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TURKISH JOURNAL OF OBSTETRICS AND GYNECOLOGY

CONTENTS

Clinical Investigations

TURKISH JOURNAL OF OBSTETRICS AND GYNECOLOGY

CONTENTS

TURKISH JOURNAL OF OBSTETRICS AND GYNECOLOGY

LETTER FROM THE PRESIDENT

Dear TJOD Family,

We are delighted to present the first issue of our magazine for the year 2025 to you in March. In the March issue, 12 articles, which have been meticulously reviewed and deemed suitable for publication through the rigorous efforts of our editors and reviewers, have been presented to the opinions of scientists on national and international platforms. I believe that the published studies will contribute to the current knowledge of literature.

As the TJOD Family, we place great importance on scientific studies, meetings, and symposiums. We carry out our webinars and regional meetings, held online, with great enthusiasm. Our TJOD Assistant School project, which we believe plays a crucial role in the training of our young colleagues, continues in full swing. The high number of participants in the assistant schools we held in January and February at Ankara Gulhane Faculty of Medicine and Izmir Ege University has greatly motivated us. I would like to mention that these courses are also planned for March, April, and May.

I would also like to mention that we plan to hold the 22nd National Gynecology and Obstetrics Congress, which we have been working on for a long time, in Cyprus from May 14 to 18, 2025. Looking forward to meeting all our colleagues at Turkiye's largest and most intensely attended congress.

Best Regards
İsmail Mete Itil, Prof. MD
President of TJOD

TURKISH JOURNAL OF OBSTETRICS AND GYNECOLOGY

EDITORIAL

Dear Colleagues,

We are with you with the first issue of Turkish Journal of Gynecology and Obstetrics 2025. Our March issue includes 12 original research articles. Five of these studies have been submitted to our journal from international institutions, while the remaining articles are studies conducted at leading centers in our country.

We believe that the international study examining artificial intelligence and biomarker values in the prenatal screening of Down syndrome, a significant concern for obstetricians, will attract great interest from our readers.

We extend our gratitude to our reviewers who contributed to the preparation of the March issue and express our love and respect to our physicians.

Sincerely

Ercan Yılmaz, Prof. MD
Fatih Şendağ, Prof. MD

Clinical Investigation / Araştırma

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Turk J Obstet Gynecol 2025;22:1-12

Association of *TAB2* gene polymorphism with endometrial cancer susceptibility and clinical analysis

TAB2 gen polimorfizminin endometriyal kanser duyarlılığı ve klinik analizle ilişkisi

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Abstract

Objective: Transforming growth factor-β-activated kinase 1 binding protein 2 (TAB2) plays a vital role in inflammatory pathways. It has also been considered a potential target for the enhancement of the the antiestrogen effects. Previous evidence has indicated that *TAB2* gene variants are associated with several diseases, whereas their potential correlation with endometrial cancer (EC) is unclear. This study aims to initially explore the association between *TAB2* gene polymorphisms (rs237028 /AG, rs521845 T/G, and rs652921 T/C) and EC.

Materials and Methods: Polymerase chain reaction-restriction fragment length polymorphism was applied to determine the genotype composition and the allele frequencies of *TAB2* gene variant polymorphisms in 270 EC patients and 294 healthy controls.

Results: The G allele of rs521845 was related to the increase of EC risk [p=0.08, odds ratio (OR): 0.72, 95% confidence interval (CI): 0.56-0.91]. Moreover, EC risk was associated with rs521845 in different genetic models (p=0.017, OR: 0.63, 95% CI: 0.44-0.91 in the codominant model; p=0.0051, OR: 0.61, 95% CI: 0.43-0.87 in the dominant model). For rs237028, the percentage of AG genotype in patients with highly differentiated tumours (G1) was significantly higher than that in moderately, poorly differentiated patients (G2/G3) (p=0.031, OR: 0.77, 95% CI: 0.45-1.30).

Conclusion: Our results showed that the rs521845 polymorphism of TAB2, was associated with EC risk, suggesting that TAB2 may play a crucial role in EC prognosis.

Keywords: Endometrial cancer, TAB2, polymorphisms, risk

Öz

Amaç: Dönüştürücü büyüme faktörü-β ile aktive olan kinaz 1 bağlayıcı protein 2 (TAB2), enflamatuvar yollarda hayati bir rol oynar. Ayrıca anti-östrojen etkilerinin artırılması için potansiyel bir hedef olarak da kabul edilmiştir. Önceki kanıtlar, *TAB2* gen varyantlarının çeşitli hastalıklarla ilişkili olduğunu gösterirken, endometriyal kanser (EK) ile potansiyel ilişkisi belirsizdir. Bu çalışma, *TAB2* gen polimorfizmleri (rs237028 /AG, rs521845 T/G ve rs652921 T/C) ile EK arasındaki ilişkiyi araştırmayı amaçlamaktadır.

Gereç ve Yöntemler: Polimeraz zincir reaksiyonu-restriksiyon parça uzunluk polimorfizmi, 270 EK'li hastada ve 294 sağlıklı kontrolde *TAB2* gen varyantı polimorfizmlerinin genotip kompozisyonunu ve alel frekanslarını belirlemek için uygulandı.

Bulgular: Rs521845'in G aleli, EK riskinde artışla ilişkiliydi [p=0,08, olasılık oranı (OR): 0,72, %95 güven aralığı (GA): 0,56-0,91]. Dahası, EK riski farklı genetik modellerde rs521845 ile ilişkiliydi [kodominant modelde (p=0,017, OR: 0,63, %95 GA: 0,44-0,91); dominant modelde (p=0,0051, OR: 0,61, %95

PRECIS: This study investigates the association between *TAB2* gene polymorphisms and endometrial cancer (EC). The rs521845 G allele was linked to increased EC risk, while the rs237028 AG genotype correlated with tumor differentiation. Findings suggest TAB2 may play a key role in EC prognosis.

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GA: 0,43-0,87). Rs237028 için, iyi farklılaşmış tümörleri olan hastalarda (G1) AG genotipinin yüzdesi, orta derecede farklılaşmış tümörleri olan hastalardan (G2/G3) önemli ölçüde daha yüksekti (p=0,031, OR: 0,77, %95 GA: 0,45-1,30).

Sonuç: Sonuçlarımız TAB2'nin rs521845 polimorfizminin EK riskiyle ilişkili olduğunu gösterdi ve bu da TAB2'nin EK prognozunda önemli bir rol oynayabileceğini düşündürmektedir.

Anahtar Kelimeler: Endometrial kanser, TAB2, polimorfizmler, risk

Introduction

Endometrial cancer (EC) is ranked as one of the significant gynecologic malignancies, with an estimated 420,242 new cases diagnosed and 97,704 deaths worldwide in 2022[1,2]. Among malignant tumours of the female reproductive system in China, the incidence of EC is only lower than that of cervical cancer, and it mainly occurs in postmenopausal women[3]. However, in the past decade, the onset age of this disease has tended to become younger, and the incidence rate in young women has increased steadily year by year[4]. EC risk factors include persistent estrogen stimulation without progesterone antagonism[5], obesity, diabetes, and hypertension, and infertility[6]. However, the molecular pathogenesis of EC remains unclear.

Previous research have shown that in the process of tumour formation and progression, in addition to the activation of related proto-oncogenes and inactivation of tumour suppressor genes, inflammatory stimulation and avoidance of immune surveillance are also important pathogenic factors[7]. Therefore, the tumour microenvironment[8] has become a research hotspot, as it is composed of tumour-related cells, inflammatory cells, immune cells, various cytokines secreted by related cells, and extracellular matrix[9]. Evidence from multiple sources has suggested that a critical factor in the occurrence and development of EC is the inflammatory microenvironment, and various inflammatory immune responses jointly promote the angiogenesis, proliferation, and invasion of EC[10].

Transforming growth factor-β-activated-activated kinase 1 (TAK1) binding protein 2 (TAB2) is crucial to tumour necrosis factor (TNF) receptor-associated factor 6 (TRAF6) as a K63-polyubiquitin-binding TAK1 adaptor protein[11], which is critical to TAK1 activation and downstream interleukin (IL)-1β induced nuclear factor-κB and mitogen-activated protein kinase pathway activation[12,13]. TNF and IL-1-induced signal pathway activation is vital to inflammation, immunity, and cancer development[14]; TAB2 has been suggested to be meaningful in several diseases. Moreover, the *TAB2* gene, which encodes TAB2 protein, is identified to be significantly correlated with diseases such as coronary heart disease[15], dilated cardiomyopathy (DCM)[16], congenital heart disease[17], breast cancer[18], and epithelial ovarian cancer[19]. Endometrial, breast, and ovarian cancers share some hormonal and epidemiologic risk factors[20]. Currently, no studies have been conducted on EC and *TAB2* gene variation.

With the implementation of the Human Genome Project, single nucleotide polymorphism (SNP) research has become an essential approach in studying disease-related genes[21]. Single nucleotide polymorphisms are the most common genetic variations in the human genome, and they can affect the expression of a gene, leading to certain changes in cells. To date, genome-wide association studies have confirmed multiple SNPs associated with EC[22,23]. Based on the above, we hypothesized that *TAB2* gene polymorphism was associated with EC risk. To test our hypothesis, we conducted the following studies to assess the role of rs237028 (A/G), rs521845 (T/G), and rs652921(T/C). To our knowledge, this study would be the first to evaluate the correlation between *TAB2* gene polymorphism and EC susceptibility.

Materials and Methods

Study Subjects

In this retrospective study, 270 EC women (mean age: 51.63±9.79 years) were recruited from the West China Second Hospital of Sichuan University from July 2010 to July 2016. All patients were diagnosed with EC by pathologists after histologic examination of biopsy tissue. Patients with autoimmune diseases or other malignancies were excluded to avoid multifactorial effects. The tumour stage is defined by the International Federation of Gynecology and Obstetrics (FIGO) surgical staging system for EC. The control group consisted of 294 healthy women (mean age: 51.86±12.70 years) who had no abnormal EC clinical symptoms and other underlying diseases and were randomly selected from routine physical examinations.

The present study was approved by the Medical Ethical Review Committee of West China Second Hospital of Sichuan University (approval number: 038, date: 03.03.2022), and all participants gave informed consent.

DNA Extraction and Genotyping

The DNA isolation kit (BioTeke, Peking, China) was used to extract subjects' genomic DNA from blood samples per the manufacturer's instructions and stored the DNA at -20 °C. The rs237028 (A/G), rs521845 (T/G), and rs652921 (T/C) SNPs in the *TAB2* gene were genotyped using polymerase chain reaction (PCR)-restriction fragment length polymorphism analysis. Primer3.0 software was used to design primer sequences, which are shown in Table 1. The total volume of the PCR reaction was 10 mL, including 100 ng of the genomic DNA, 5 mL 2× Power Taq PCR MasterMix (BioTeke, Peking, China), 0.15 mL each primer, 3.7 mL ddH_2O. The PCR cycle conditions of all SNPs were 95 °C for 4 minutes, followed by 30 cycles at 94 °C for 30 seconds, 60 °C for 30 seconds, 72 °C for 30 seconds and a final extension at 72 °C for 10 minutes. Then, PCR products were digested by restriction enzymes (shown in Table 1).

Table 1. Information on PCR-RFLP in enrolled subjects

SNPs	Primer sequence	Major/minor gene	Annealing temperature (°C)	Restriction enzyme	Product size (Bp)
rs237028	F:5′-GCAGACTTGGAAAAGCAAACA-3′	A/G	58.0	Hpy188I	A: 138
	R: 5′-CCAGCCTGAGCAACAAGAG-3′				G: 32 + 106
rs521845	F: 5′-TAGGGCGGTTGAGAAGTGAA-3′	T/G	60.0	AclI	T: 120
	R: 5′-CCTGGGTGACTGAGCTCTTA-3′				G: 20 + 120
rs652921	F: 5’-GGCCATTTGGCTCAGAAAT-3′	T/C	62.0	BsaJI	T: 104
	R: 5’-GAGGGAGCTCAGTGGAATTG-3′				C: 21 + 83

PCR: Polymerase chain reaction, RFLP: Restriction fragment length polymorphisms, SNP: Single nucleotide polymorphism, Bp: Base pair

Finally, the genotype of the sites was determined through the Fragment Analyzer 96 Automated CE system (AATI, America); about 10% of the samples were randomly selected for repeated determination, and the results were 100% consistent.

Statistical Analysis

Version 20.0 of the SPSS software package for Windows (SPSS Inc., Chicago, IL, USA) was used to analyze the data. The genotype associations between the *TAB2* gene and EC were calculated by SNPstats online analysis software (https://www.snpstats.net/start.htm), which assessed the frequency distributions of four genetic models (codominant, dominant, recessive, and overdominant) in EC women and healthy controls. Chi-square tests were conducted to compare allele frequencies, genotype distributions, and the Hardy-Weinberg equilibrium between the two groups. Odds ratios (ORs) and respective 95% confidence intervals (CIs) were used to assess the influences of different alleles and genotypes. The primary parameters compared included allele frequency, genotype distribution, OR, and 95% CI between EC patients and controls. P<0.05 was considered statistically significant.

Results

Clinical Characteristics and Hardy-Weinberg Equilibrium Test

Between the EC patients and control subjects, there was no statistically significant difference (p>0.05) in general characteristics, such as mean age, body mass index (BMI), menopausal ratio, pregnancy history, etc. (shown in Table 2). Among the 270 EC patients, 247 patients had abnormal uterine bleeding, and 268 had received surgical treatment. The primary histopathological type of these patients was endometrioid adenocarcinoma. The genotypes of the three SNPs (rs237028, rs521845, rs652921) in the two groups all conformed to Hardy-Weinberg equilibrium.

TAB2 Gene Polymorphisms and EC Susceptibility

The differences in the allele frequencies and genotypes of the three SNPs between patients with EC and controls are presented in Table 3. The genotype distributions of T/T, T/G, and G/G for rs521845 were 32.1%, 52.2%, and 15.7% in the case group and 43.5%, 44.9%, and 11.6% in the control group, respectively. Significance had been observed in the codominant model (p=0.017). In the dominant model, compared with the T/T genotype, T/G or G/G genotypes were associated with a significantly decreased risk of EC (p=0.0051, OR: 0.61, 95% CI: 0.43-0.87). Similarly, the frequency of G allele of rs521845 was higher in patients (42%) than in controls (34%) (p=0.08, OR: 0.72, 95% CI: 0.56-0.91). Meanwhile, similar results were observed between patients with endometrioid adenocarcinoma and controls. No significant correlation was found between EC and rs237028 or rs652921.

Association Between *TAB2* Gene Polymorphisms and Clinical Features

To learn more about the association between the three SNPs and EC, we stratified these EC patients according to age, BMI, family history of cancer, menopausal status, histological types, FIGO grades, myometrium invasion, parametrial invasion, cervical invasion, and lymph nodes status (Tables 4, 5, and 6). It was observed that for rs237028, the percentage of AG genotype in patients with highly differentiated tumours (G1) was significantly higher than that in moderately differentiated patients (G2/G3) (p=0.031, OR: 0.77, 95% CI: 0.45-1.30). In addition, there were no statistically significant differences among subgroups of other SNPs.

