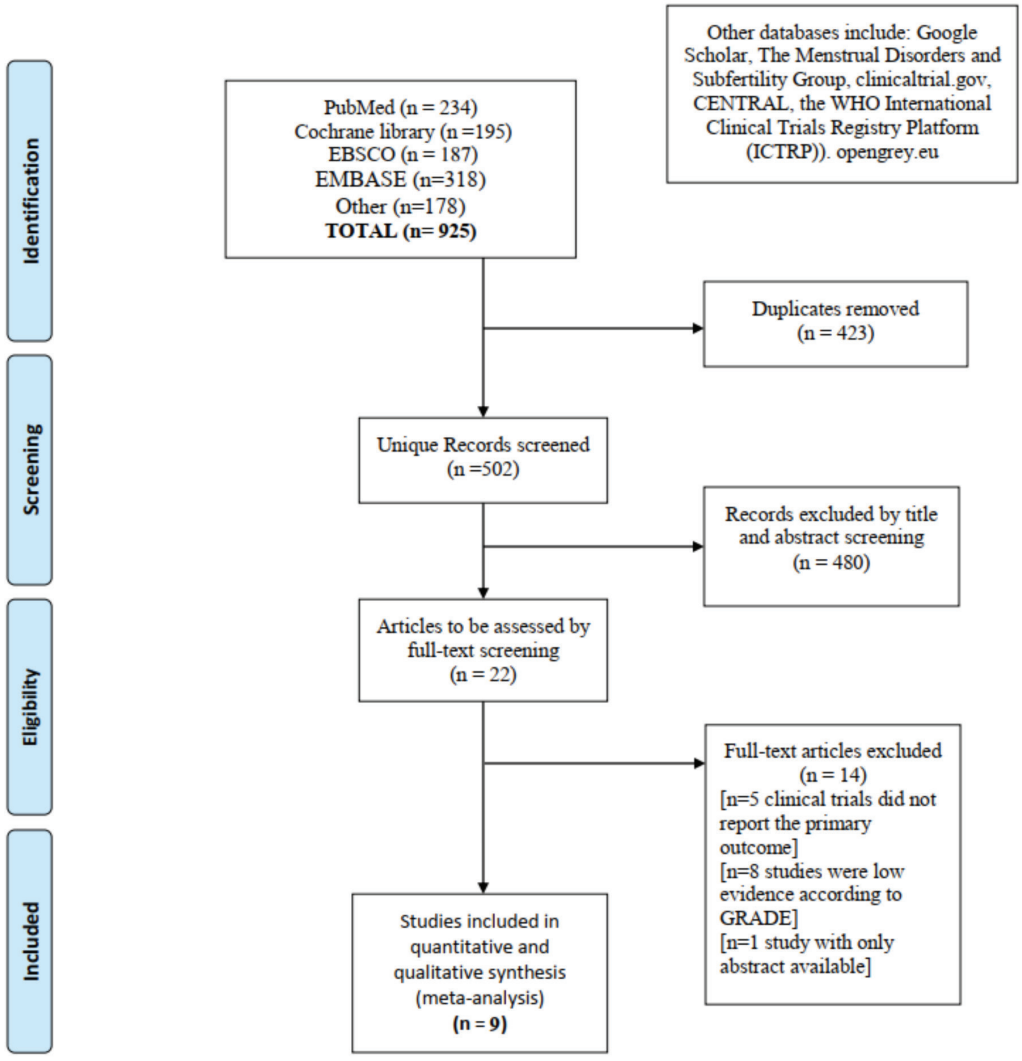
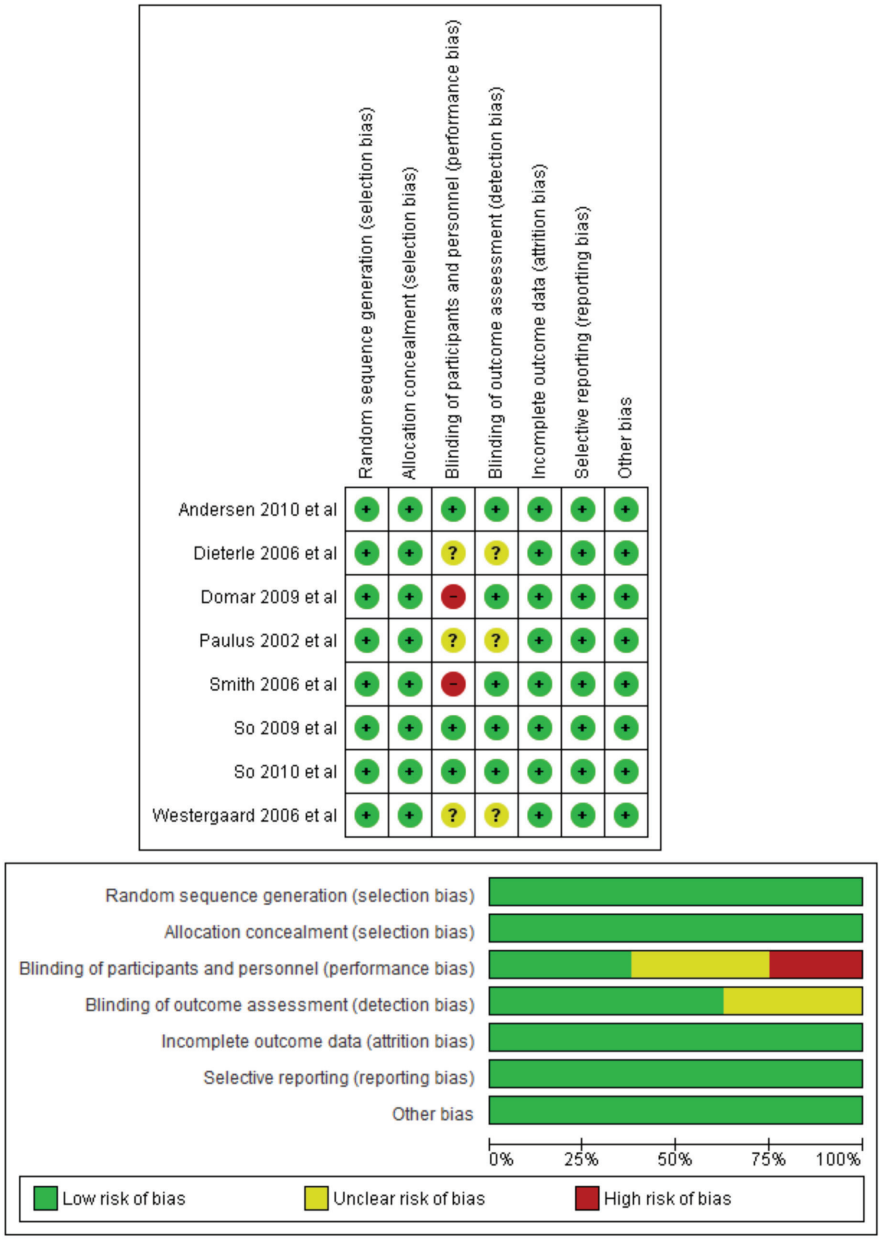