Discussion

The *TAB2* gene maps on chromosome 6q25.1 and encodes a scaffold protein that forms a kinase complex that links TRAF6 and TAK1, thus determining TAK1 activation[24]. TAK1 has been implicated in regulating various cellular processes, including embryonic development, differentiation, autophagy, apoptosis, and cell survival has been thought to be related to the occurrence and development of cancer[25]. TAB2, as an essential protein for TAK1 activation, has been studied in many diseases. Initially, Sanjo et al.[26] found that TAB2 was necessary for embryonic development by preventing

Table 2. Baseline characteristics of endometrial cancer patients and health controls

Characteristics	Number of case (%)	Number of controls (%)	p
Sample size	270	294	
Age mean ± SD (range) (year)	51.63±9.79 (25-81)	51.86±12.70 (28-75)	0.81
BMI mean ± SD (kg/m^2)	24.14±3.42	24.32±3.26	0.59
History of pregnancy			
Yes	254 (94.1%)	277 (94.2%)	0.54
No	16 (5.9%)	17 (5.8%)	
Menopausal status			
Premenopausal	131 (48.5%)	128 (43.5%)	0.14
Postmenopausal	139 (51.5%)	166 (56.5%)	
Family history of cancer			
Yes	21 (7.8%)	19 (6.5%)	0.33
No	249 (92.2%)	275 (93.5%)	
Abnormal uterine bleeding			
Yes	254 (94.1%)		
No	13 (4.8%)		
FIGO grade			
G1	97 (35.9%)		
G2	98 (36.3%)		
G3	75 (27.8%)		
FIGO stage			
I	205 (75.9%)		
II	22 (8.1%)		
III	29 (10.7%)		
IV	12 (4.4%)		
Unknown	2 (0.7%)		
Histology			
Endometrioid adenocarcinoma	228 (84.4%)		
Non-endometrioid adenocarcinoma	42 (15.6%)		
Myometrial invasion			
<1/2	167 (61.9%)		
≥1/2	60 (22.2%)		
No	43 (15.9%)		
Parametrial invasion			
Yes	23 (8.5%)		
No	245 (90.7%)		
Cervical invasion			
Yes	43 (15.9%)		
No	225 (83.3%)		

Table 2. Continued

Characteristics	Number of case (%)	Number of controls (%)	p
Vascular invasion			
Yes	23 (8.5%)		
No	216 (80.0%)		
Lymph node status			
Yes	23 (8.5%)		
No	216 (80.0%)		
IHC			
ER (+)	198/222		
PR (+)	188/221		
P53 (+)	132/212		

SD: Standard deviation, BMI: Body mass index, FIGO: International Federation of Gynecology and Obstetrics, IHC: Immunohistochemistry, ER: Estrogen, PR: Progesterone

hepatocyte apoptosis. Owerbach et al.[27] thatfirst identified that the *TAB2* gene was associated with susceptibility to type 1 diabetes mellitus. It's worth noting that in Thienpont et al.'s[28] study, the *TAB2* gene was expressed in the developing heart and was mutated, deleted, or disrupted by a translocation among congenital heart defect patients. This suggested that the gene plays a vital role in the development of embryos and the formation of heart valves, a finding that has been linked to much subsequent research on the gene. Weiss et al.'s[29] report supported the association of TAB2 haploinsufficiency with various congenital heart defects. Cheng et al.[30,31] found that TAB2 microdeletion is a risk factor for hypoplastic left heart syndrome (HLHS) and proposed the necessity of SNP microarray analysis and molecular testing for a TAB2 loss of function variant in HLHS patients.

TAB2 is not just part of the inflammatory pathway, but also considered a potential target to potentiate antiestrogen action[32]. Evidence provided by Reineri et al.[18] suggested that TAB2, in conjunction with the nuclear receptor corepressor complex and its novel functional domain, interacts with estrogen receptors in breast cancer cells. EC's leading risk factor is exposure to endogenous and exogenous oestrogens, and estrogen requires estrogen to function physiologically through estrogen receptor α (ERS1). The *ESR1* gene, encoding the estrogen receptor 1, is an identified oncogene for EC[33]. It is worth noting that the *TAB2* gene is close to the *ESR1* gene on the chromosome. This suggests that the *TAB2* gene may be interlinked with the *ESR1* gene, jointly affecting the occurrence and development of EC.

In the latest study, Shen et al.[16] confirmed that the *TAB2* gene polymorphism is associated with susceptibility to DCM in a Chinese population. Furthermore, their results showed that the risk of DCM was higher among G (A/G-G/G) carriers of rs237028, C carriers (C/T-C/C) of rs652921, and G carriers (T/G-G/G) of rs521845. Interestingly, Huang et al.[19] showed that only rs237028 polymorphism in the *TAB2* gene was significantly associated with ovarian cancer susceptibility.

Although *TAB2* gene variants are significantly associated with various diseases, the association with EC remains unclear. Accordingly, we decided to investigate whether the *TAB2* gene polymorphism has an impact on EC. We selected the three SNPs located on the intron of the *TAB2* gene, may influence the regulation of gene expression. In the present study, we first confirmed that the rs521845 polymorphism in TAB2 is significantly associated with EC susceptibility. Our data showed that the decrease in EC risk was related to the T allele of rs521845. Moreover, in both codominant and dominant models, the TT genotype of rs521845 was correlated with a lower EC risk. Therefore, TAB2 might be a potential protective factor for EC. However, the other two SNPs (rs237028, rs652921) had no significant link with EC.

Conclusion

According to the degree of differentiation of EC tissue, EC tissue differentiation can be classified as the grade 1 (highly differentiated, G1), the grade 2 (moderately differentiated, G2), and the grade 3 (poorly differentiated, G3). The lower the degree of differentiation, the more malignancy is exhibited. For rs237028, patients with A/G genotype had lower-grade tumours (G1), compared to patients with genotype A/A or G/G. Stratified analysis of the three SNP genotypes of the *TAB2* gene showed no significant differences in age, BMI, family history, and menopause history. Although rs521845 variation in TAB2 was associated with EC susceptibility, there were no significant differences in the clinicopathological features of EC such as pathological stage, histological grade, and histological type among patients with different genotypes.

Table 3. Distribution of SNPs in TAB2 between patients and controls as well as their association with endometrial cancer risk

		Genotype	Cases		Controls n=294 (%)	Logistic regression			
						Cases vs. controls		EA vs. control	
			Total n=270 (%)	EA n=228 (%)		OR (95% CI)	p	OR (95% CI)	p
rs237028									
Genetic model	Codominant	A/A	142 (55.9%)	116 (54%)	177 (60.2%)	1.00	0.36	1.00	0.15
		A/G	99 (39%)	89 (41.4%)	98 (33.3%)	0.79 (0.56-1.13)		0.72 (0.50-1.05)	
		G/G	13 (5.1%)	10 (4.7%)	19 (6.5%)	1.17 (0.56-2.46)		1.25 (0.56-2.77)	
	Dominant	A/A	142 (55.9%)	116 (54%)	177 (60.2%)	1.00	0.31	1.00	0.16
		A/G-G/G	112 (44.1%)	99 (46%)	117 (39.8%)	0.84 (0.60-1.18)		0.77 (0.54-1.11)	
	Recessive	A/A-A/G	241 (94.9%)	205 (95.3%)	275 (93.5%)	1.00	0.50	1.00	0.38
		G/G	13 (5.1%)	10 (4.7%)	19 (6.5%)	1.28 (0.62-2.65)		1.42 (0.64-3.11)	
	Overdominant	A/A-G/G	155 (61%)	126 (58.6%)	196 (66.7%)	1.00	0.17	1.00	0.06
		A/G	99 (39%)	89 (41.4%)	98 (33.3%)	0.78 (0.55-1.11)		0.71 (0.49-1.02	
Allele		A	383 (75%)	321 (75%)	452 (77%)	1.00	0.57	1.00	0.41
		G	125 (25%)	109 (25%)	136 (23%)	0.92 (0.70-1.22)		0.89 (0.66-1.18)	
rs521845									
Genetic model	Codominant	T/T	86 (32.1%)	72 (31.7%)	128 (43.5%)	1.00	**0.017**	1.00	**0.017**
		T/G	140 (52.2%)	118 (52%)	132 (44.9%)	0.63 (0.44-0.91)		0.63 (0.43-0.92)	
		G/G	42 (15.7%)	37 (16.3%)	34 (11.6%)	0.54 (0.32-0.92)		0.52 (0.30-0.89)	
	Dominant	T/T	86 (32.1%)	72 (31.7%)	128 (43.5%)	1.00	**0.0051**	1.00	**0.0057**
		T/G-G/G	182 (67.9%)	155 (68.3%)	166 (56.5%)	0.61 (0.43-0.87)		0.60 (0.42-0.87)	
	Recessive	T/T-T/G	226 (84.3%)	190 (83.7%)	260 (88.4%)	1.00	0.16	1.00	0.12
		G/G	42 (15.7%)	37 (16.3%)	34 (11.6%)	0.70 (0.43-1.14)		0.67 (0.41-1.11)	
	Overdominant	T/T-G/G	128 (47.8%)	109 (48%)	162 (55.1%)	1.00	0.17	1.00	0.11
		T/G	140 (52.2%)	118 (52%)	132 (44.9%)	0.78 (0.55-1.11)		0.75 (0.53-1.07)	
Allele		T	312 (58%)	262 (58%)	388 (66%)	1.00	**0.008**	1.00	**0.007**
		G	224 (42%)	192 (42%)	200 (34%)	0.72 (0.56-0.91)		0.70 (0.55-0.91)	

Table 3. Continued

		Genotype	Cases		Controls n=294 (%)	Logistic regression			
						Cases vs. controls		EA vs. control	
			Total n=270 (%)	EA n=228 (%)		OR (95% CI)	p	OR (95% CI)	p
rs652921									
Genetic model	Codominant	T/T	74 (29.5%)	59 (28.1%)	98 (33.3%)	1.00	0.60	1.00	0.45
		C/T	126 (50.2%)	104 (49.5%)	137 (46.6%)	0.82 (0.56-1.21)		0.79 (0.53-1.20)	
		C/C	51 (20.3%)	47 (22.4%)	59 (20.1%)	0.87 (0.54-1.41)		0.76 (0.46-1.25)	
	Dominant	T/T	74 (29.5%)	59 (28.1%)	98 (33.3%)	1.00	0.33	1.00	0.21
		C/T-C/C	177 (70.5%)	151 (71.9%)	196 (66.7%)	0.84 (0.58-1.20)		0.78 (0.53-1.15)	
	Recessive	T/T-C/T	200 (79.7%)	163 (77.6%)	235 (79.9%)	1.00	0.94	1.00	0.53
		C/C	51 (20.3%)	47 (22.4%)	59 (20.1%)	0.98 (0.65-1.50)		0.87 (0.57-1.34)	
	Overdominant	T/T-C/C	125 (49.8%)	106 (50.5%)	157 (53.4%)	1.00	0.40	1.00	0.52
		C/T	126 (50.2%)	104 (49.5%)	137 (46.6%)	0.87 (0.62-1.21)		0.89 (0.62-1.27)	
Allele		T	274 (55.0%)	222 (53.0%)	333 (57.0%)	1.00	0.50	1.00	0.25
		C	228 (45.0%)	198 (47.0%)	255 (43.0%)	0.92 (0.72-1.17)		0.86 (0.67-1.10)	

SNP: Single nucleotide polymorphism, TAB2: Transforming growth factor-β-activated kinase 1 binding protein 2, EA: Endometrioid adenocarcinoma, OR: Odds ratio, CI: Confidence interval

Table 4. Association between the genotype distribution of rs237028 polymorphism of *TAB2* gene and clinical features

Clinical characteristics		Genotype			Genetic model							
		AA	AG	GG	Codominant (AA vs. AG vs. GG)		Dominant (AA vs. AG/GG)		Recessive (AA/AG vs. GG)		Overdominant (AA/GG vs. AG)	
					OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
Age	<50	57			AG: 0.95 (0.56-1.60)	0.76	1.00 (0.60-1.65)	1.00	1.54 (0.46-5.15)	0.47	0.92 (0.55-1.53)	0.74
	≥50	85	58	9	GG: 1.51 (0.44-5.13)							
BMI	<27	112	81	11	AG: 0.83 (0.43-1.59)	0.78	0.81 (0.43-1.52)	0.51	0.73 (0.16-3.41)	0.68	0.85 (0.45-1.62)	0.63
	≥27	30	18	2	GG: 0.68 (0.14-3.23)							
Family history of cancer	Negative	135	92	10	AG: 1.47 (0.50-4.32)	0.11	1.89 (0.70-5.14)	0.21	4.86 (1.20-19.70)	0.049	1.10 (0.41-3.00)	0.85
	Positive	7	7	3	GG: 5.79 (1.29-25.86)							
Menopausal status	Premenopausal	68	49	5	AG: 0.94 (0.56-1.57)	0.75	0.99 (0.60-1.62)	0.96	1.51 (0.48-4.75)	0.48	0.91 (0.55-1.50)	0.71
	Postmenopausal	74	50	8	GG: 1.47 (0.46-4.71)							
Pathological type	EA	116	89	10	AG: 0.50 (0.23-1.09)	0.15	0.59 (0.29-1.20)	0.14	1.71 (0.45-6.51)	0.45	0.49 (0.23-1.05)	0.05
	Non-EA	26	10	3	GG: 1.34 (0.34-5.21)							
FIGO grade	G1	50	41	1	AG: 0.77 (0.45-1.30)	**0.031**	0.91 (0.54-1.52)	0.71	7.28 (0.93-56.84)	**0.014**	0.69 (0.41-1.17)	0.17
	G2-G3	92	58	12	GG: 6.52 (0.83-51.55)							
FIGO stage	I	105	78	10	AG: 0.81 (0.44-1.49)	0.79	0.82 (0.45-1.48)	0.50	0.98 (0.26-3.69)	0.98	0.81 (0.44-1.49)	0.51
	II-IV	35	21	3	GG: 0.90 (0.23-3.46)							
Myometrial invasion	<1/2	117	84	12	AG: 0.80 (0.42-1.51)	0.58	0.76 (0.41-1.41)	0.38	0.56 (0.12-2.63)	0.44	0.84 (0.44-1.58)	0.59
	≥1/2	84	64	10	GG: 0.51 (0.11-2.45)							
Parametrial invasion	Negative	125	92	12	AG: 0.63 (0.25-1.62)	0.61	0.64 (0.26-1.57)	0.32	0.82 (0.10-6.62)	0.85	0.65 (0.26-1.65)	0.35
	Positive	15	7	1	GG: 0.69 (0.08-5.72)							
Cervical invasion	Negative	119	82	11	AG: 1.17 (0.58-2.36)	0.90	1.16 (0.59-2.28)	0.67	0.96 (0.20-4.51)	0.96	1.17 (0.59-2.32)	0.65
	Positive	21	17	2	GG: 1.03 (0.21-4.98)							
Vascular invasion	Negative	117	84	12	AG: 0.91 (0.45-1.84)	0.66	0.85 (0.42-1.70)	0.64	0.44 (0.06-3.49)	0.39	0.96 (0.48-1.94)	0.91
	Positive	23	15	1	GG: 0.42 (0.05-3.42)							
Lymph node status	Negative	112	80	10	AG: 0.60 (0.22-1.63)	0.44	0.71 (0.29-1.77	0.46	1.92 (0.39-9.38)	0.45	0.57(0.21-1.52)	0.25
	Positive	14	6	2	GG: 1.60 (0.32-8.06)							

TAB2: Transforming growth factor-β-activated kinase 1 binding protein 2, OR: Odds ratio, CI: Confidence interval, BMI: Body mass index, EA: Endometrioid adenocarcinoma, FIGO: International Federation of Gynecology and Obstetrics

Table 5. Association between the genotype distribution of rs652921 polymorphism of *TAB2* gene and clinical features