Supplemental Figure S1. Shows a PRISMA flow chart for literature search of included studies

PRISMA: Preferred reporting items for systematic reviews and meta-analyses



Supplemental Figure S2. Shows a detailed risk of bias assessment and risk of bias graph of included trials

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Efficacy of lidocaine local anesthesia on pain perception during amniocentesis: A meta-analysis of randomized controlled trials

Lidokain lokal anestezisinin amniyosentez sırasında ağrı algısı üzerindeki etkinliği: Randomize kontrollü çalışmaların bir meta-analizi

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Abstract

To evaluate the efficacy of lidocaine local analgesia on maternal pain reduction during amniocentesis. Web of Science, Scopus, PubMed, and CENTRAL databases were screened from inception and updated in July 2022. The included randomized controlled trials (RCTs) were evaluated for the risk of bias via the Cochrane tool. The primary outcome was pain perception using the 10 cm visual analog scale, and was summarized as mean difference (MD) with 95% confidence interval (CI) in a random-effects model. Subgroup analysis was performed according to the mode of administration. Meta-analysis was done via Review Manager software. We included five RCTs totaling 1004 women (lidocaine arm n=502 patients and control arm n=502 patients). Overall, there was no significant difference between both arms [MD=-0.21, 95% CI (-0.48, 0.07), p=0.80]. The pooled analysis showed homogeneity (p=0.13, I^2=43%). Subgroup analysis according to the mode of administration showed that pain perception did not significantly differ between both arms when lidocaine was employed as injection [n=3 RCTs, MD=-0.26, 95% CI (-0.76, 0.23), p=0.29] or non-injection [n=2 RCTs, MD=-0.18, 95% CI (-0.55, 0.18), p=0.33]. The pooled analyses showed heterogeneity (p=0.05, I^2=66%) and homogeneity (p=0.27, I^2=19%), respectively. There was no noteworthy change concerning maternal pain perception between the lidocaine and control arms. Most women reported just minimal discomfort during amniocentesis. Counseling should educate patients that the pain they might experience during amniocentesis is comparable to venous blood sampling.

Keywords: Amniocentesis, local anesthesia, pain, pregnancy, analgesia

Öz

Bu çalışmada amaç, lidokain ile lokal analjezinin amniyosentez sırasında annenin ağrısının azaltılması üzerindeki etkinliğini değerlendirmektir. PubMed, Scopus, Web of Science ve CENTRAL veritabanları başlangıçtan itibaren arandı ve Temmuz 2022'de güncellendi. Dahil edilen randomize kontrollü araştırmalar (RKÇ'ler), Cochrane aracı aracılığıyla bias hatası riski açısından değerlendirildi. Primer sonlanım, 10 cm'lik görsel analog skala kullanılarak ağrı algısının ölçümü idi ve rastgele etkiler modelinde %95 güven aralığı (GA) ile ortalama fark (MD) olarak özetlendi. Uygulama şekline göre alt grup

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analizi yapıldı. Meta-analiz, Review Manager yazılımı aracılığıyla yapıldı. Toplam 1.004 kadını kapsayan beş RKÇ'yi dahil ettik. Bunlardan 502 hasta lidokain grubuna, 502 hasta ise kontrol grubuna ayrıldı. Genel olarak, her iki grup arasında anlamlı bir fark yoktu [n=5 RKÇ, MD=-0,21, %95 GA (-0,48, 0,07), p=0,80]. Birleştirilmiş analiz homojendi (ki-kare p=0,13, I^2=%43). Uygulama şekline göre alt grup analizi yapıldığında, lidokainin enjeksiyon olarak kullanması ile [n=3 RKÇ, MD=-0,26, %95 GA (-0,76, 0,23), p=0,29] enjeksiyon olarak kullanılmaması [n=2 RKÇ, MD=-0,18, %95 GA (-0,55, 0,18), p=0,33] durumlarında ağrı algısının her iki grup arasında anlamlı bir şekilde farklı olmadığı gösterilmiştir. Birleştirilmiş analizler sırasıyla heterojen (ki-kare p=0,05, I^2=%66) ve homojendi (ki-kare p=0,27, I^2=%19). Lidokain lokal anestezi grubu ile kontrol grubu arasında ağrı algısı açısından anlamlı fark yoktu. Kadınların çoğu, amniyosentez sırasında çok az rahatsızlık bildirdi. Danışmanlık ile hastaların amniyosentez sırasında yaşayabilecekleri ağrının venöz kan örneklemesi ile benzer olduğu konusunda eğitim verilmelidir.

Anahtar Kelimeler: Amniyosentez, lokal anestezi, ağrı, gebelik, analjezi

Introduction

Amniocentesis is an invasive procedure employed primarily in prenatal diagnosis[1]. Pain is a common concern among pregnant women undergoing amniocentesis[2]. The most popular approach for evaluating pain perception with high reliability during and after procedures is the visual analog scale (VAS)[2,3].

Currently, there are two main approaches for pain relief during amniocentesis, namely, pharmacological agents and non-pharmacological methods[2]. Among the pharmacological agents, lidocaine is a common local anesthetic agent for pain relief[4].

Several randomized controlled trials (RCTs) explored the capacity of lidocaine-mediated pain relief among pregnant women undergoing amniocentesis[5-9]. However, the findings of these RCTs were limited by various shortcomings, such as small sample sizes, relatively poor quality of studies, different routes of administration, and inconsistent reported results. All in all, the analgesic efficacy of lidocaine among pregnant women undergoing amniocentesis remains poorly delineated. Moreover, no meta-analysis report has been published to assess the clinical utility of lidocaine during amniocentesis. Such research is enormously imperative to generate evidence-based recommendations that will inform obstetric practice.