Clinical characteristics		Genotype			Genetic model							
		TT	TG	GG	Codominant (TT vs. TG vs. GG)		Dominant (TT vs. TG/GG)		Recessive (TT/TG vs. GG)		Overdominant (TT/GG vs.TG)	
					OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
Age	<50	36	56	16	TG: 1.08 (0.63-1.86)	0.92	1.10 (0.65-1.85)	0.72	1.12 (0.57-2.20)	0.75	1.03 (0.63-1.67)	0.92
	≥50	50	84	26	GG: 1.17 (0.55-2.49)							
BMI	<27	67	117	32	TG: 0.69 (0.35-1.37)	0.43	0.78 (0.41-1.47)	0.45	1.37 (0.62-3.00)	0.44	0.67 (0.36-1.23)	0.2
	≥27	19	23	10	GG: 1.10 (0.46-2.64)							
Family history of cancer	Negative	80	129	38	TG: 1.14 (0.40-3.19)	0.88	1.20 (0.45-3.20)	0.72	1.29 (0.41-4.06)	0.67	1.01 (0.41-2.46)	0.99
	Positive	6	11	4	GG: 1.40 (0.37-5.27)							
Menopausal status	Premenopausal	42	67	21	TG: 1.04 (0.61-1.78)	0.97	1.02 (0.61-1.70)	0.94	0.93 (0.48-1.80)	0.83	1.06 (0.65-1.71)	0.82
	Postmenopausal	44	73	21	GG: 0.95 (0.46-2.00							
Pathological type	EA	72	118	37	TG: 0.96 (0.46-1.99)	0.79	0.90 (0.44-1.81)	0.76	0.71 (0.26-1.94)	0.49	1.07 (0.55-2.08)	0.84
	Non-EA	14	22	5	GG: 0.69 (0.23-2.08)							
FIGO grade	G1	25	56	15	TG: 0.61 (0.35-1.09)	0.25	0.64 (0.37-1.11)	0.11	1.01 (0.51-2.00)	0.99	0.68 (0.41-1.13)	0.13
	G2-G3	61	84	27	GG: 0.74 (0.34-1.62)							
FIGO stage	I	60	108	36	TG: 0.69 (0.37-1.27)	0.14	0.62 (0.34-1.11)	0.11	0.50 (0.20-1.25)	0.11	0.89 (0.50-1.57)	0.68
	II-IV	25	31	6	GG: 0.40 (0.15-1.07)							
Myometrial invasion	<1/2	53	83	30	TG: 0.77 (0.41-1.47)	0.24	0.68 (0.37-1.26)	0.23	0.51 (0.20-1.30)	0.14	0.97 (0.53-1.75)	0.91
	≥1/2	24	29	6	GG: 0.44 (0.16-1.20							
Parametrial invasion	Negative	74	129	40	TG: 0.52 (0.21-1.29	0.21	0.48 (0.20-1.13)	0.09	0.48 (0.11-2.14)	0.3	0.68 (0.29-1.61)	0.38
	Positive	11	10	2	GG: 0.34 (0.07-1.59)							
Cervical invasion	Negative	70	115	38	TG: 0.97 (0.48-1.98)	0.40	0.85 (0.43-1.70)	0.65	0.50 (0.17-1.48)	0.18	1.19 (0.62-2.29)	0.61
	Positive	15	24	4	GG: 0.49 (0.15-1.58)							
Vascular invasion	Negative	71	121	35	TG: 0.75 (0.35-1.61)	0.71	0.81 (0.40-1.66)	0.57	1.20 (0.49-2.93)	0.69	0.75 (0.38-1.48)	0.41
	Positive	14	18	7	GG: 1.01 (0.38-2.74)							
Lymph node status	Negative	62	118	35	TG: 0.43 (0.17-1.09)	0.13	0.41 (0.17-0.98)	0.048	0.51 (0.11-2.30)	0.35	0.57 (0.23-1.39)	0.21
	Positive	11	9	2	GG: 0.32 (0.07-1.54)							

TAB2: Transforming growth factor-β-activated kinase 1 binding protein 2, OR: Odds ratio, CI: Confidence interval, BMI: Body mass index, EA: Endometrioid adenocarcinoma, FIGO: International Federation of Gynecology and Obstetrics

Table 6. Association between the genotype distribution of rs5652921 polymorphism of *TAB2* gene and clinical features

Clinical characteristics		Genotype			Genetic model							
		TT	TC	CC	Codominant (TT vs.TC vs.CC)		Dominant (TT vs. TC/CC)		Recessive (TT/TC vs. CC)		Overdominant (TT/CC vs. TC)	
					OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
Age	<50				TC: 1.08 (0.60-1.95)	0.63	0.99 (0.57-1.72)	0.96	0.75 (0.40-1.39)	0.36	1.20 (0.72-1.99)	0.49
	≥50	45	79	28	CC: 0.78 (0.38-1.62)							
BMI	<27	58	105	39	TC: 0.72 (0.35-1.50)	0.50	0.83 (0.42-1.62)	0.59	1.36 (0.65-2.84)	0.43	0.69 (0.37-1.30)	0.25
	≥27	16	21	12	CC: 1.12 (0.48-2.61)							
Family history of cancer	Negative	69	118	46	TC: 0.94 (0.29-2.97)	0.73	1.09 (0.38-3.19)	0.87	1.56 (0.53-4.61)	0.43	0.78 (0.30-2.05)	0.61
	Positive	5	8	5	CC: 1.50 (0.41-5.47)							
Menopausal status	Premenopausal	35	57	28	TC: 1.09 (0.61-1.93)	0.5	0.97 (0.56-1.67)	0.92	0.70 (0.38-1.30)	0.26	1.23 (0.75-2.02)	0.41
	Postmenopausal	39	69	23	CC: 0.74 (0.36-1.51)							
Pathological type	EA	59	104	47	TC: 0.83 (0.40-1.73)	0.13	0.68 (0.34-1.37)	0.28	0.37 (0.13-1.11)	0.049	1.18 (0.60-2.31)	0.63
	Non-EA	15	22	4	CC: 0.33 (0.10-1.08							
FIGO grade	G1	25	47	18	TC: 0.86 (0.47-1.57)	0.88	0.88 (0.50-1.56)	0.66	1.03 (0.54-1.96)	0.93	0.88 (0.53-1.48)	0.63
	G2-G3	49	79	33	CC: 0.94 (0.44-1.98)							
FIGO stage	I	54	92	42	TC: 1.02 (0.53-1.97)	0.42	0.89 (0.48-1.67)	0.72	0.60 (0.27-1.32)	0.19	1.23 (0.69-2.19)	0.48
	II-IV	19	33	9	CC: 0.61 (0.25-1.48)							
Myometrial invasion	<1/2	49	69	35	TC: 1.42 (0.70-2.87)	0.41	1.24 (0.63-2.41)	0.53	0.70 (0.32-1.53)	0.36	1.50 (0.82-2.75)	0.19
	≥1/2	16	32	10	CC: 0.87 (0.36-2.16)							
Parametrial invasion	Negative	65	114	47	TC: 0.78 (0.30-2.05)	0.82	0.69 (0.20-2.43)	0.55	0.80 (0.26-2.47)	0.69	0.90 (0.38-2.13)	0.81
	Positive	8	11	4	CC: 0.69 (0.20-2.43)							
Cervical invasion	Negative	62	101	44	TC: 1.34 (0.61-2.92)	0.6	1.21 (0.57-2.55)	0.62	0.74 (0.31-1.78)	0.49	1.40 (0.72-2.73)	0.32
	Positive	11	24	7	CC: 0.90 (0.32-2.50)							
Vascular invasion	Negative	62	107	44	TC: 0.95 (0.42-2.14)	0.98	0.93 (0.43-2.01)	0.86	0.93 (0.38-2.26)	0.87	0.99 (0.49-2.01)	0.98
	Positive	11	18	7	CC: 0.90 (0.32-2.50)							
Lymph node status	Negative	56	100	43	TC: 0.77 (0.29-2.03)	0.78	0.73 (0.29-1.83)	0.51	0.76 (0.25-2.36)	0.63	0.91 (0.38-2.15)	0.83
	Positive	8	11	4	CC: 0.65 (0.18-2.31)							

TAB2: Transforming growth factor-β-activated kinase 1 binding protein 2, OR: Odds ratio, CI: Confidence interval, BMI: Body mass index, EA: Endometrioid adenocarcinoma, FIGO: International Federation of Gynecology and Obstetrics

In summary, polymorphisms found in the rs521845 site of the *TAB2* gene may serve as a novel genetic marker of susceptibility to EC in Chinese women. The findings of other independent research groups suggest that the *TAB2* gene may play an essential role in EC's molecular pathogenesis. However, the function and the underlying signal transduction mechanisms of TAB2 in EC development need to be clarified. This study has certain limitations. Although these deficiencies were random, due to incomplete clinical information of some patients, they may affect the accuracy and objectivity of stratified analysis results. Therefore, these findings need to be further confirmed in a larger cohort. Secondly, the functions of the three SNPs studied in this research and the potential mechanism of rs521845 SNP in the development of EC are still unclear. Further investigations are warranted.

Ethics

Ethics Committee Approval: The present study was approved by the Medical Ethical Review Committee of West China Second Hospital of Sichuan University (approval number: 038, date: 03.03.2022).

Informed Consent: Informed consent was obtained from all participants.

Footnotes

Authorship Contributions

Concept: Y.W., Design: Y.W., Data Collection or Processing: S.L., Analysis or Interpretation: S.L., Literature Search: S.L., Writing: S.L.

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

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Impact of sympathetic denervation via paraaortic lymphadenectomy on blood pressure in endometrial cancer patients

Paraaortik lenfadenektomi ile sempatik denervasyonun kan basıncı üzerine etkisi

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Abstract

Objective: To evaluate the effect of para-aortic lymphadenectomy on blood pressure changes in endometrial cancer patients.

Materials and Methods: This retrospective study included patients with endometrial cancer treated surgically between 2017 and 2023. Patients undergoing para-aortic lymphadenectomy, up to the renal artery, in a non-nerve-sparing fashion, were compared with those undergoing pelvic lymphadenectomy or sentinel lymph node mapping. Data collected included age, body mass index, comorbidities including hypertension, diabetes mellitus, coronary artery disease, operative time, number of lymph nodes removed, tumor size, and postoperative complications. Preoperative blood pressure was recorded during outpatient visits, and postoperative measurements were collected daily during hospitalization and at follow-up visits. Statistical analyses assessed differences in systolic and diastolic blood pressure changes, operative outcomes, and complications.

Results: A total of 264 patients were analyzed. Patients in the para-aortic group had significantly longer operative times. Tumor size was larger in the para-aortic group than in another group. Systolic blood pressure decreased significantly in the para-aortic group compared to the control group (para-aortic: -17 mmHg *vs.* non-para-aortic: -1.10 mmHg, $p<0.05$), with a similar trend for diastolic pressure (-8.00 mmHg *vs.* -0.80 mmHg, $p<0.05$). Chylous ascites (15.6% *vs.* 5.6%) and ileus (0% *vs.* 12%) were more common in the para-aortic group, along with the administration of radiotherapy and chemotherapy. Both systolic and diastolic blood pressures were significantly lower in paraaortic group, in both early and late postoperative follow-up measures ($p<0.005$).

Conclusion: Aortic lymphadenectomy is associated with decreased blood pressure and may have therapeutic potential for hypertensive patients, highlighting the need for prospective randomized studies to explore this effect further.

Keywords: Cancer of endometrium, hypertension, lymph node excison

Öz

Amaç: Bu çalışmanın amacı sinir koruyucu olmayan paraaortik lenfadenektominin paraaortik ve renal bölgedeki sempatik sinir liflerini etkileyerek kan basıncı üzerindeki değişimlerini değerlendirmektir.

PRECIS: We evaluated the impact of para-aortic lymphadenectomy on blood pressure levels in endometrial cancer patients, demonstrating significant reductions linked to sympathetic nerve disruption.

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Gereç ve Yöntemler: Bu retrospektif çalışma, 2017-2023 yılları arasında cerrahi tedavi uygulanan endometrium kanseri hastalarını içermektedir. Renal arter seviyesine kadar sinir koruyucu olmayan paraaortik lenfadenektomi yapılan hastalar, pelvik lenfadenektomi veya sentinel lenf nodu haritalaması yapılan hastalarla karşılaştırılmıştır. Toplanan veriler arasında yaş, vücut kitle indeksi, hipertansiyon, diyabet, koroner arter hastalığı gibi komorbiditeler, operasyon süresi, çıkarılan lenf nodu sayısı, tümör boyutu ve postoperatif komplikasyonlar yer almaktadır. Preoperatif kan basıncı ölçümleri ameliyat öncesi poliklinik değerlendirmesinde, postoperatif ölçümler ise hastanede yatış süresince ve onkolojik takip sırasında kaydedilmiştir. Kan basıncı değişimleri, operasyon sonuçları ve komplikasyonlar arasındaki farklar istatistiksel olarak analiz edilmiştir.

Bulgular: Toplam 264 hasta çalışmaya dahil edilmiştir. Paraaortik grupta operasyon süresi daha uzun ve tümör boyutları daha büyük bulunmuştur. Sistolik kan basıncı paraaortik grupta kontrol grubuna kıyasla anlamlı olarak daha fazla düşmüştür (-17 mmHg *vs.* -1,10 mmHg, p<0,05). Benzer şekilde, diyastolik kan basıncı da paraaortik grupta daha fazla düşmüştür (-8,00 mmHg *vs.* -0,80 mmHg, p<0,05). Şilöz asit (%15,6 *vs.* %5,6), ileus (%12 *vs.* %0) ve adjuvan radyoterapi/kemoterapi uygulaması paraaortik grupta daha sık gözlenmiştir. Postoperatif erken dönemde ve uzun vadeli takiplerde sistolik ve diyastolik kan basınçlarındaki düşüşler anlamlı şekilde korunmuştur (p<0,005).

Sonuç: Paraaortik lenfadenektomi, sistolik ve diastolik kan basınçlarında anlamlı düşüşlerle ilişkilidir ve hipertansif hastalar için tedavi edici potansiyele sahip olabilir.

Anahtar Kelimeler: Endometriyum kanseri, hipertansiyon, lenf nodu diseksiyonu

Introduction

Hypertension is a common condition in the general population and a significant cause of morbidity, particularly among older adults[1]. In patients with endometrial cancer, hypertension is a frequently associated comorbidity, observed both in the presence and absence of obesity[2]. While obesity is a well-known risk factor for hypertension, the coexistence of metabolic syndrome further increases the prevalence of both hypertension and endometrial cancer[2]. This suggests that metabolic factors may create a common underlying pathway between these two conditions.

Hypertension is a major comorbidity among patients with endometrial cancer, with approximately 40% of these patients requiring antihypertensive medication[3,4]. The most common type of hypertension is essential hypertension and underlying factors include genetic factors, age, lifestyle factors and stress[5]. Initial treatment strategies typically involve dietary modifications and antihypertensive medications[5]. Numerous studies have demonstrated the involvement of the adrenergic system in blood pressure regulation. Blocking this system is a fundamental mechanism underlying antihypertensive therapy[6]. However, sympathetic fibers located in the paraaortic region, particularly in the perirenal area, have been implicated as a potential cause of hypertension by increasing sodium uptake, renin secretion, and renal arterial vasoconstriction[7]. Based on this knowledge, angiographic radiofrequency ablation has been explored as a treatment option for patients unresponsive to antihypertensive therapies[8]. Studies have reported that this method can lead to an average reduction in blood pressure by approximately 20 mmHg. In some cases, patients no longer required antihypertensive medications[9]. The majority of the studies accumulated after 2020. In patients with endometrial cancer, especially in those at higher risk, retroperitoneal lymphadenectomy is a standard component of surgical management when sentinel lymph node mapping is not available. The cranial boundary of the lymphadenectomy is typically defined at the level of the renal artery. While nerve-sparing approaches exist, periaortic neural structures within the lymphatic tissue are often excised or damaged during systematic lymphadenectomy.

This study is based on the following hypothesis: if blocking neural structures in the para-aortic region can lead to a reduction in blood pressure, it is plausible that para-aortic lymphadenectomy in endometrial cancer patients, may similarly result in decreased postoperative blood pressure levels. Our aim was to evaluate the changes in blood pressure measurements between the preoperative and postoperative periods in patients undergoing para-aortic lymphadenectomy up to the level of the renal arteries.

Materials and Methods

Our study received approval from the Institutional Review Board (İzmir Democracy University Buca Seyfi Demirsoy Training and Research Hospital, Non-Interventional Research Ethics Committee - no: 2023/211, date: 27.12.2023). The study had been reviewed by the appropriate ethics committee and had been performed in accordance with the ethical standards described in an appropriate version of the 1975 Declaration of Helsinki, as revised in 2000. Patients treated for endometrial cancer were identified through the hospital database. The records of patients who underwent surgery between 2017 and 2023 were reviewed. A total of 289 patients with endometrial cancer were initially included in the study. Seven patients who underwent bulky lymph node dissection for cytoreduction in advanced-stage endometrial cancer, and ten patients who underwent para-aortic lymphadenectomy up to the level of the inferior mesenteric artery were excluded from the study. Additionally, eight patients with missing postoperative blood pressure monitoring data were excluded. Figure 1 shows the flowchart of the study. Data collected from patient files included age, gravida, parity, history of previous surgeries, presence of diabetes, coronary artery disease, body mass index, endometrial biopsy results, findings from preoperative imaging studies, pelvic lymphadenectomy, and paraaortic lymphadenectomy. Additionally, those with conditions affecting blood pressure, such as bleeding, hypovolemia, and arrhythmia were excluded from the study. All persons gave their informed consent prior to their inclusion in the study.