Therefore, the purpose of this contemporary investigation is to determine whether lidocaine administration has any analgesic effect on reducing maternal pain during amniocentesis when contrasted with a control treatment. The hypothesis is that the lidocaine administration will correlate with better maternal analgesia than the control treatment during amniocentesis.

In this study, we followed the steps of the Cochrane Handbook for Systematic Reviews of Interventions[10] as well as the Preferred Reporting Items for Systematic Reviews and Meta-Analyses[11]. Ethical approval was exempted.

Search Approach

Web of Science, Scopus, PubMed and Cochrane Central Register of Controlled Trials were searched until July 2022. The search approach comprised (amniocentesis OR amniocenteses) AND (anesthesia OR "local anesthesia" OR lidocaine OR xylocaine OR EMLA OR "lidocaine-prilocaine" OR lignocaine OR prilocaine OR dalcaine OR xylocitin OR xylesthesin OR xyloneural OR "2-2EtN-2MePhAcN" OR otocaine).

Inclusion and Exclusion Criteria

The inclusion criteria comprised (*a*) patients: Females undergoing amniocentesis, (*b*) intervention: Local analgesia using lidocaine, (*c*) comparison: Placebo or no treatment, (*d*) outcome: Pain perception, (*e*) study design: RCTs. The exclusion criteria comprised non-RCT study designs and studies published in languages other than English.

Screening and Study Selection

The retrieved citations were sequentially subjected to removal of duplicates, title/abstract examination, and lastly full-text inspection to determine final eligibility. Two independent authors completed the task and resolved the conflicts.

Quality Assessment

Quality assessment was completed using the revised version of the Cochrane Risk of Bias assessment tool[12]. Two authors performed the quality assessment independently for all RCTs to assess the risk of bias of the included studies according to the second version of the Cochrane Risk of Bias assessment tool[12]. "Low," "some concerns," or "high" risk of bias judgments were assigned to each domain. Two independent authors completed the task and resolved the conflicts.

Data Extraction and Outcome

Data extraction of studies comprised country, trial period, total number of patients, the intervention arm, the control arm, and type of administration. Data extraction of patients comprised the number of patients per arm, age, gestational age (weeks), parity, body mass index (kg/m^2), weight (kg), and height (in). The primary outcome included pain perception by using the 10 cm VAS. Two independent authors completed the task and resolved the conflicts.

Meta-analysis

The primary outcome was analyzed via the Inverse-Variance method and reported as mean difference (MD) with 95% confidence interval (CI). The random-effects model of statistical analysis was employed. I^2 values of more than 50% and the chi-square test ($p<0.1$) were indicative of high heterogeneity. Forest plots were generated through the Review Manager software, version 5.4.

Results

Literature Search

Overall, 203 citations were retrieved after the omission of duplicates. Additionally, nine articles progressed to full-text screening, of which five studies met the eligibility criteria and were included in the quantitative synthesis (Figure 1)[5-9].

Summary of the Included Studies

We included five RCTs[5-9] with 1004 patients (lidocaine arm n=502 patients and control arm n=502 patients). Three RCTs used lidocaine as injection[5-7] and two RCTs used it as non-injection [spray[8] and cream[9]]. Table 1 and Table 2, respectively, summarize the major features of the included studies and participants.

Quality Assessment

Three RCTs achieved an overall low risk of bias[6,8,9]. One RCT[7] was evaluated as "some concerns" in the domain of randomization because it provided no information about the randomization process and allocation concealment. Lastly, one RCT[5] was judged as high risk of bias in the domain of randomization because it provided no information about the randomization process and allocation concealment, and baseline imbalance suggested a problem in the randomization process (Figure 2).

Meta-Analysis of Pain Perception (VAS)

All RCTs reported pain perception[5-9]. Overall, there was no significant difference between both arms [MD=-0.21, 95% CI (-0.48, 0.07), p=0.80]. The pooled analysis showed homogeneity (p=0.13, I^2=43%). Subgroup analysis according to the mode of administration showed that pain perception did not significantly differ between both arms when lidocaine was employed as injection [n=3 RCTs, MD=-0.26, 95% CI (-0.76, 0.23), p=0.29] or non-injection [n=2 RCTs, MD=-0.18, 95% CI (-0.55, 0.18), p=0.33]. The pooled analyses showed heterogeneity (p=0.05, I^2=66%) and homogeneity (p=0.27, I^2=19%), respectively (Figure 3).

Discussion

Summary of the Main Findings

During amniocentesis, this meta-analysis of five RCTs showed no significant difference concerning maternal pain perception between the local analgesia group with lidocaine and the control group.

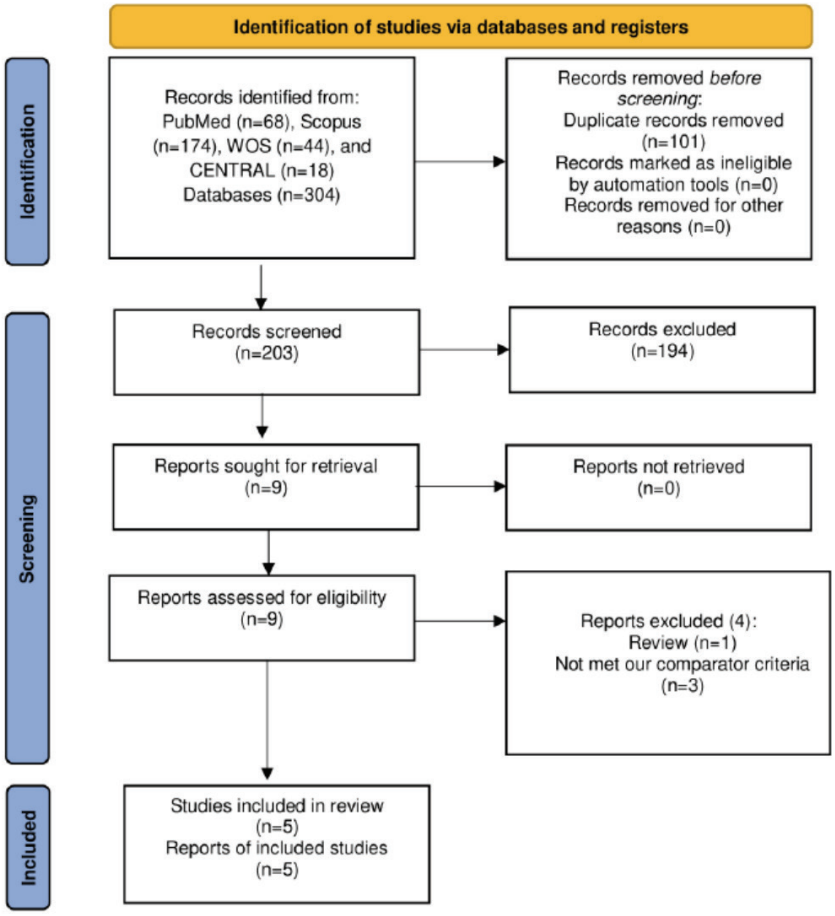

Figure 1. The preferred reporting items for systematic reviews and meta-analyses flowchart for literature search