The study group comprised patients with endometrial cancer who underwent para-aortic lymphadenectomy up to the level of the renal vein via either laparoscopic transperitoneal, extraperitoneal, or laparotomy approaches. Patients who did not undergo lymphadenectomy; and who underwent pelvic lymphadenectomy, pelvic lymph node sampling, or sentinel lymph node mapping were included in the control group. Preoperative blood pressure measurements were taken during outpatient visits, which typically occurred two weeks prior to surgery. Blood pressure measurements were taken under ideal conditions, with patients seated comfortably in a quiet environment, their back supported, legs uncrossed, and arms at heart level. A properly calibrated and validated blood pressure monitor was used, and measurements were obtained after a 5-minute rest period, avoiding recent physical activity, caffeine, or smoking. Postoperative blood pressure readings were collected daily from the hospital system. On the first postoperative day, measurements were taken hourly, while on subsequent days, they were recorded every six hours unless an unusual situation arose. The daily postoperative blood pressure values reported in our analysis was presented as the average of all measurements taken throughout each day. Patients' blood pressure data were collected throughout their hospitalisation and at follow-up outpatient clinic visits.

Statistical Analysis

Statistical analyses were conducted using the Statistical Package for Social Sciences (SPSS) version 21.0 (IBM Corp., Armonk, NY, USA). The Kolmogorov-Smirnov test was used to assess the normality of the data distribution. Comparisons between groups for normally distributed continuous variables were performed using the independent samples t-test, while the Mann-Whitney U test was used for variables without normal distribution. Categorical variables were analyzed using the chi-square test or Fisher's exact test, as appropriate. The mean differences in systolic and diastolic blood pressure changes between the groups were calculated, and their effect sizes were assessed using Cohen's d. Effect sizes were interpreted as small (0.2), medium (0.5), or large (0.8), based on Cohen's criteria. A p-value of <0.05 was considered statistically significant.

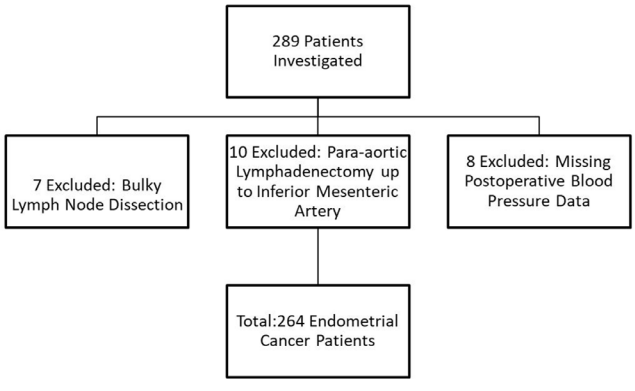

Figure 1. Patient selection process for the study, showing exclusions and final cohort of 264 patients

Results

The mean age of the patients was similar between the groups (61±12 *vs.* 62±10 years, p=0.528), and there was no significant difference in terms of gravida, platelet count or CA-125 levels (p>0.05).

The operation time was longer in paraaortic group (4±1 *vs.* 3±1 hours, p<0.001) and a greater number of pelvic lymph nodes removed (39±17 *vs.* 21±20, p<0.001) as well as number paraaortic lymph nodes collected (42±24 *vs.* 0±0, p<0.001). Tumor size at final pathology was larger in the paraaortic group (4±2 *vs.* 3±2, p<0.001), and hospital stays were longer (p<0.001).

Suspicious lymph nodes were more frequent in the paraaortic group (22.9% *vs.* 9.7%, p=0.016). Additionally, the paraaortic group had significantly higher rates of radiotherapy (65.6% *vs.* 31.9%, p<0.001); chemotherapy (36.5% *vs.* 11.1%, p<0.001). While overall complications like evisceration were not significantly different, chylous ascites was more frequent in the paraaortic group (15.6% *vs.* 5.6%, p=0.030). Similarly, ileus was more frequent in the paraaortic group. Demographic and clinical characteristics of the patients were given in Table 1 and Table 2.

Blood pressure changes were significantly greater in the paraaortic group, with systolic blood pressure showing a mean change of -17.20 compared to -1.10 in the no paraaortic group (p<0.001, Cohen's d=3.52); and diastolic blood pressure a mean change of -8.00 compared to -0.80 (p<0.001, Cohen's d=2.89). These findings indicate that paraaortic lymphadenectomy is associated with longer operative times, greater lymph node

Table 1. Demographic and laboratory parameters of patients underwent paraaortic lymphadenectomy and no paraaortic lymphadenectomy

	No paraaortic (n=72)	Paraaortic (n=192)	p-value
Age	61±12	62±10	0.528
Gravida	2 (0-12)	2 (0-8)	0.916
BMI	33±7	32±6	0.167
Plt	288±71	298±83	0.358
CA-125	32±70	63±304	0.393
Preop tumor size	3±2	4±6	0.055
Op. time	3±1	4±1	0.000
Pelvic LN	21±20	39±17	0.000
Paraaortic LN	0±0	42±24	0.000
Tumor size	3±2	4±2	0.000
Hospital stay	5 (2-31)	9 (2-65)	0.000

BMI: Body mass index, Plt: Platelet, Op.: Operation, LN: Lymph node

dissection, higher utilization of adjuvant therapies, significant changes in blood pressure, and an increased risk of developing chylous ascites (Table 3).

For systolic blood pressure, the paraaortic group showed a greater reduction compared to the no paraaortic group throughout the 10 days, as depicted by the downward trend in the blue line in Figure 1. The mean systolic blood pressure in the no paraaortic group remained relatively stable around the preoperative mean, while the paraaortic group showed significant decreases. These differences reflect the earlier reported mean changes (-1.10 *vs.* -17.20, p<0.001, Cohen's d=3.52).

Similarly, the diastolic blood pressure graph indicates a more prominent reduction in the paraaortic group compared to the no paraaortic group, consistent with the reported mean changes (-0.80 *vs.* -8.00, p<0.001, Cohen's d=2.89). The preoperative mean diastolic pressure is marked as a reference, and the trends show that the paraaortic group deviates significantly from this baseline over time. Figure 2 and Figure 3 showed blood pressure changes over time.

Table 2. Comorbidities and postoperative complications in paraaortic and no paraaortic lymphadenectomy groups

	No paraaortic (n=72)	Paraaortic (n=192)	p-value
Hypertension			
No	30 (41.7%)	68 (35.4%)	**0.349**
Yes	42 (58.3%)	124 (64.6%)	
Diabetes mellitus			
No	50 (69.4%)	128 (66.7%)	**0.668**
Yes	22 (30.6%)	64 (33.3%)	
Suspicious lymph node			
No	65 (90.3%)	148 (77.1%)	**0.016**
Yes	7 (9.7%)	44 (22.9%)	
Radiotherapy			
No	49 (68.1%)	66 (34.4%)	**0.000**
Yes	23 (31.9%)	126 (65.6%)	
Chemotherapy			
No	64 (88.9%)	122 (63.5%)	**0.000**
Yes	8 (11.1%)	70 (36.5%)	
Evisceration			
No	72 (100%)	187 (97.4%)	**0.167**
Yes	0 (0.0%)	5 (2.6%)	
Ileus			
No	72 (100%)	186 (93.8%)	**0.001**
Yes	0 (0.0%)	12 (6.2%)	
Chylous ascites			
No	68 (94.4%)	162 (84.4%)	**0.030**
Yes	4 (5.6%)	30 (15.6%)	

Table 3. The comparison of sistolic and diastolic blood changes in paraaortic and no paraaortic groups

	No paraaortic	Paraaortic	Mann-Whitney U statistic	Cohen's d
Systolic change	-1.10	-17.20	p<0.001	3.52
Diastolic change	-0.80	-8.00	p<0.001	2.89

Discussion

This study demonstrates that para-aortic lymphadenectomy, performed up to the level of the renal artery in patients with endometrial cancer, is associated with a significant reduction in postoperative blood pressure levels. The findings align with existing evidence on the role of neural structures within the para-aortic region in blood pressure regulation. Extending lymphadenectomy to include the para-aortic area may disrupt sympathetic fibers that contribute to renal vasoconstriction, sodium uptake, and renin secretion, thereby reducing blood

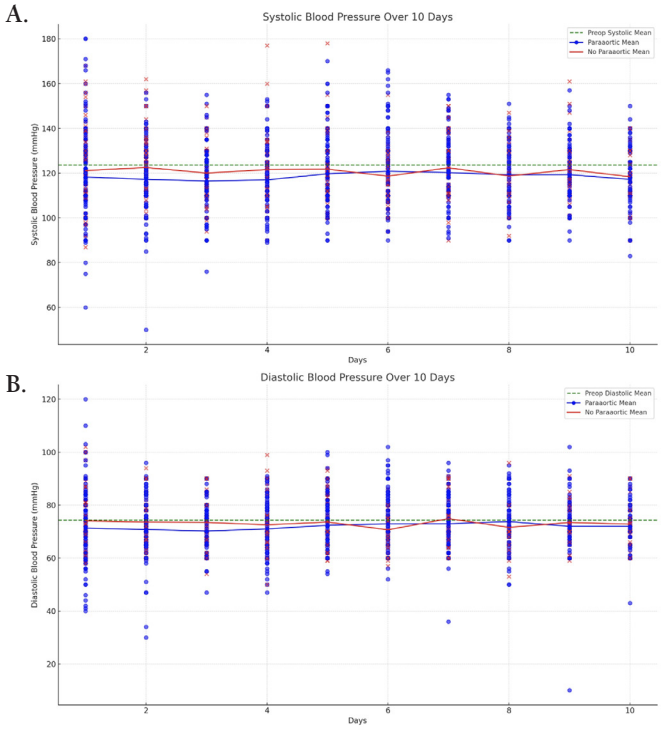

Figure 2. A. Systolic blood pressure trends over 10 days in patients undergoing para-aortic lymphadenectomy compared to controls. Preoperative systolic blood pressure levels are indicated by the green dashed line, **B.** Diastolic blood pressure trends over 10 days for the same groups, showing a similar pattern to systolic pressure. Preoperative diastolic levels are marked with a green dashed line

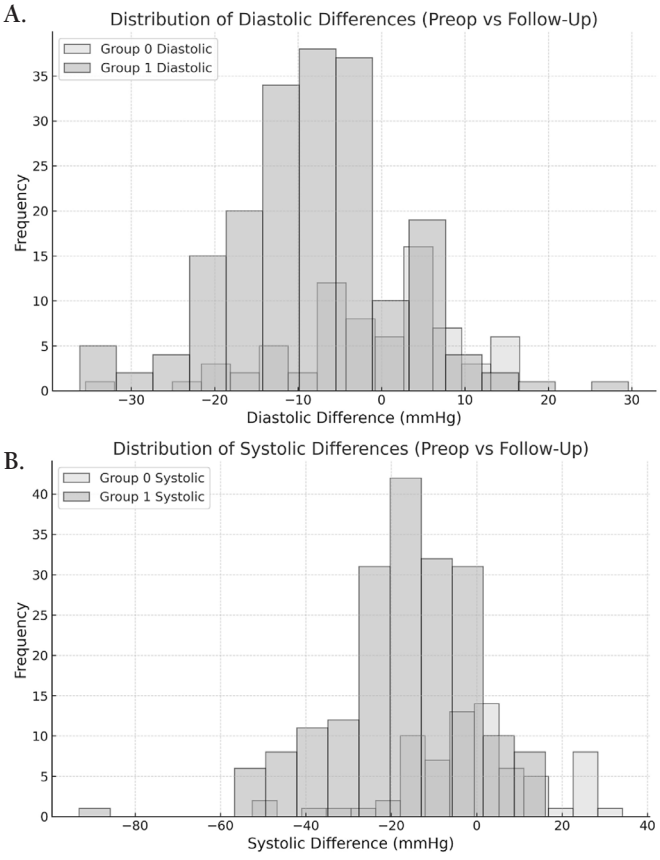

Figure 3. A. Distribution of diastolic blood pressure differences (preoperative *vs.* follow-up) in patients undergoing para-aortic lymphadenectomy and controls, **B.** Distribution of systolic blood pressure differences (preoperative *vs.* follow-up) in the same groups, highlighting significant reductions in the para-aortic group

pressure. These results are consistent with prior studies evaluating interventions such as renal sympathetic denervation, which have similarly shown significant blood pressure reductions through targeted disruption of neural pathways.

The aorticorenal splanchnic nerves are constituted by the least and lesser splanchnic nerves, which play a critical role in regulating renal vascular tone and renin secretion[10]. Sympathetic activation via these nerves induces renal vasoconstriction, reducing blood flow and glomerular filtration rate to maintain systemic hemodynamic stability. Additionally, their adrenergic signaling stimulates renin release, activating the renin-angiotensin-aldosterone system to support blood pressure and fluid balance[11]. The sympathetic nerves give fibers to the renal plexus and reach the kidney, traversing the renal artery. Renal denervation has emerged as a promising interventional therapy for hypertension, targeting the renal sympathetic nervous system to achieve blood pressure reduction. This procedure disrupts sympathetic efferent and sensory afferent fibers, reducing renin secretion, sodium reabsorption, and systemic sympathetic outflow, which collectively contribute to regulation[12]. Similar mechanisms may explain blood pressure reductions observed following para-aortic lymphadenectomy, where the renal nerves could usually be affected due to their anatomical proximity to the para-aortic lymphatic structures. Evidence from experimental studies indicates that ablative or inhibitory interventions targeting renal nerves can significantly alter sympathetic activity, suggesting that such surgical approaches could share mechanistic similarities with renal denervation procedures in modulating blood pressure[13,14].

We observed in our study a significant reduction in blood pressure following paraaortic lymphadenectomy, with systolic blood pressure showing a mean decrease of 17.20 mmHg in the paraaortic group. This reduction exceeds the systolic blood pressure decrease of approximately 13 mmHg reported in renal denervation studies[15], which involve angiographic ablation of sympathetic nerves as a treatment for resistant hypertension. While renal denervation has been widely studied, with long-term follow-up data demonstrating sustained blood pressure reductions, there is currently no comparable data in the literature to show the impact of paraaortic lymphadenectomy on blood pressure changes. The lack of studies exploring the disruption of sympathetic nerves during paraaortic lymphadenectomy and its effects on blood pressure highlights the originality of our findings. This suggests that surgical interruption of sympathetic pathways during lymphadenectomy may play a role in postoperative blood pressure regulation, offering a new area for future research.

The findings from the study conducted Wen et al.[16] highlight the potential adverse effects of para-aortic lymphadenectomy, particularly in non-nerve-sparing procedures. Complications such as lymphorrhea, lymphocele, and acute intestinal obstruction were observed more frequently in the para-aortic group compared to the nerve sparing para-aortic lymphadenectomy group, consistent with our study's findings of increased rates of ileus, chylous ascites, and longer hospital stays in the para-aortic lymphadenectomy cohort. Despite these complications, our study revealed a significant reduction in systolic blood pressure following para-aortic lymphadenectomy. These results indicate a dual perspective: while para-aortic lymphadenectomy carries a risk of postoperative complications, it also offers a potential therapeutic benefit for hypertension management.

In our study, significant reductions in both systolic and diastolic blood pressure were observed following para-aortic lymphadenectomy, with consistent effects seen in both short-term and long-term follow-ups. The changes in blood pressure align with findings from renal denervation studies, where sustained systolic blood pressure reductions of approximately 12.7 mmHg were reported over 36 months, and similar long-term decreases were observed over 10 years[17]. However, the magnitude of blood pressure reduction in our study, particularly during the early postoperative period, was more pronounced, with systolic blood pressure showing a median decrease of approximately 17 mmHg. This suggests that the

surgical disruption of para-aortic sympathetic nerves during lymphadenectomy may result in both immediate and sustained antihypertensive effects. While renal denervation has been widely studied as a therapy for resistant hypertension, our findings highlight a potential additional benefit of para-aortic lymphadenectomy in reducing blood pressure.