Interpretation of Findings

Lidocaine is among the most potent anesthetic substances frequently used in medical procedures[4]. Lidocaine spray is a practical approach that is simple to employ in clinical practice for relieving pain in the skin or mucosa. A previous study showed that women who received lidocaine spray experienced less pain than those who received a placebo[8]. Women who are highly fearful of the procedure or have low pain tolerance may be given the option of lidocaine spray[8]. Some restrictions apply to this investigation; although the lidocaine spray starts immediately, the one-minute wait before the procedure may not have been long enough to provide the required anesthetic effect[8]. However, waiting for just a minute would prevent any intervening fetal movement that would change the targeted puncture site on the mother's abdomen[8].

In another trial on lidocaine cream[9], the results showed that patients' perceptions of worry and pain were mild before amniocentesis. There were no discernible variations in the VAS values between the two arms for anxiety (before procedure), anticipated pain, and pain (after procedure). Based on the distinction between the VAS pain levels before and after the procedure, the findings showed that lidocaine-prilocaine cream did not significantly reduce amniocentesis-related pain[8]. The local anesthetic effect can explain this result as it lowers cutaneous pain but not peritoneal discomfort. The peritoneum and uterus are the primary sources of pain during the procedure[8].

According to data from a previous study by Van Schoubroeck and Verhaeghe[5], most patients (59%) believed that the pain induced by amniocentesis was analogous to that induced by venipuncture. After injecting a local anesthetic into the dermis and subcutaneous tissues, they noticed no variance

Table 1. The summary of the included studies

Study ID	Country	Trial duration	Total sample size, n	Trial arms		Type of administration
				Lidocaine	Control	
Van Schoubroeck and Verhaeghe[5] 2000	Belgium	Between April 1998 and November 1998	n=220	Lignocaine (1%)	Nothing	Injection
Gordon et al.[6] 2007	USA	Between January 1995 and March 2001	n=204	Lidocaine (1%)	Nothing	Injection
Pongrojpaw et al.[8] 2007	Thailand	Between October 2006 and April 2007	n=120	Lidocaine-prilocaine	Placebo	Cream
Elimian et al.[7] 2013	USA	Between October 2007 and September 2009	n=76	Lidocaine (1%)	Placebo	Injection
Homkrun et al.[9] 2019	Thailand	Between June 2017 and January 2018	n=384	Lidocaine (10%)	Placebo	Spray

Table 2. The baseline characteristics of the included studies

Study ID	Group	Sample size, n	Age (years)	Gestational age (weeks)	Parity	BMI (kg/m²)	Weight (kg)	Height (in)
Van Schoubroeck and Verhaeghe[5] 2000	Lidocaine	n=114	34.1	15.9	1.1	NA	62.7	NA
	Control	n=106	33	15.8	1.1	NA	67.7	NA
Gordon et al.[6] 2007	Lidocaine	n=101	33.7±5.7	19.6±6.1	1.2±1	26.4±3.8	71.4±11.3	64.8±2.7
	Control	n=103	33.3±5.9	19.3±5.5	1.2±1.1	27.3±5.1	72.5±15.4	64.0±2.7
Pongrojpaw et al.[8] 2007	Lidocaine	n=60	36.8±3.79	17.6±1.6	0.7±0.8	24.4±4.2	NA	NA
	Control	n=60	36.9±3.41	19.9±6.6	0.6±0.7	24.1±3.6	NA	NA
Elimian et al.[7] 2013	Lidocaine	n=36	31.3±6.5	19.8±2.6	NA	NA	167.1±30.7 (Ibs)	64.9±2.6
	Control	n=40	30.1±7.5	20.1±2.4	NA	NA	170.2±37.7 (Ibs)	65.6±3.1
Homkrun et al.[9] 2019	Lidocaine	n=191	36±3	17±1	NA	NA	NA	NA
	Control	n=193	36±3	17±2	NA	NA	NA	NA

BMI: Body mass index, NA: Not available

in pain or distress during amniocentesis[5]. This finding is crucial since skipping local anesthetics saves both time and money. It takes time to aspirate the local anesthesia, slowly inject it and wait for it to take effect. Also, it is possible to avoid paying 3.26 EUR ($3.41) for each patient for a single syringe, two needles, and local anesthesia material (lidocaine)[5].

In the investigations by Van Schoubroeck and Verhaeghe[5] and Gordon et al.[6], lidocaine was locally injected before amniocentesis, but neither group reported that this technique reduced pain. The verbal rating scale of 1 to 4 was employed in the study by Van Schoubroeck and Verhaeghe[5], however without blinding. In the study by Gordon et al.[6], 66% of the local anesthetic arm and 53% of the control arm had the procedure performed by maternal-fetal medicine staff. They also discovered that women felt less discomfort when staff members performed the procedure. This result might be confusing since the women experienced pain due to local penetration. Although the study by Van Schoubroeck and Verhaeghe[5] was a quasi-randomized trial, the results of the investigation by Gordon et al.[6] showed no evidence of considerable heterogeneity. The amniocentesis procedures were not wholly carried out by doctors with the exact clinical expertise. Still, because in the Gordon et al.[6] study, maternal-fetal medicine staff carried out more operations involving an anesthetic, this might have influenced the study in favor of an affirmative conclusion regarding the utility of local anesthesia.

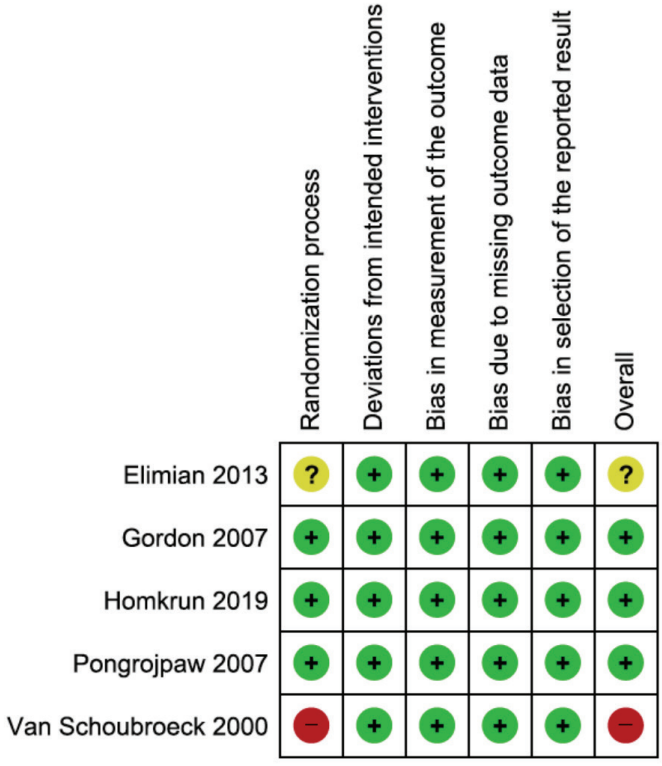

Figure 2. The summary of risk of bias assessment of the included randomized controlled trials

Study Limitations

The usage of LA for pain management is not yet supported by sufficient evidence (i.e., small number of trials and sample sizes). Our study only evaluated post-procedural pain and did not analyze post-procedural anxiety. Also, publication bias was not explored secondary to the few studies included.

Conclusion

There was no noteworthy change concerning maternal pain perception between the lidocaine and control arms. Most women reported just minimal discomfort during amniocentesis. Counseling should educate patients that the pain they might experience throughout the procedure is comparable to discomfort during venipuncture.

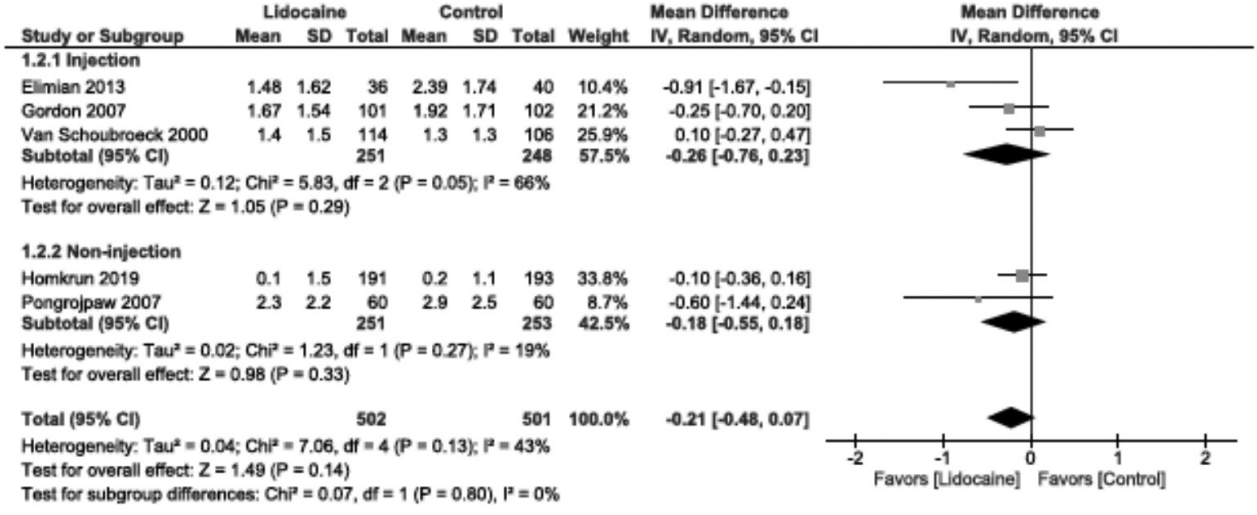

Figure 3. Meta-analysis of the post-procedural pain of amniocentesis

Ethics

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: E.A., A.S., A.A., Design: E.A., A.S., A.A., Data Collection or Processing: E.A., R.S., M.A., Ab.A., F.A., G.R., H.A., Analysis or Interpretation: E.A., R.S., A.S., Literature Search: E.A., R.S., M.A., Ab.A., F.A., G.R., H.A., M.G.B., A.A., Writing: E.A., R.S., M.G.B., A.A.

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

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Case Report / Olgu Sunumu

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Prenatally diagnosed fetal thoraco-lumbar spine duplication associated with lipomyelomeningocele: An extremely rare case of split cord malformation

Prenatal dönemde tanı koyulan lipomyelomeningosel ile ilişkili torakolomber omurga duplikasyonu: Nadir bir split kord malformasyon olgusu

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Abstract

Spine duplication is considered rare, a more serious form of split cord malformation. Ultrasonographic evaluation of the spine in the second trimester is central to the antenatal diagnosis of spinal malformations. Here, we report a case of thoraco-lumbar spine duplication associated with lipomyelomeningocele diagnosed by ultrasonography at 19 weeks of gestation. To the best of our knowledge, this is the first case report of spine duplication diagnosed by antenatal ultrasonography.

Keywords: Congenital spine defect, spinal dysraphism, spine duplication, split cord malformations, split notochord syndrome

Öz

Vertebra duplikasyonu, bölünmüş kord malformasyonlarının nadir ve daha ciddi bir şekli olarak kabul edilir. Vertebranın ikinci trimesterde ultrasonografik değerlendirilmesi spinal malformasyonların antenatal tanısında önemli rol oynar. Bu olgu sunumunda, 19. gebelik haftasında ultrasonografi ile tanı koyulan lipomyelomeningosel ile ilişkili torakolomber vertebra duplikasyonu olgusunu sunuyoruz. Bildiğimiz kadarıyla bu olgu, antenatal ultrasonografi ile tanı koyulan ilk vertebra duplikasyonu olgusudur.