Study Limitations

The limitation of the study is it's retrospective design inherently introduces potential biases and limits the ability to establish causal relationships. The variability in follow-up periods among patients may have influenced the consistency of the results. Additionally, the absence of ambulatory blood pressure monitoring, such as Holter measurements, restricts the ability to evaluate more detailed fluctuations and patterns in blood pressure changes over time. However, the study also has notable strengths. The inclusion of a strictly defined cohort of patients who underwent para-aortic lymphadenectomy up to the renal vein level ensures a high degree of consistency in the surgical approach. This uniformity strengthens the validity of the observed blood pressure changes as an independent effect of para-aortic lymphadenectomy on the sympathetic nerves, enhancing the reliability of the findings in demonstrating the direct impact of the procedure on blood pressure regulation

Conclusion

Para-aortic lymphadenectomy has a blood pressure-lowering effect and may reduce the need for antihypertensive medication in hypertensive patients.

Ethics

Ethics Committee Approval: Our study received approval from the Institutional Review Board (İzmir Democracy University Buca Seyfi Demirsoy Training and Research Hospital, Non-Interventional Research Ethics Committee - no: 2023/211, date: 27.12.2023).
Informed Consent: All persons gave their informed consent prior to their inclusion in the study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: S.E., B.Ö., C.A., İ.Ç., Concept: S.E., S.Ö., U.A., C.A., H.A.A., T.B.B., İ.Ç., Design: S.E., S.C.İ., S.Ö., B.Ö., U.A., T.B.B., Data Collection or Processing: S.C.İ., U.A., H.A.A., T.B.B., İ.Ç., Analysis or Interpretation: S.E., S.C.İ., S.Ö., B.Ö., H.A.A., İ.Ç., Literature Search: S.E., B.B., Writing: S.E., S.Ö., C.A.
Conflict of Interest: No conflict of interest was declared by the authors.
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Glutathione S-transferase polymorphisms and their role in recurrent pregnancy loss: A genetic risk assessment

Glutatyon S-transferaz polimorfizmleri ve bunların tekrarlayan gebelik kaybındaki rolü: Genetik bir risk değerlendirmesi

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Abstract

Objective: The frequency of recurrent pregnancy loss in society is 3-5%. Experts suggest that genetics account for over 80% of unexplained recurrent pregnancy loss. Glutathione S-transferase (GST) enzymes, regulated by GST genes, facilitate the detoxification of a variety of naturally occurring metabolites as well as environmentally derived chemicals. This research aimed to investigate *GST* gene polymorphisms as a potential risk factor in recurrent pregnancy loss etiology in the Turkish population.

Materials and Methods: This study involved 107 recurrent pregnancy loss patients who sought treatment at the Sivas Cumhuriyet University Faculty of Medicine, Department of Medical Genetics, along with a control group of 107 individuals who had a successful birth and no previous history of miscarriage. The multiplex polymerase chain reaction and restriction fragment length polymorphism techniques were employed to analyze *GSTM1, GSTT1 and GSTP1* gene polymorphisms in these cases.

Results: GSTT1 null genotype (X^2=4.74; p=0.029) and GSTT1/GSM1 null genotype (X^2=3.333; p=0.047) were associated with statistically significant differences between the study groups. No statistical significance was detected when considering the GSTM1 null genotype (X^2=3.326; p=0.068) or the *GSTM1/GSTP1* and *GSTT1/GSTP1* gene polymorphisms.

Conclusion: A statistically significant association was observed between the GSTT1 null genotype and the diseased group. Our research demonstrated a substantial increase in the risk of recurrent pregnancy loss in the Turkish population, specifically among individuals with the GSTM1-null genotype. No statistical correlation was found between the *GSTM1* and *GSTP1* gene polymorphisms and recurrent pregnancy loss. Furthermore, no statistical significance was observed when they were assessed together.

Keywords: Glutathione S-transferase, GSTM1, GSTT1, GSTP1, recurrent pregnancy loss

Öz

Amaç: Toplumda tekrarlayan gebelik kaybı sıklığı %3-5'tir. Uzmanlar, açıklanamayan tekrarlayan gebelik kayıplarının %80'inden fazlasının genetiğe bağlı olduğunu ileri sürmektedir. GST genleri tarafından kodlanan glutatyon s-transferaz (GST) enzimleri, çevresel olarak türetilen kimyasalların yanı sıra çeşitli doğal olarak oluşan metabolitlerin detoksifikasyonundan sorumludur. Bu çalışma, Türk toplumunda tekrarlayan gebelik kayıplarının etiyolojisinde bir risk faktörü olarak *GST* gen polimorfizmlerini araştırmayı amaçlamaktadır.

PRECIS: In the investigation of GSTM1, GSTT1, GSTP1 polymorphisms of the Glutathione S-Transferase (GST) genes in Patients with Recurrent Pregnancy Loss, statistically significant differences were detected indicating an increased risk ratio in the groups with GSTT1 and GSTT1/GSM1 null genotype.

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Gereç ve Yöntemler: Bu çalışmaya Sivas Cumhuriyet Üniversitesi Tıp Fakültesi, Tıbbi Genetik Bölümü'nde tedavi gören 107 tekrarlayan gebelik kaybı hastası ve daha önce düşük yapmamış ve başarılı bir doğum yapmış 107 kişiden oluşan bir kontrol grubu dahil edildi. Bu olgulardaki *GSTM1, GSTT1* ve *GSTP1* gen polimorfizmlerini multipleks polimeraz zincir reaksiyonu ve kısıtlama parça uzunluğu polimorfizmi yöntemi kullanarak araştırdık.

Bulgular: GSTT1 null genotipi (X^2=4,74; p=0,029) ve GSTT1/GSM1 null genotipi (X^2=3.333; p=0,047) çalışma grupları arasında istatistiksel olarak anlamlıydı. GSTM1 null genotipi (X^2=3.326; p=0,068), ***GSTT1/GSTP1*** ve ***GSTM1/GSTP1*** gen polimorfizmleri dikkate alındığında istatistiksel olarak anlamlı bir fark saptanmadı.

Sonuç: GSTT1 null genotipi hasta grubunda istatistiksel olarak anlamlıydı. Araştırmamız Türk popülasyonunda, özellikle GSTM1 ve null genotiplere sahip bireyler arasında tekrarlayan gebelik kaybı riskinde önemli bir artış olduğunu gösterdi. ***GSTM1*** ve ***GSTP1*** genlerinin polimorfizmleri ile tekrarlayan gebelik kaybı arasında istatistiksel bir korelasyon bulunamadı. Ayrıca, birlikte değerlendirildiğinde istatistiksel olarak anlamlı bir fark saptanamadı.

Anahtar kelimeler: Glutatyon S-transferaz, GSTM1, GSTT1, GSTP1, tekrarlayan gebelik kaybı

Introduction

Recurrent pregnancy loss is characterized by two or more antecedent pregnancy losses before 24 weeks gestation[1,2]. The most commonly identified cause of explained pregnancy loss is fetal chromosomal abnormalities, which are responsible for about 70% of spontaneous abortions and 30-50% of recurrent losses[1-6].

In at least 50% of recurrent pregnancy loss, the reason cannot be determined by any diagnostic test and is thought to be idiopathic. Environmental and lifestyle-related risk factors cause genetic susceptibility to recurrent pregnancy loss[3-6]. Despite the proposal of numerous etiological factors, the etiology of recurrent pregnancy loss remains elusive. In recurrent pregnancy loss, the balance between phase I and II enzyme systems in response to endogenous and exogenous substances is influenced by the genetic variability of individual metabolic detoxification activation[7]. Many genetic studies have recently discovered a link between genetic polymorphisms relating to metabolic enzymes and recurrent pregnancy loss. Phase I enzymes like cytochrome P450-1A1 (CYP-1A1) activate many potentially toxic compounds, including those found in cigarettes, coffee, and alcohol, to form the final reactive compound[8,9]. Phase II enzymes, particularly glutathione-S-transferases (GST), have a key impact on detoxifying these active forms. Activation of toxins by phase I enzymes increases oxidative stress, and these toxins are then eliminated by the phase II detoxification system utilizing glutathione. Cytosolic GST is categorized into four primary classes: pi (P), alpha (A), mu (M), and theta (T)[10]. Each consists of one or more isoenzymes. Genetic differences in the GST and CYP-1A1 enzymes may impact the equilibrium between the phase I and II biotransformation pathways. This could explain why some people are more or less likely to get diseases linked to smoking, alcohol, coffee, or other toxins[7]. In this regard, phase I and II biotransformation enzymes are believed to be relevant in individuals with recurrent pregnancy loss. GSTT1 and GSTM1, glutathione detoxification pathway enzymes, shield the embryo against oxidative stress[8].

Recurrent pregnancy loss is a multifactorial disease affected by various epidemiological risk factors such as diabetes, genetic mutations, uterine structural anomalies, genital infections, maternal age, coffee, smoking, alcohol, and chemical use. An abnormal placenta causes the production of reactive oxygen products and results in harmful effects which invade the embryo. It is hypothesized that oxidative stress contributes to the etiopathogenesis of abortion[10]. This study aimed to determine if *GST* gene polymorphisms increase the risk of recurrent pregnancy loss in Turkish females.

Materials and Methods

Study Population

This prospective cohort study recruited 214 participants from January 15 to March 2015. The study group included 107 patients who had been admitted to Sivas Cumhuriyet University Faculty of Medicine, Department of Medical Genetics. They experienced three or more consecutive miscarriages. The control group comprised 107 healthy female controls with no history of miscarriage or infertility. Patients with two or more of the unexplained recurrent pregnancy loss diagnoses were included in the study. Female patients with less than 2 abortions and those patients with abortions due to anatomical or endocrine reasons were excluded from the study. The healthy control group included women with no history of abortion.

Patients with anatomical, infectious, or systemic diseases that could contribute to recurrent pregnancy loss, those with chromosomal abnormalities in themselves or their spouses, and those who either declined participation or did not provide written informed consent were excluded from the study.

This study design was based on the principles of the Declaration of Helsinki. The study received ethical approval from the Sivas Cumhuriyet University Research Ethics Committee (approval number: 2013-09/15, date: 24.09.2013). Verbal and written consent of all participants were obtained.

DNA Extraction

Restriction fragment length polymorphism (RFLP) and polymerase chain reaction (PCR) techniques were used to analyze deletions in the *GSTM1* and *GSTT1* genes and the exon 5 Ile105Val polymorphism in the *GSTP1* gene. Peripheral venous blood samples of 3 mL were collected from all volunteers in ethylenediaminetetraacetic acid tubes and stored at -20 °C

until analysis. For total genomic DNA isolation, the UltraClean BloodSpin DNA (Mo Bio Laboratories, Carlsbad, CA, USA) kit was used.

Polymerase Chain Reaction and Genotyping

The PCR mixture prepared for these genes contained 100 ng template DNA, 2.5 mmol/L dNTP, 0.4 µmol/L GSTM1 primer, 0.8 µmol/L GSTT1 primer, 0.8 µmol/L albumin primer, 5 µL 10x buffer (Complete, Bioron GmbH, Ludwigshafen, Germany), 5 U DNA Taq polymerase (New England BioLabs, Ipswich, USA), and dH_2O in a total reaction volume of 25 µL. Then, the conditions of the thermal cycler (2720, Applied Biosystems, Foster City, USA) were programmed as 15 minutes at 95 °C, 35 cycles of 60 seconds at 94 °C, 60 seconds at 58 °C, and 60 seconds at 72 °C, followed by 10 min at 72 °C. The PCR amplification product was confirmed by 2% agarose gel electrophoresis. Band lengths of the PCR products obtained for *GSTT1*, *GSTM1,* and *albumin* were 459 bp, 219 bp, and 350 bp, respectively. The primer sequences used for the *GSTM1*, *GSTT1* and *GSTP1* genes are shown in Table 1.

GSTP1 Ile105Val polymorphism was determined by the PCR-RFLP method. Then 25 µL PCR reaction mixture consisted of: 100 ng template DNA, 5 µL of 10x buffer (Complete, Bioron GmbH, Ludwigshafen, Germany), 2.5 mmol/L dNTP, 10 U Taq polymerase (New England Biolabs, Ipswich, MA, USA), 0.3 µM GSTP1 primers (Table 1), and dH_2O. Then, the conditions of the thermal cycler were programmed as follows: for 5 minutes at 94 °C, 5 cycles of 30 seconds at 94 °C, 30 seconds at 64 °C, and 30 seconds at 72 °C, (annealing temperature decreased 1 °C in each cycle) and 25 cycles of 30 seconds at 94 °C, 30 seconds at 59 °C, and 30 seconds at 72 °C. The length of the PCR products obtained was 433 bp. The PCR product was incubated for 16 hours at 37 °C with 5 units of BsmAI (Fermentas, Lithuania) restriction endonuclease. After the restriction, 2 bands formed in the sizes of 328 and 105 bp in the wild type (AA), 4 bands in the sizes of 328, 222, 106, and 105 bp in the heterozygous genotype (AG), and 3 bands in the sizes of 222, 106, and 105 bp in the homozygous mutant genotype.

Table 1. Primer sequences of GST

Gene	Primer sequences (5'->3')
GST T1- forward	TTCCTTACTGGTCCTCACATCTC
GST T1- reverse	TCACCGGATCATGGCCAGCA
GST M1- forward	GAACTCCCTGAAAAGCTAAAGC
GST M1- reverse	GTTGGGCTCAAATATACGGTGG
Albumin- forward	GCCCTCTGCTAACAAGTCCTAC
Albumin- reverse	GCCCTAAAAAGAAAATCCCCAATC
GST P1, Ile105Val forward	GTAGTTTGCCCAAGGTCAAG
GST P1, Ile105Val reverse	AGCCACCTGAGGGGTAAG

GST: Glutathione S-transferase

Statistical Analysis

The SPSS 22.0 software package (SPSS Inc., Chicago, IL, USA) was employed. The chi-square test for 2x2 tables and the chi-square test for multi-way contingency tables were used to analyze categorical variables, while the independent samples t-test was used to compare group means. Statistical significance was defined as a p-value of 0.05.

Results

The patient group included 107 female recurrent pregnancy loss patients who had never given birth and had experienced two or more abortions. The control group comprised 107 women who were in good health and had successfully given birth without any previous instances of abortion. The patient group had a mean age of 26.54±6.67, whereas the control group's mean age was 38.32±9.23. We examined the GST polymorphisms in diseased and control groups. Individuals in the patient and control groups did not engage in smoking or alcohol consumption.

The number of patients with GSTT1 null genotype is 35 (32.7%). The number of individuals with null genotype in the control group is 21 (19.6%). The number of patients with GSTT1 positive genotype is 72 (67.3%). GSTT1 positive genotype was determined in 80.4% (n=86) of the control group. Our results showed a significant difference between the study groups (X^2=4.74; p=0.029) (Figure 1).

There were 48 patients with the GSTM1 null genotype (44.9%), and 35 individuals with the null genotype in the control group (32.7%); 59 patients had the GSTM1 positive genotype (55.1%), and 72 individuals in the control group had the positive genotype (67.3%). There was no significant difference between the study groups (X^2=3.326; p=0.068) (Figure 2).

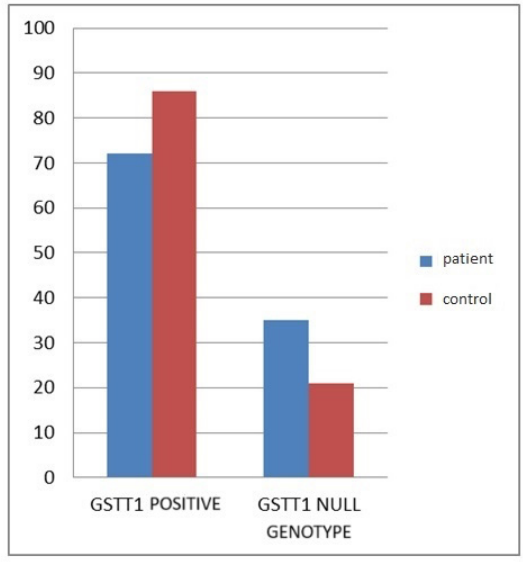

Figure 1. GSTT1 genotype in patient and control groups

The genotype frequency of GSTP1 exon 5 I105V polymorphism was found to be I allele 165, V allele 49 in the patient group; and I allele 166 and V allele 48 in the control group (X^2=0.013; p>0.90).

The GSTP1 wild genotype was 64 (59.5%) in the diseased group and 65 (60.7%) in the healthy group. The heterozygous genotype was 37 (34.6%) among patients and 36 (33.6%) in healthy individuals. The homozygous genotype was 6 (5.6%) in the patient group and 6 (5.6%) in the control group (X^2=0.021; p=0.989) (Table 1). The difference between the groups in terms of gene polymorphisms was not statistically significant (Figure 3).

The patient group exhibited significantly higher null genotypes for GSTM1 and GSTT1 positive and null genotypes combined (X^2=5.57; p=0.018) (Figure 4).

The comparison of the *GSTP1* and *GSTM1* gene polymorphisms across the study groups revealed no statistically significant difference (X^2=4.07, p=0.131).

GSTP1 and *GSTT1* gene polymorphisms were not significantly different between the study groups (chi-squared statistic, X^2=5.02, p=0.081).

GSTT1 null and GSTT1/GSTM1 genotypes are considered risk factors because they were significantly higher in recurrent pregnancy loss patients than in the healthy group.

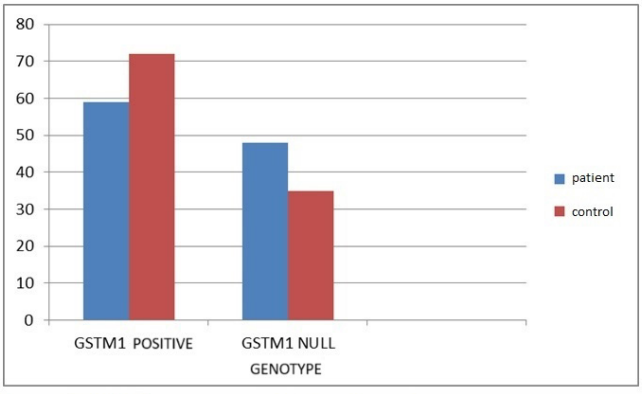

Figure 2. GSTM1 genotype in patient and control groups

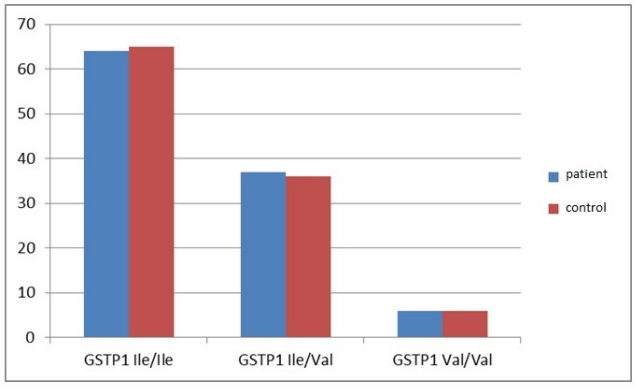

Figure 3. *GSTP1* gene polymorphism in patient and control groups

Discussion

GSTT1 and GSTT1/GSM1 null genotypes were significantly more frequent in recurrent pregnancy loss patients compared to the healthy group. Our study suggests that GSTT1 and GSTT1/GSTM1 null genotypes may increase the risk of recurrent pregnancy loss.

The oxidative state of cells plays a critical role in embryonic growth and endometrial differentiation by inducing angiogenesis. Oxidative stress causes functional changes in idiopathic recurrent pregnancy loss. This situation causes cellular and endometrial damage and destroys placental vascularization[11]. We investigated the GST gene polymorphism in recurrent pregnancy loss for the first time in our society.

According to epidemiological studies, recurrent pregnancy loss, a common pregnancy complication, is a multifactorial disease with a genetic predisposition[12]. The reason for recurrent pregnancy loss has been identified in only 30-50% of the cases[13]. The true cause of recurrent pregnancy loss is controversial, and the pathophysiological and etiological mechanisms are not fully understood. Oxidative stress increases the risk of recurrent pregnancy loss. An elevated oxidative load on the placenta supports the process of embryonic differentiation and development during pregnancy. An imbalance in the oxidant-antioxidant system can lead to an excessive oxidative load or an inadequate antioxidant defense to clear the oxidative load[14]. Phase I and II metabolic enzymes, which eliminate the destructive agents of oxidative stress, primarily determine a cell's ability to manage oxidative stress. According to several studies, genetic polymorphisms of antioxidant enzymes are implicated in an elevated risk of oxidative stress-linked diseases[15,16]. GST is an enzyme in the phase II detoxification system. The GST system and glutathione represent two of the most critical mechanisms in detoxifying and metabolizing carcinogens, xenobiotics, and reactive oxygen products[17].

In studies investigating the involvement of GST genes in recurrent pregnancy loss pathology, researchers have analyzed three different functional variants of GST. We examined the

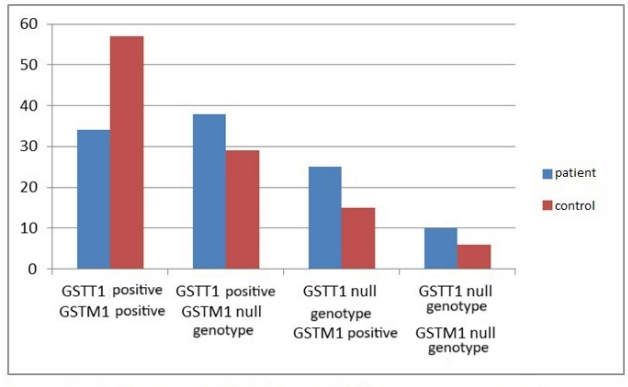

Figure 4. Evaluation of GSTT1 and GSTM1 genotypes together in patient and control groups

I105V substitution in the *GSTP1* gene to distinguish individuals with GSTT1 positive genotypes from those with positive/null genotypes of GSTM1, GSTT1, and null genotypes; as well as to distinguish non-identical carriers[17-21].

Research studies have suggested a potential association between genetic polymorphisms in metabolic enzymes and recurrent pregnancy loss[17-19]. In particular, GSTM1 null genotype may pose a risk for recurrent pregnancy loss, study in Japan suggests[20]. Conversely, an Indian study revealed a potential association between the GSTT1 null genotype and recurrent pregnancy loss[17-21]. A study conducted in the Netherlands observed a significant relationship between GSTP1 I105V polymorphism and the risk of recurrent pregnancy loss[21]. In light of the above, GST enzymes may be considered crucial for maintaining antioxidant defense, especially during pregnancy. It has been shown that a decrease in the activity and expression of enzymes can increase the risk of experiencing recurrent pregnancy loss by increasing sensitivity to oxidative stress. GST genes may provide a new perspective on recurrent pregnancy loss genetics. However, further investigation is required to validate this hypothesis[12].

Sata et al.[20], in their study of the Japanese population in 2003, found a higher rate of GSTM1 null genotype in the recurrent pregnancy loss group than in the control group [65.2%, 45.6%; odds ratio (OR)=2.23, 95% confidence interval (CI)=1.36-3.66]. The GSTM1 null genotype frequency in both groups with primary and secondary recurrent pregnancy loss was significantly high in comparison with the control group. Females with a history of three or more recurrent pregnancy losses were found to have a significant increase in the risk of recurrent pregnancy loss associated with the GSTM1 null genotype (OR=2.90, 95% CI=1.58-5.34). The International Project on Genetic Susceptibility to Environmental Carcinogens data indicates that the GSTM1 null genotype is 47.5% prevalent in the Japanese population, with a higher rate in Caucasians, 54.3% in America, and 50.4% in the Netherlands. In the Japanese population, the lower frequency of the GSTM1 null genotype makes the recurrent pregnancy loss risk even more statistically significant.

In the study conducted by Nair et al.[22] in 2013, the GSTT1 null genotype was compared between early, recurrent pregnancy loss and control groups. Individuals with pregnancy loss demonstrated a significantly higher frequency of GSTT1 null genotype compared to the other groups (10.92%, p=0.004; 10.77%, p=0.006). The GSTM1 null allele frequency was 37.36% among early pregnancy loss (EPL) individuals, 36.15% in recurrent pregnancy loss, and 28.89% in the control group. Nevertheless, no significant relationship was detected between the GSTM1 null genotype and recurrent pregnancy loss and EPL. When analyzed together, the combined GSTT1 and GSTM1 null genotypes had a 4.74-fold higher risk for EPL and a 5.67-fold higher risk for recurrent pregnancy loss.

In 2010, Parveen et al.[23] conducted a study on northern Indian women. They observed that the recurrent pregnancy loss group exhibited a significant increase in the GSTT1 null genotype (26%) compared to the controls (15%) (p=0.0034, OR=1.99, CI=1.27-3.12). The analysis of Phase II genes revealed a 4-fold increase in disease risk among north Indian women who had either the GSTP1 variant alleles or the GSTM1 null genotype. The fact that the risk goes up seven times when GSTM1 and GSTT1 null genotypes are combined with GSTP1 variant alleles suggests a strong link with recurrent pregnancy loss.

In 2011, Nonaka et al.[9] reported the presence of a statistically significant elevated frequency of GSTM1 null genotype in patients who consumed coffee daily compared to healthy individuals (61%; OR=2.25; 95% CI=1.13-4.49; p=0.025). It is estimated that increased embryonic exposure to endogenous or exogenous toxins due to the deficiency in the decidual and placental detoxification systems significantly contributes to the pathophysiology of recurrent pregnancy loss. GST catalyzes the transport and binding of many harmful substances, as well as the detoxification of oxygen radicals, by attaching a wide range of electrophilic compounds to the sulfhydryl group of glutathione[23]. Importantly, placental GSTs are essential for both fetal and maternal detoxification. Previous research in the Japanese population has documented the presence of a link between the GSTM1 null genotype and impaired caffeine detoxification in individuals experiencing recurrent pregnancy loss[7].

In 2004, Ada et al.[24] conducted a study on Turkish society and documented that the GSTM1 null genotype prevalence was 51.9%, whereas the GSTT1 null genotype prevalence was 17.3%. In 2001, Törüner et al.[25] found that the frequencies of GSTM1 and GSTT1 null genotypes in Turkish society were 45.5% and 17.4% respectively. According to Aktas et al.[26], the GSTM1 null genotype frequency was 34.7%. While in a study by Pinarbasi et al.[27], the GSTM1 null genotype frequency was 16%. Karaca et al.[28] found that the GSTM1 and GSTT1 null genotype frequencies were 52% and 23% respectively.

Factors such as genetic heterogeneity between societies, differences in susceptibility to some diseases, differences in exposure to toxins, selection based on different lifestyles, and differences in the evolutionary history of each society explain the distribution difference in the GSTT1 and GSTM1 null phenotype frequencies[29].

Study Limitations

In our study, the statistically significant difference observed between patients with the GSTT1 null genotype and the control group shows that we have identified it as a risk factor in the diagnosis of recurrent pregnancy loss. However, to obtain more accurate results about other variants, comprehensive studies on these genes should be conducted by expanding the sample size.

Conclusion

We genotyped the GSTM1, GSTT1, and GSTP1 biotransformation enzymes in both recurrent pregnancy

loss and healthy individuals. The GSTT1 null genotype was statistically significant in the recurrent pregnancy loss group, which shows how important biotransformation enzymes are in etiopathogenesis. It is thought that genetic polymorphism studies on biotransformation enzymes may shed light on the recurrent pregnancy loss pathogenesis. The link between GSTT1 and GSTT1/GSM1 null genotypes and recurrent pregnancy loss as risk factors should be supported by studies on larger patient groups and functional studies.

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Ethics

Ethics Committee Approval: This study design was based on the principles of the Declaration of Helsinki. The study received ethical approval from the Sivas Cumhuriyet University Research Ethics Committee (approval number: 2013-09/15, date: 24.09.2013).

Informed Consent: Verbal and written consent of all participants were obtained.

Footnotes

Authorship Contributions

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

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

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Predictive value of homocysteine levels in embryo culture media for embryo selection in infertile patients with endometriosis

Endometriozisli infertil hastalarda embriyo kültür ortamındaki homosistein düzeylerinin embriyo seçimi için prediktif değeri

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Abstract

Objective: To investigate the possible ability of homocysteine (Hcy) levels in embryo culture media for estimating better invitro fertilization outcomes in endometriosis patients.

Materials and Methods: Nineteen women with endometriosis who were admitted to Cerrahpaşa Medical Faculty, Department of Obstetrics and Gynecology, Infertility Outpatient Clinic with the diagnosis of infertility were included in the study. The results of intracytoplasmic sperm injection treatments were recorded and Hcy levels in the embryo culture were evaluated. The results were compared with those of the control patients without endometriosis, who had previously been admitted to our clinic for assisted reproductive technology.

Results: Mean Hcy levels in the culture media of the endometriosis group and non-endometriosis group were 4.31±0.48 µmol/L and 4.15±1.44 µmol/L, respectively (p>0.05). Pregnancy was achieved in 3 patients in the endometriosis group, while 13 pregnancies were obtained in the non-endometriosis group (p>0.05). When all cases were evaluated, the mean value of Hcy in the culture medium was found to be 3.60±0.84 µmol/L in the patients with a pregnancy and 4.21±0.84 µmol/L in the group that failed to achieve a pregnancy, and this difference was statistically significant (p<0.05).

Conclusion: Difference between mean Hcy levels in the culture media of the endometriosis group and non-endometriosis group was statistically non-significant. Further studies with larger groups are needed for evaluating the association of Hcy with infertility in endometriosis patients. Mean Hcy levels in the group of patients who succeeded in conceiving were statistically higher than the group of patients who failed to conceive. It may be suggested that Hcy levels in the embryo culture media can predict the achievement of a pregnancy independently from some conditions which may adversely affect the embryo quality, such as endometriosis.

Keywords: Infertility, embryo, culture, homocysteine, endometriosis

Öz

Amaç: Endometriozis hastalarında embriyo kültür ortamındaki homosistein (Hcy) düzeylerinin infertilite hastalarında embriyo seçiminde belirleyiciliğini araştırmaktır.

Gereç ve Yöntemler: Cerrahpaşa Tıp Fakültesi, Kadın Hastalıkları ve Doğum Anabilim Dalı İnfertilite Polikliniği'ne infertilite tanısıyla başvuran 19 endometriozis hastası kadın çalışmaya dahil edildi. İntrastoplazmik sperm enjeksiyonu tedavilerinin sonuçları kaydedildi ve embriyo kültüründeki

PRECIS: Homocysteine levels in the embryo culture media can serve as an independent predictor of pregnancy success and embryo quality, regardless of conditions like endometriosis that may negatively impact infertility treatment outcomes.

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homosistein düzeyleri değerlendirildi. Sonuçlar, daha önce kliniğimize başvuran ve endometriozis tanısı olmayan kontrol hastalarının sonuçlarıyla karşılaştırıldı.

Bulgular: Endometriozis grubu ve endometriozis olmayan grubun kültür ortamındaki ortalama Hcy düzeyleri sırasıyla 4,31±0,48 µmol/L ve 4,15±1,44 µmol/L idi. Bu değerler arasındaki fark istatistiksel olarak anlamlı değildi (p>0,05). Endometriozis grubunda 3 hastada gebelik elde edilirken, endometriozis olmayan grupta 13 hastada gebelik elde edildi (p>0,05). Tüm olgular değerlendirildiğinde; gebelik oluşan grupta kültür ortamındaki Hcy değerinin ortalaması 3,60±0,84 µmol/L, gebelik oluşmayan grupta ise 4,21±0,84 µmol/L olarak bulundu ve bu fark istatistiksel olarak anlamlıydı (p<0,05).

Sonuç: Endometriozis grubu ve endometriozis olmayan grubun kültür ortamındaki ortalama Hcy düzeyleri arasındaki fark istatistiksel olarak anlamlı değildi. Endometriozis hastalarında Hcy'nin infertilite ile ilişkisini değerlendirmek için daha büyük gruplarla yapılacak çalışmalara kesinlikle ihtiyaç vardır. Gebe kalmayı başaran hastaların grubundaki ortalama Hcy düzeyleri gebe kalmayı başaramayan hastaların grubuna kıyasla istatistiksel olarak daha yüksekti. Embriyo kültür ortamındaki Hcy düzeylerinin, endometriozis gibi embriyo kalitesini olumsuz etkileyebilecek bazı durumlardan bağımsız olarak gebelik oluşumunu öngörebileceği ileri sürülebilir.