Anahtar Kelimeler: Konjenital spinal defect, spinal disrafizm, spinal duplikasyon, split kord malformasyonu, split notokord sendromu

Introduction

Split cord malformations are defined as the formation of two hemicords along with segmental duplication of the spinal cord because of sagittal separation by a bony, fibrous, or cartilaginous spur[1,2]. Each hemicord formed because of this split contains a central canal and a series of ventral and dorsal nerve roots. Spine duplication is considered a more serious form of split cord malformations. In the spine duplication, unlike other split cord malformations, each cord has its own spinal canal in its own set of lumbar vertebrae. Other terms refer to the spine duplication such as "split notochord syndrome", "spinal duplication syndrome" and "fetal vertebral cleft". Spine duplication may be a component of caudal duplication syndrome. However, in caudal duplication syndrome, vertebral anomalies are accompanied by gastrointestinal and genitourinary system anomalies[3-6].

Spine duplication is a very rare malformation and only a few cases diagnosed in childhood or adult patients have been reported. With the widespread use of ultrasonography, spinal malformations can be diagnosed more frequently in the antenatal period. Most spinal malformations diagnosed on prenatal ultrasonography are neural tube defects. However, rarer malformations such as hemivertebrae, diastematomyelia and

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tethered cord, can be diagnosed by prenatal ultrasonography. Here, we present a case of thoraco-lumbar duplication of the spine with lipomyelomeningocele diagnosed in the prenatal period. This case, which included prenatal ultrasonographic findings, fetal radiographic images and fetal autopsy findings, is an extremely rare spinal malformation case. To the best of our knowledge, this is the first case of spine duplication with lipomyelomeningocele diagnosed by prenatal ultrasonography in the literature.

Case Report

A 32-year-old pregnant woman was referred to our tertiary center at the 19th week of gestation with a preliminary diagnosis of lumbar meningomyelocele. The pregnant woman had one healthy child and no known disease in her history. There was no known genetic disease in the first- or second-degree relatives of the pregnant woman and her husband. The marriage between the parents was not consanguineous. She did not have first trimester ultrasonographic fetal anatomical screening and serum screening tests. In fetal ultrasonography, lateral ventricles were measured as 6.1 mm and 5.2 mm and had a normal appearance. When the posterior fossa was evaluated, the cerebellum had a normal shape and the cisterna magna was measured as 3.9 mm. In the fetal dorsal coronal section, a 29x26 mm mass containing cystic and solid components was observed in the thoracolumbar vertebral region and there was no flow in this mass on color Doppler examination. In the evaluation of the spine, duplication and separation of the thoracolumbar spine and rotoscoliosis were observed (Figure 1). In the axial and sagittal sections, it was observed that the cervical and upper thoracic parts of the spine had normal shape, but separated from the eighth thoracic spine and the lower thoracic and lumbar spines were duplicated (Figure 2). Spinal duplication with lipomyelomeningocele was considered in the prenatal ultrasonographic diagnosis. In ultrasonographic examination of other systems, fetal biometric measurements were consistent with gestational age and there was no accompanying fetal malformation. Fetal magnetic resonance imaging (MRI) was not performed due to early gestational age.

Parents were informed about the fetal, neonatal and childhood prognosis of spine duplication with lipomyelomeningocele and the option of termination of pregnancy and prenatal genetic diagnostic tests were offered to the parents. The pregnancy was terminated after obtaining the informed consent of the parents. A female fetus, weighing 320 g, was delivered with vaginal misoprostol treatment. Postnatal fetal examination revealed a protruding cystic mass on the thoracolumbar vertebra of the fetus and duplication and separation of the thoracolumbar spine and rotoscoliosis was observed on fetal radiography (Figure 3). External genitourinary system examination of the fetus was normal. In fetal autopsy, when the spine was evaluated after abdominal dissection, two separate spinal process lines were palpated under the thoracolumbar junction. No malformations were detected in the thoracic, abdominal and pelvic organs in the fetal autopsy. No duplication was detected in the gastrointestinal tract or genitourinary system. During retroperitoneal dissection of the fetus, spine duplication was confirmed and lipomyelomeningocele was observed between the duplicated vertebrae. The cystic mass located in the midline between the doubled vertebrae and surrounded by adipose tissue was evaluated as lipomyelomeningoceles because it originated from the spinal cord and contained neural tissue. The karyotype and microarray results of the genetic diagnostic tests were reported as normal.

Discussion

Spine duplication is considered a more serious form of split cord malformations. Split cord malformation is the general term used to describe malformations involving two spinal cords, including traditionally used definitions such as diplomyelia and diastematomyelia. In 1992, Pang et al. [1,2] described the embryogenesis of the currently accepted split cord malformations and proposed a classification. This "unified theory of embryogenesis" suggested that all split cord malformations resulted from a fundamental ontogenetic error that occurred at the time of closure of the primordial neuroenteric duct. According to this theory, an "accessory neuroenteric canal" is formed through the midline embryonic disc, which provides contact between the ectoderm and the endoderm. The accessory neuroenteric canal, which is covered with mesenchyme to form the endomesenchymal pathway, causes regional separation of the notochord and the overlying neural plate. The final state of the resulting split neural tube and the constituent components of the endomesenchymal pathway ultimately determine the configuration and orientation of the hemicords, the median septum, and the coexistence of various vascular, lipomatous, neural, and fibrous anomalies. According to the classification proposed by Pang et al.[1,2] split cord malformations are classified as type 1 if the hemicords are in separate dural sacs separated by a rigid osseo-cartilaginous spur, and type 2 if they are in a single dural sac separated by a fibrous midline septum. However, this classification excludes the spine duplication, in which the bone elements are completely and separately copied. Some authors consider spine duplication a type 1 split cord malformation, while others suggest that it is unclassified[7].

Spine duplication is an extremely rare malformation and has been published as a limited number of case reports in the literature. Moreover, all of these cases were diagnosed during the postnatal period. To the best of our knowledge, the case we report is the first case diagnosed in the prenatal period, and it demonstrates that the spine duplication can be diagnosed by prenatal ultrasonography. Some of the reported cases were neurologically asymptomatic patients diagnosed between the ages of 1 and 44 years[7-11]. Among the reported cases, the one that was most similar to our case is a neurologically

asymptomatic female who did not require surgical treatment and was followed up for 6 years[8]. Here, the patient had a mass protruding from her back, and the authors did not report any symptoms other than cosmetic problems. However, in this study, unlike this case, there was a large lipomyelomeningocele sac that may cause possible neurological symptoms in the early stages of life and may require surgical treatment. In another reported case of spine duplication accompanied by lipomyelomengocele, the patient was diagnosed at the age of 14 and was neurologically asymptomatic[7]. In that case report, due to concerns about the clinical consequences of the tethered cord, the authors planned fusion for scoliosis and a surgical operation to release the tethered cord and instrumentation; but the operation was refused by the patient. Since it has been observed that the risk of neurological deficits increases with age in patients with split cord malformations, the necessity of

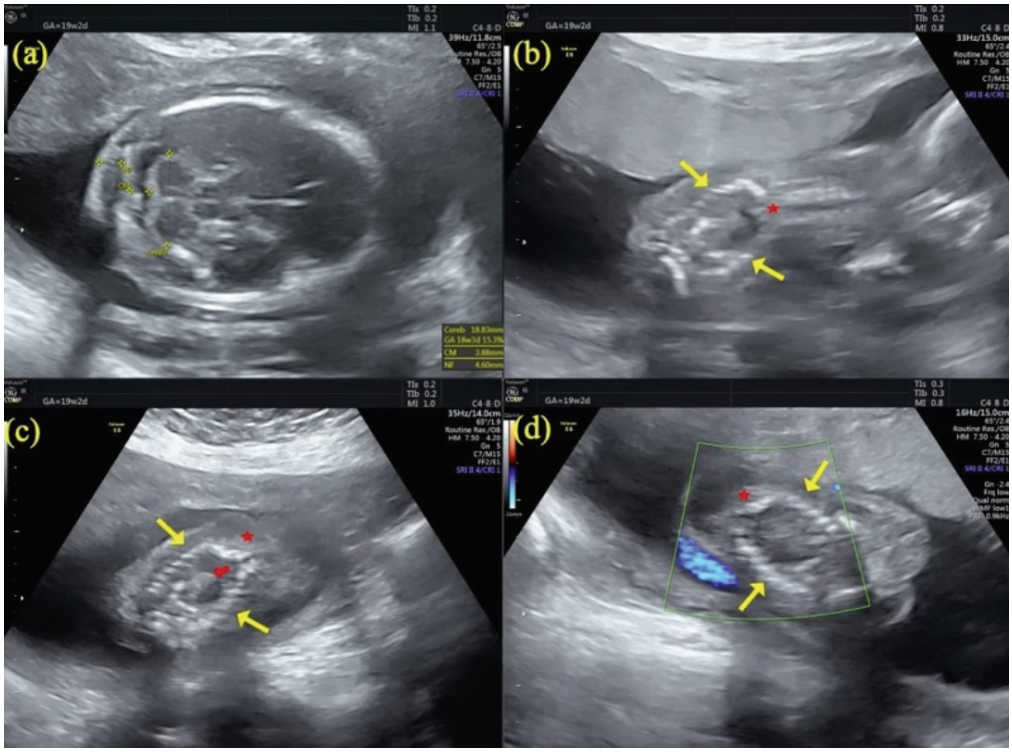

Figure 1. Ultrasonographic images of fetal spine duplication with lipomyelomeningocele. In the axial transcerebellar section, the cerebellum is in normal shape and the posterior fossa is open (a). Ultrasonographic images of the duplication and separation of the thoracolumbar spine distal to the T8 vertebra (Red star) in the evaluation of the spine in the dorsal coronal section (Yellow arrows mark right and left vertebrae) (b, c). The hyperechoic appearance (Red arrow) between the duplicated vertebrae was diagnosed as the lipomatous part of the lipomyelomeningocele at fetal autopsy (c). In color Doppler examination, no flow is observed in the mass between the vertebrae (d)

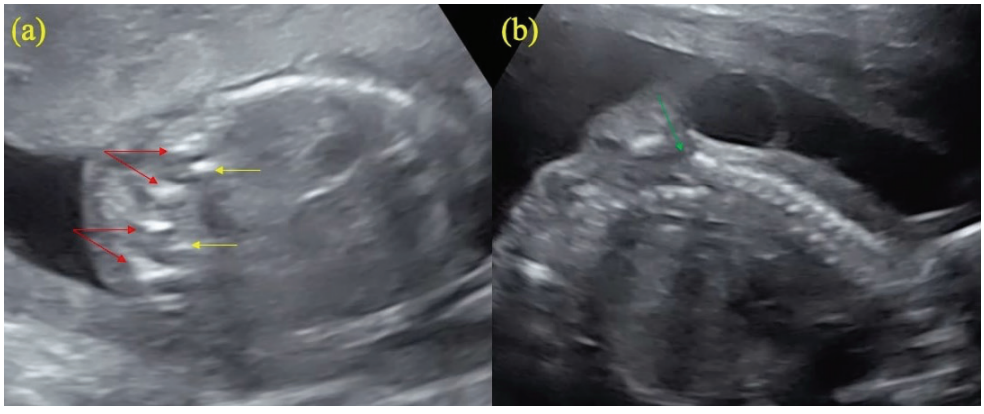

Figure 2. Ultrasonographic images of fetal spine duplication with lipomyelomeningocele in axial and sagittal section. Ultrasonographic image of spine duplication in axial section distal to T8 vertebra (Yellow arrows mark the vertebral bodies and red arrows mark the vertebral laminae) (a). Ultrasonographic image of spine duplication with lipomyelomeningocele in sagittal section (Green arrow marks the meningomyelocele sac at the level of the T8 spinal bifurcation) (b)

prophylactic surgical treatment has been suggested even if the patients are asymptomatic[12,13]. However, it is still controversial whether neurologically asymptomatic cases require surgery. In a case report of a 44-year-old neurologically intact patient with spine duplication, the authors suggested that asymptomatic patients without associated abnormalities should be followed up before planning surgical intervention[9]. Furthermore, none of the previously reported cases of asymptomatic isolated spine duplication were treated surgically and none had neurological symptoms.

Spine duplication may be a component of the caudal duplication syndrome, which includes gastrointestinal, genitourinary, and distal neural tube malformations[3-7]. In this syndrome, unlike split cord malformations, duplication is not limited to the distal spine and clinical manifestations are present in all three germ layers, including the hindgut structures. In this study, there was an isolated spine duplication with lipomyelomeningocele, and caudal duplication syndrome was not diagnosed since fetal autopsy did not detect any malformations in other abdominal organs. In caudal duplication syndrome, gastrointestinal anomalies usually include duplicated colons, genitourinary anomalies include duplication of external genitalia, urethra and bladder, and duplication of cervix or vagina in female patients[14]. Therefore, patients with caudal duplication syndrome may have worse outcomes compared to split cord malformations due to the presence of concomitant anomalies.