Anahtar Kelimeler: İnfertilite, embriyo, kültür, homosistein, endometriosis

Introduction

Endometriosis is estimated to affect around 10-15% of reproductive-aged women and is a well-known factor in the etiology of infertility[1]. It is marked by the presence of tissue resembling the endometrial epithelium and/or stroma outside the endometrium and myometrium, often accompanied by an inflammatory response[2]. A wide spectrum of mechanisms has been thought to be involved in infertility related to endometriosis, including impaired oocyte quality and oxidative stress[3].

Enhancing the current embryo assessment methods is imperative, prompting numerous researchers to concentrate on developing the most effective methodology for gauging an individual embryo's reproductive potential. Lately, there has been significant interest in evaluating the metabolic parameters of developing embryos and analyzing the residual embryo culture media. Metabolomics refers to the comprehensive detection and measurement, without specific targeting, of all small molecular weight byproducts, known as metabolites, resulting from metabolic processes[4]. Metabolomics offers an overview of the levels of all metabolites present in cell's metabolic or environmental conditions. Thus, the metabolome, representing the array of small molecule metabolites within a biological sample, serves as a reliable indicator of cellular activity[5]. Metabolomics finds utility in reproductive medicine due to the potential association between subfertility causes and disruptions in typical metabolism. Consequently, there is a hypothesis that gaining deeper insights into the metabolic implications of different infertility causes could enhance reproductive outcomes. Moreover, it is expected that advancements in this domain would facilitate the discovery of non-invasive biomarkers for diagnostic and prognostic applications[6].

In a state of normalcy, where the body isn't experiencing heightened oxidative stress, a delicate equilibrium exists at the cellular level, regulating reactive oxygen species (ROS) to low levels through diverse antioxidant mechanisms[7]. Homocysteine (Hcy) is an amino acid formed during the metabolism of methionine, yet it doesn't become part of protein structures. Furthermore, Hcy plays a role in producing the thiol glutathione, which serves as a crucial endogenous antioxidant, vital for preserving the balance between pro-oxidants and antioxidants in human tissues[8].

Hyperhomocysteinemia can significantly impact reproductive processes in multiple ways[9]. The negative effects of hyperhomocysteinemia on reproductive processes at various levels are well-documented in numerous studies. These effects include poor oocyte quality, male infertility due to abnormal morphology, low sperm concentrations, and loss of motility, as well as congenital malformations, miscarriages, preeclampsia, and low birth weight[10,11].

Elevated endogenous oxidative stress, which is characterized by increased production of ROS and nitric oxide, along with changes in ROS detoxification pathways, is well documented[12]. In addition, high Hcy levels are shown in the follicular fluid of patients with endometriosis. However, endometriosis-related infertility, and Hcy stand as a field that warrants further investigation.

In this study, we aimed to evaluate the levels of Hcy in the embryo culture media in patients with endometriosis undergoing infertility treatment and its possible predictive value in predicting embryo quality and treatment outcomes.

Materials and Methods

Total number of 57 women with infertility who were seen at the in vitro fertilization (IVF) unit of a tertiary care center between May 2011-September 2014 were included. The study was approved by the Clinical Research Ethics Committee of İstanbul University-Cerrahpaşa, Cerrahpaşa Medical Faculty (date: 07.03.2013, number: 83045809/5579). Informed consent was obtained from all participants. The study group comprised 19 patients with documented endometriosis with surgical pathology results. The control group included 38 patients without an endometriosis diagnosis. The initial gynecological exam included infertility tests and a detailed reproductive history. Hormone profile included follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), anti-Müllerian hormone (AMH) and estradiol (E2) levels (Roche Diagnostics Corporation, Indianapolis, IN, USA). Exclusion criteria were as follows: Age ≥42, presence of hydrosalpinx, serum levels of FSH ≥15 and LH ≥15.

Conventional long agonist protocol was initiated for all patients. On the 21st day of the last cycle, gonadotropin-releasing hormone (GnRH) analogues were started. rec-FSH 75 unit,: Gonal-f® (Merck Serono, Türkiye), or Puregon® (Schering-Plough, Türkiye) was initiated on the 3rd day of the cycle, following the confirmation of the absence of any corpus luteum or an ovarian cyst larger than 1.5 cm with transvaginal ultrasound (5MHz sector probe, Siemens Adara). The step down protocol was used and the gonadotropin dose was adjusted according to the body mass index (kg/m^2) values of the patients as either 275, 325, or 375 international unit (IU) ultrasonographic evaluations were initiated on the 8th day of the cycle and conducted daily. 10,000 IU of human chorionic gonadotropin (HCG, Pregnyl®, Schering-Plough) was administered when the largest follicle was seen to be at 18 mm in diameter and with the presence of two follicles >16 mm.

Oocyte retrieval was conducted 36-38 hours post, HCG. Day 3 transfer was performed in each case and intravaginal 600 mg/day micronized progesterone (Progestan® tb, Kocak, Türkiye) was started on the night of procedure and continued for 12 days when the pregnancy test was performed. Pregnancy was diagnosed by the visualization of a gestational sac on ultrasound examination five weeks after embryo transfer.

Follicular fluids from mature follicles (>17 mm) were aspirated, and clear samples were pooled for each patient. Any aspirates that were not clear or contaminated with blood were discarded. Retrieved oocytes underwent rinsing, grading, and placement in bicarbonate-buffered human tubal fluid (Lonza, Verviers, Belgium, with a 10% protein solution, Sanquin, Amsterdam, The Netherlands) at 37 °C under 5% CO_2 in air. Oocyte insemination commenced approximately 40 hours after HCG injection using standard IVF or intracytoplasmic sperm injection (ICSI) procedures. Following the 40th hour of the ICSI, the dividing embryos were observed, and embryo grading was performed. This process was managed by the same embryologist for all cases. Embryos were categorized based on blastomere size and cytoplasmic fragmentation: “grade 1” for equally-sized blastomeres without cytoplasmic fragmentation, “grade 2” for equally-sized blastomeres with minor cytoplasmic fragmentations, “grade 3” for embryos without equally-sized blastomeres and cytoplasmic fragmentations, “grade 4” for embryos with or without equally-sized blastomeres and major cytoplasmic fragmentations, and “grade 5” for embryos with blastomeres that cannot be distinguished and major cytoplasmic fragmentations .

Embryo transfer took place at the two-cell stage or later on the third day after oocyte collection. After removing the embryos for transfer, 65 µL of Vitrolife G-2 v5 (Vitrolife Sweden AB, Göteborg, Sweden) medium was added to 35 µL of spent medium for each embryo. Each sample was brought up to 100 µL for laboratory evaluation. Samples, including a control sample incubated under the same conditions without an embryo, were immediately sent to the laboratory. Hcy levels in the collected samples were estimated using the enzyme cycling method with the Diazyme enzymatic Hcy assay kit (Diazyme Laboratories, CA, USA) on Beckman CX (Beckman Coulter Inc., CA, USA) automated chemistry analyzer . The intra, and inter-assay coefficients of variation values were <5.9%.

Statistical Analysis

Analyses were performed using the statistical package for the social sciences (SPSS) version 20.0 (Chicago, IL, USA). The Kolmogorov-Smirnov test was used to assess the normality of the distribution of variables. Using the independent samples t-test, we compared the variables with normal distributions; data were presented as mean ± standard deviation. Continuous variables in more than two groups were analyzed using either the Kruskal-Wallis test or analysis of variance and were represented as median and interquartile range or mean ± standard deviation, respectively. Spearman’s rank correlation coefficient was used to calculate correlations between continuous variables. A two-tailed p-value of less than 0.05 was considered statistically significant.

Results

The demographic characteristics, hormone values, and embryologic parameters of the patients enrolled in this study are presented in Table 1. Mean age in the endometriosis group was 32±8.31, whereas it was found to be 31.21±3.72 in the control group. The average infertility duration for the endometriosis group was 5.89±4.3 years. For the control group, it was found to be 5.93±3.42 years. In terms of total administered gonadotropin dose, the mean dose of the endometriosis group was 2673.6±722.3 IU, and in the control group, it was 2284.3±785.7 IU. Hormonal parameters on the 3rd day of menstruation were compared between the groups. The average FSH value was found to be 7.34±2.57 ng/mL in the endometriosis group and 5.4±1.59 ng/mL in the control group. A statistically significant difference was found ($p<0.05$). LH, E2, TSH, and PRL values were 5.93±2.42 ng/mL, 51.67±24.44 ng/mL, 2.42±1.15 ng/mL, and 15.04±6.18 ng/mL in the endometriosis group. In the control group, the concentrations were found to be 4.96±4.64 ng/mL, 54.97±49.58 ng/mL, 2.19±1.34 ng/mL, and 17.58±8.30 ng/mL. No significant difference was detected among all these parameters ($p>0.05$). AMH levels were found to be 2.98±2.79 ng/mL in the endometriosis group and 3.90±4.33 ng/mL in the control group, with no significant difference between the groups ($p>0.05$).

The average number of the total oocytes obtained on the day of aspiration, the number of oocytes subjected to ICSI, and the number of fertilized oocytes were 7.32±3.11, 5.16±2.29, and 4.32±24 in the endometriosis group, respectively. In the control group, the values were found to be 8.5±3.94, 5.42±2.18, and 3.06±2.15. No statistical significance was observed ($p>0.05$).

In the group with endometriosis, the average culture medium Hcy value was 4.31±0.48 µmol/L; in the control group, it was 4.15±1.44 µmol/L (Table 1). The difference was not found to be

statistically significant. (p>0.05). In terms of embryos obtained, a significant difference was found only in the number of grade 2 embryos on the 3rd day, but no significant difference could be detected in terms of other grades.

While 5 pregnancies were achieved in the endometriosis group, 14 pregnancies were achieved in the control group, and no statistically significant difference was found (p>0.05).

In the 2nd stage of the statistical analysis, patients were

Table 1. Clinical characteristics and serum hormone values of all patients

	Endometriosis		Control		
	n	Mean ± SD	n	Mean ± SD	p-value
Age (years)	19	32±8.31 32	38	31.21±3.72 31	0.486
Total gonadotropin dose (IU)	19	2673.6±722.3 2775	38	2284.3±785.7 2100	0.026
Duration of infertility (years)	19	5.89±4.3 5	38	5.93±3.42 5	0.970
FSH (mIU/mL)	19	7.34.1±2.57 6.8	38	5.4±1.59 5.17	**0.001**
LH (mIU/mL)	19	5.93±2.42 5.69	38	4.96±4.64 4.27	0.022
E2 (mIU/mL)	19	51.67±24.44 50	38	54.97±49.58 39.5	0.531
TSH (mIU/mL)	19	2.42±1.15 2.3	38	2.19±1.34 1.8	0.275
AMH (ng/mL)	19	2.98±2.79 2.1	38	3.90±4.33 2.56	0.472
Prolactin (mIU/mL)	19	15.04±6.18 13.09	38	17.58±8.30 15.6	0.400
Total oocystes	19	7.32±3.11 7	38	8.5±3.94 9	0.287
ICSI oocytes	19	5.16±2.29 5	38	5.42±2.18 5	0.601
Fertilized oocytes	19	4.32±24 4	19	3.06±2.15 3	0.475
Day 2nd embryo grade 1	12	2.50±1.83 3	26	2.54±1.52 2	0.946
Day 2nd embryo grade 2	7	1.43±1.39 1	23	2.30±1.22 2	0.098
Day 2nd embryo grade 3	3	1.33±0.57 1	7	1.57±0.78 1	0.696
Day 3rd embryo grade 1	13	3±2.58 3	24	2.88±1.62 3	0.871
Day 3rd embryo grade 2	11	1.64±0.67 2	22	2.55±1.22 2	**0.031**
Day 3rd embryo grade 3	6	1.50±1.22 1	12	1.67±0.65 2	0.227
Homocysteine (µmol/L)	19	4.31±0.48 4.47	38	4.15±1.44 3.08	0.123
Pregnancy (pregnant/total)	19	5/14	38	14/24	0.326

p<0.05 statistically significant
FSH: Follicle stimulating hormone, LH: Luteinizing hormone, E2: Estradiol, TSH: Thyroid stimulating hormone, AMH: Anti-Müllerian hormone, ICSI: Intra cytoplasmic sperm injection, SD: Standard deviation

distributed into three separate groups: all patients, patients with endometriosis, and patients without an endometriosis diagnosis. All the above-mentioned parameters were compared again each group based on pregnancy diagnosis.

The patient data in the endometriosis group (n=19) according to pregnancy diagnosis are shown in Table 2. Among all parameters, a significant difference was found only in terms of the number of grade 2 embryos on the 3rd day (p<0.05). No

Table 2. Clinical characteristics of patients with endometriosis based on pregnancy outcomes

	Pregnancy (+)		Pregnancy (-)		
	n	Mean ± SD median	n	Mean ± SD median	p-value
Age (years)	5	33.40±3.20 32	14	31.50±3.7 32	0.512
Total gonadotropin dose (IU)	5	2650±871.06 2775	14	2682.14±699.13 2765.50	0.765
Duration of infertility (years)	5	3.80±1.78 4	14	6.64±4.73 5	0.258
FSH (mIU/mL)	5	7.46±2.03 7.9	14	7.30±2.81 6.32	0.817
LH (mIU/mL)	5	5.61±1.38 5.8	14	6.04±2.74 5.49	0.488
E2 (mIU/mL)	5	49.43±12.42 57	14	52.47±27.87 49	0.711
TSH (mIU/mL)	5	2.91±1.52 3	14	2.25±1.01 2.25	0.459
AMH (ng/mL)	5	2.73±2.56 2.5	14	3.07±2.97 2.01	0.588
Prolactin (mIU/mL)	5	16.90±9.33 15.95	14	14.51±5.33 13.09	0.750
Total oocystes	5	8.4 ± 4.39 7	14	6.93±2.61 6.5	0.637
ICSI oocytes	5	6.40 ± 3.43 5	14	4.71±1.68 5	0.397
Fertilized oocytes	5	6 ± 3.53 5	14	3.71±1.63 3.5	0.158
Day 2nd embryo grade 1	2	3.50±0.70 3.5	10	2.3±1.94 2.5	0.269
Day 2nd embryo grade 2	1	2 3.5	6	1.33±1.5 1	0.441
Day 2nd embryo grade 3	0	0 0	3	1.33±0.57 1	0.696
Day 3rd embryo grade 1	4	4.50±3.78 3	9	2.33±1.73 3	0.389
Day 3rd embryo grade 2	4	2 2	7	1.43±0.787 1	**0.009**
Day 3rd embryo grade 3	1	1 1	5	1.60±1.34 1	0.677
Homocysteine (µmol/L)	5	4.23±0.68 4.47	14	4.29±0.57 4.43	0.853

p<0.05 statistically significant
FSH: Follicle stimulating hormone, LH: Luteinizing hormone, E2: Estradiol, TSH: Thyroid stimulating hormone, AMH: Anti-Müllerian hormone, ICSI: Intra cytoplasmic sperm injection, SD: Standard deviation

significant difference was detected in Hcy levels between the groups or conditions studied.

The patients' data in the group without endometriosis diagnosis (n=38) are shown in Table 3. A significant difference was detected between Hcy levels in the two groups, with levels significantly lower in the pregnancy group ($p<0.05$).

When all cases were evaluated, the mean value of Hcy in the culture medium was found to be 3.60±0.84 μmol/L in the patients with a pregnancy and 4.21±0.84 μmol/L in the group in which failed to achieve a pregnancy (Table 4) and this difference was statistically significant ($p<0.05$).