Split notochord syndrome is caused by the persistent communication of the embryonic endoderm and ectoderm layers and is accompanied by vertebral anomalies[15]. Almog et al.[16] were the first to publish prenatal ultrasonographic findings of two fetuses with split notochord syndrome. In a literature review of fetuses with split notochord syndrome diagnosed prenatally, most of the fetuses had a thoracic cystic mass[15]. In this study, there was no thoracic or abdominal cystic mass or anomaly in the visceral organs, and these ultrasonographic findings differentiated it from split notochord syndrome.

In this study, the diagnosis was made by 2D ultrasonography. With 3D ultrasonography, more demonstrative images could be obtained and thus, the vertebrae could be evaluated more clearly. Multi-planner evaluation of the vertebrae in 3D ultrasonography may be useful in determining the level and characteristic features of the malformation. Therefore, we recommend the use of multi-planner 3D ultrasonography for evaluating spinal malformations. Additionally, fetal MRI in

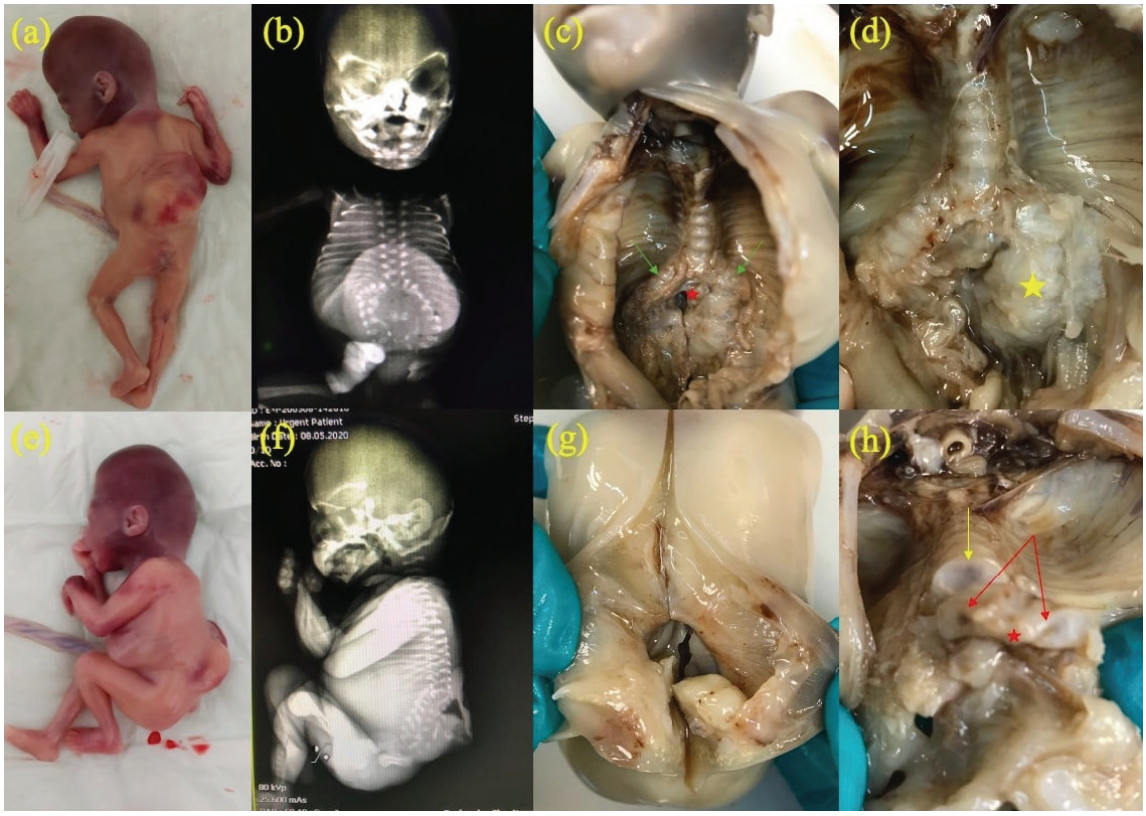

Figure 3. Fetal autopsy images and radiographic images of fetus with spine duplication and lipomyelomeningocele

Images of a cystic mass protruding from the thoracolumbar region on the fetal back (a,e). Antero-posterior and lateral radiographic images of spine duplication with lipomyelomeningocele (b,f). Fetal autopsy images of spine duplication with lipomyelomeningocele (c,d,g and h). Green arrows (c) and red arrows (h) marks right and left vertebrae. Red star marks the lipomyelomeningocele sac originating from the vertebral bifurcation (c,h). Yellow star marks the lipomatous tissue between the right and left vertebrae (d). Posterior dissection of the lipomyelomeningocele sac (g)

the late second trimester or third trimester may contribute to the diagnosis of vertebral anomalies. Moreover, performing fetal MRI may contribute to the detection of genitourinary and gastrointestinal system anomalies that cannot be detected by ultrasonography. We recommend performing fetal MRI in the late second trimester or the third trimester in complex vertebral anomalies.

Split cord malformations are not associated with chromosomal abnormalities[17]. In this study, the karyotype and microarray results were reported as normal. However, an associated gene could be detected if advanced genetic diagnostic tests, such as whole-exome sequencing were performed. The *AXIN1* gene located in the 16p13.3 chromosome region has been associated with caudal duplication syndrome[18]. Therefore, we recommend performing advanced genetic testing in fetuses with complex vertebral anomalies if no genetic abnormality is detected in karyotype and microarray analysis. Thus, genetic counseling can be provided to the parents for a subsequent pregnancy.

In conclusion, our case report is the first case to reveal that the spine duplication can be diagnosed by prenatal ultrasonography. Spine duplication is a rare malformation and has clinical consequences ranging from asymptomatic to severe neurological dysfunction. Therefore, we suggest a multidisciplinary approach that includes neonatology specialists, pediatric neurosurgeons, geneticists and radiology specialists in the prenatal and postnatal management of these cases. Although it is not associated with a known chromosomal anomaly, microarray and, if necessary, whole-exome sequencing can be recommended in cases diagnosed in the prenatal period.

Ethics

Informed Consent: Informed consent of the parents was obtained.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: M.A., O.D., E.K., Concept: M.A., O.D., Design: M.A., G.E.D., Data Collection or Processing: E.K., Analysis or Interpretation: E.K., G.E.D., Literature Search: M.A., G.E.D., Writing: M.A., O.D.

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

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