Table 3. Clinical characteristics of control patients based on pregnancy outcomes

	Pregnancy (+)		Pregnancy (-)		
	n	Mean ± SD median	n	Mean ± SD median	p-value
Age (years)	15	30.67±3.26 30	23	31.57±4.02 32	0.376
Total gonadotropin dose (IU)	15	2220±871.07 2100	23	2326.30±742.27 2250	0.580
Duration of infertility (years)	15	6.96±4.17 6	23	5.26±2.71 5	0.291
FSH (mIU/mL)	15	5.96±1.54 6	23	5.03±1.54 5.06	0.100
LH (mIU/mL)	15	3.78±1.76 3.39	23	5.73±5.71 4.4	0.210
E2 (mIU/mL)	15	48.73±25.44 40	23	59.04±60.65 39	0.858
TSH (mIU/mL)	15	2.19±1.16 1.79	23	2.19±1.47 1.81	0.709
AMH (ng/mL)	15	4.55±5.63 2.6	23	3.48±3.30 2.52	0.881
Prolactin (mIU/mL)	15	17.36±8.20 16	23	17.73±8.54 15.3	0.917
Total oocystes	15	8.87±3.72 9	23	8.26±4.14 9	0.787
ICSI oocytes	15	5.87±2.20 6	23	5.13±2.18 5	0.342
Fertilized oocytes	8	5.07±1.87 5	10	4.17±1.82 4	0.141
Day 2nd embryo grade 1	11	3.09±1.57 3	15	2.13±1.40 2	0.095
Day 2nd embryo grade 2	9	2.44±1.50 2	14	2.21±1.05 2	0.895
Day 2nd embryo grade 3	5	1.60±0.89 1	2	1.50±0.70 1.5	0.898
Day 3rd embryo grade 1	10	3±1.49 3	14	2.79±1.76 2.5	0.632
Day 3rd embryo grade 2	8	2.63±1.30 2	14	2.50±1.22 2	0.859
Day 3rd embryo grade 3	4	1.50±0.57 1.5	8	1.75±0.70 2	0.571
Homocysteine (μmol/L)	15	3.39±0.94 3.13	23	4.16±0.97 4.24	**0.022***

FSH: Follicle stimulating hormone, LH: Luteinizing hormone, E2: Estradiol, TSH: Thyroid stimulating hormone, AMH: Anti-Müllerian hormone, ICSI: Intra cytoplasmic sperm injection, SD: Standard deviation, *: Statistically significant

When receiver operating characteristic (ROC) analysis of Hcy level in culture medium is used to predict inability to conceive, the area under the curve was found to be 0.675. Since the ROC curve did not intersect the threshold of 0.5, the relationship was found to be significant ($p<0.05$) (Figure 1). For 3.145 μmol/L, which was chosen as the most appropriate cut-off value, the sensitivity was determined as 81.8%, the specificity as 76.1%, the positive predictive value as 0.45, and the negative predictive value as 0.94.

Discussion

Endometriosis and its possible effects on embryo quality have been investigated in many studies. An experimental study by Da Broi et al.[16], demonstrated impaired oocyte quality in bovine oocytes that were subjected to follicular fluid obtained from patients with endometriosis. Similar results were confirmed by Giorgi et al.[17] In a subsequent study, Da Broi et al.[18] were successful to identify elevated levels of oxidative stress markers in serum and follicular fluid of endometriosis patients. Yanushpolsky et al.[19] compared the IVF results of 45 endometriosis patients and 55 normal patients. They revealed that early pregnancy losses were significantly higher in the endometriosis group, and the number of embryos reaching the four-cell stage 48 hours after the IVF procedure was significantly lower. They suggested that there was a negative relationship between endometriosis and embryo quality. Studies on patients who have undergone oocyte donation are particularly noteworthy. Hauzman et al.[20] compiled five studies on patients who underwent oocyte donation and were diagnosed with endometriosis. In this review, it was concluded that relatively negative pregnancy outcomes were obtained if embryos from oocytes taken from patients with endometriosis were transferred to patients with endometrial receptivity, which was determined to be appropriate by morphological and molecular analyses.

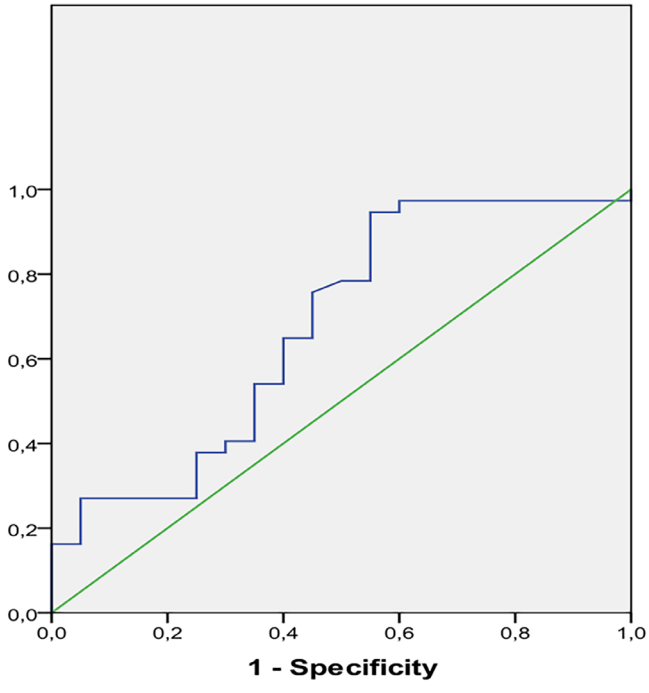

Figure 1. Receiver operative characteristic curve analysis for Homocysteine to predict inability to conceive

Our results revealed an inverse association between Hcy levels in embryo culture and pregnancy. Hcy levels were significantly lower in the group of patients with a positive pregnancy test, regardless of endometriosis diagnosis. The same association was found in the patients without endometriosis; no clear association was found in patients with endometriosis.

When we look at previous studies on Hcy, most studies are on serum, seminal plasma, and follicular fluid. These studies indirectly support our results by emphasizing the inverse relationship between high Hcy values and quality embryos, and pregnancy. In an old study conducted on mice, which is similar to our study, it was shown that L-Hcy was embryotoxic and that the rate of embryos reaching the blastocyst stage in these mice decreased in inverse proportion to Hcy levels. In the same study, it was stated that the other form of Hcy, D-Hcy, and its oxidation product, L-Hcy, were not embryotoxic[21]. Based on this, it can be suggested that the impaired embryo cannot progress to the blastocyst stage because it cannot clear Hcy from the environment. Hansen et al.[22] found that D,L-Hcy added to the culture medium during the early organogenesis stage was not embryotoxic in mouse embryos at an average concentration of 1.5 mM, and argued that these mouse embryos metabolized Hcy. This is most likely because Hcy enters the transsulfuration pathway and is metabolized. From this, it suggests that there is a certain non-toxic value range for Hcy and that the embryo metabolizes Hcy within this range. Berker et al.[23] and Aitken et al.[24] have emphasized the significance of Hcy levels in follicular fluid. Their findings indicated that elevated Hcy levels in follicular fluid were associated with reduced cell division, increased fragmentation in embryo cultures, and subsequently, diminished oocyte and embryo quality. Hcy and its levels in embryo culture media were evaluated by Aydin et al.[25] for the 1st time and their study had shown lower Hcy levels in patients with successful pregnancies, in accordance with the results of this study. They were also able to show a relationship between embryo grades and lower Hcy levels. The results of all these studies, in addition to ours, support the hypothesis that the viability and well-being of the embryo is revealed by examining the metabolic activity of that embryo, including its metabolic components, and cell residues in the culture medium.

Study Limitations

A small number of patients in the population poses a limitation, and necessitates further studies with a larger population to validate these results.

Table 4. Clinical characteristics of all patients based on pregnancy outcomes

	Pregnancy (+)		Pregnancy (-)		
	n	Mean ± SD median	n	Mean ±SD median	p-value
Age (years)	19	31.35±3.39 31	37	31.54±3.86 32	0.669
Total gonadotropin dose (IU)	19	2327.50±869.09 2112.25	37	2460.95±737.44 2475	0.389
Duration of infertility (years)	19	6.17±3.93 5	37	5.78±3.61 5	0.781
FSH (mIU/mL)	19	6.33±1.75 6.3	37	5.89±2.35 5.30	0.285
LH (mIU/mL)	19	4.24±1.83 4.12	37	5.85±4.76 4.75	0.213
E2 (mIU/mL)	19	48.90±22.57 40	37	56.55±50.39 47	0.913
TSH (mIU/mL)	19	2.37±1.26 1.95	37	2.21±1.30 2.09	0.634
AMH (ng/mL)	19	4.10±5.04 2.55	37	3.33±3.15 2.48	0.939
Prolactin (mIU/mL)	19	17.26±8.18 16	37	16.51±7.57 15.26	0.809
Total oocystes	19	8.75±3.78 8.5	37	7.76±3.66 8	0.400
ICSI oocytes	19	6±2.47 5.5	37	4.97±1.99 5	0.173
Fertilized oocytes	19	5.25±2.35 4	37	4±1.74 3	**0.037**
Day 2nd embryo grade 1	13	3.15±1.46 3	25	2.20±1.60 2	0.069
Day 2nd embryo grade 2	10	2.40±1.43 2	20	1.95±1.23 2	0.465
Day 2nd embryo grade 3	5	1.60±0.89 1	5	1.40±0.54 1	0.811
Day 3rd embryo grade 1	14	3.43±2.31 3	23	2.61±1.72 3	0.356
Day 3rd embryo grade 2	12	2.42±1.08 2	21	2.14±1.19 2	0.396
Day 3rd embryo grade 3	5	1.40±0.54 1	13	1.69±0.94 1	0.658
Homocysteine (µmol/L)	19	3.60±0.94 3.47	38	4.21±0.84 4.29	**0.030***

FSH: Follicle stimulating hormone, LH: Luteinizing hormone, E2: Estradiol, TSH: Thyroid stimulating hormone, AMH: Anti-Müllerian hormone, ICSI: Intra cytoplasmic sperm injection, SD: Standard deviation, *: Statistically significant

Conclusion

Lower Hcy levels in embryo culture media are associated with successful pregnancy outcomes, suggesting Hcy as a potential predictor of conception, independent of endometriosis. Larger studies are needed to confirm these findings and explore correlations with embryo morphology.

Ethics

Ethics Committee Approval: The study was approved by the Clinical Research Ethics Committee of İstanbul University-Cerrahpaşa, Cerrahpaşa Medical Faculty (date: 07.03.2013, number: 83045809/5579).
Informed Consent: Informed consent was obtained from all participants.

Footnotes

Authorship Contributions

Concept: M.İ., L.M.Ş., Design: M.İ., L.M.Ş., Data Collection or Processing: M.İ., Analysis or Interpretation: M.Ö., A.T., Literature Search: M.İ., Writing: M.İ., L.M.Ş.
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Effects of gonadotropin releasing hormone antagonist (GNRHant) and oral progestin-primed protocol on oocyte count over the punctured follicle number in consecutive two cycles: A comparative study

Gonadotropin salgılatıcı hormon antagonisti (GNRHant) ve oral progestin astarlı protokolün ardışık iki siklusta delinmiş folikül sayısı üzerinden oosit sayısı üzerine etkileri: Karşılaştırmalı bir çalışma

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Abstract

Objective: Controlled ovarian hyperstimulation plays a critical role in in vitro fertilization (IVF) success. However, premature luteinization and variations in oocyte yield can impact IVF outcomes. This comparative study aims to investigate the effects of gonadotropin releasing hormone antagonist (GNRHant) and oral progestin-primed protocol on the oocyte count over the punctured follicle number in the same patient group undergoing consecutive IVF cycles.

Materials and Methods: Forty-nine patients undergoing IVF were enrolled in this comparative study. Each participant underwent two consecutive IVF cycles. In the first cycle, GNRHant protocol was used. In the second cycle, the OPP protocol was used. The number of punctured follicles and oocytes retrieved was recorded and compared between the two cycles for each patient.

Results: The ratio of oocyte count per punctured follicle number was higher in the OPP group compared to the GNRHant group, without clinical significance (p>0.05). In the OPP, the ratio of oocytes retrieved over the punctured follicle number was 0.90±0.28; in the GNRHant group, it was recorded as 0.94±0.36, and the differences between the ratios were statistically insignificant.

Conclusion: Oocyte yield is a critical determinant of IVF success, and it can be influenced by various factors, including premature luteinization and follicular development. The use of GNRHant and OPP is known to prevent premature luteinization and improve follicular synchronization. This study demonstrates that neither of the protocols is superior in the success of oocyte retrieval over the punctured follicle count. Further research with larger sample sizes and randomized controlled trials is warranted to validate these results, and optimize clinical application of this combined protocol in IVF treatments.

Keywords: Infertility, gonadotropin releasing hormone antagonist, controlled ovarian hyperstimulation, oral progestin-primed protocol

PRECIS: In a comparative study of consecutive in vitro fertilization cycles, the oocyte yield relative to punctured follicles was similar between gonadotropin releasing hormone antagonist and oral progestin-primed protocols, showing no significant clinical advantage.

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Öz

Amaç: Kontrollü ovaryan hiperstimülasyon, in vitro fertilizasyon (IVF) başarısında kritik bir rol oynamaktadır. Bununla birlikte, erken luteinizasyon ve oosit verimindeki farklılıklar IVF sonuçlarını etkileyebilir. Bu karşılaştırmalı çalışma, ardışık IVF siklusları uygulanan aynı hasta grubunda Gonadotropin salgılatıcı hormon antagonisti (GNRHant) ve oral progestinle uyarılan protokolün delinmiş folikül sayısı üzerinden oosit sayısı üzerindeki etkilerini araştırmayı amaçlamaktadır.

Gereç ve Yöntemler: IVF uygulanan 49 hasta bu karşılaştırmalı çalışmaya dahil edilmiştir. Her katılımcıya iki ardışık IVF siklusu uygulanmıştır. İlk döngüde GNRHant protokolü kullanılmıştır. İkinci döngüde ise oral progestinle hazırlanan (OPH) protokol kullanılmıştır. Delinen foliküllerin ve alınan oositlerin sayısı kaydedilmiş ve her hasta için iki döngü arasında karşılaştırılmıştır.

Bulgular: Delinmiş folikül sayısına göre oosit sayısı OPH grubunda GNRHant'a kıyasla daha yüksek olup klinik olarak anlamlı değildir (p>0,05). OPH'de, alınan oositlerin delinen folikül sayısına oranı 0,90±0,28 iken GNRHant grubunda bu oran 0,94±0,36 olarak kaydedilmiştir ve korelasyon istatistiksel olarak anlamsızdır.

Sonuç: Oosit verimi IVF başarısının kritik bir belirleyicisidir ve erken luteinizasyon ve foliküler gelişim dahil olmak üzere çeşitli faktörlerden etkilenebilir. GNRHant ve OPH kullanımının erken luteinizasyonu önlediği ve foliküler senkronizasyonu iyileştirdiği bilinmektedir. Bu çalışma, her iki protokolün de delinmiş folikül sayısı üzerinden oosit alımının başarısı konusunda birbirlerine üstün olmadığını göstermektedir. Bu sonuçları doğrulamak ve IVF tedavilerinde bu kombine protokolün klinik uygulamasını optimize etmek için daha büyük örneklem boyutları ve randomize kontrollü çalışmalarla daha fazla araştırma yapılması gerekmektedir.

Anahtar Kelimeler: İnfertilite, gonadotropin salgılatıcı hormon antagonisti, kontrollü ovaryan hiperstimülasyon, oral progestin destekli protokol

Introduction

Assisted reproductive technologies (ART) have undergone significant advancements in recent years, offering a multitude of protocols to enhance the outcomes of in vitro fertilization (IVF). Among the various protocols employed, the gonadotropin releasing hormone antagonist (GNRHant) and oral progestin-primed (OPP) protocols have gained prominence for their efficacy in controlled ovarian hyperstimulation (COH). Understanding the nuanced impact of these protocols on crucial parameters such as oocyte count and punctured follicle numbers holds paramount importance for optimizing ART success.

GnRH plays a pivotal role in regulating the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), influencing the ovarian follicular development[1]. GnRH antagonists have been widely employed to prevent premature ovulation, thereby allowing precise control over the follicular maturation process during COH[2]. Conversely, the OPP protocol involves the administration of oral progestin prior to gonadotropin stimulation, exerting a modulatory effect on the endogenous gonadotropin release and ultimately impacting ovarian response[3].

While both GNRHant and OPP protocols have individually demonstrated success in promoting oocyte maturation and retrieval, there exists a paucity of studies directly comparing their effects on consecutive IVF cycles. This lacuna in the literature prompts the need for a comprehensive comparative analysis, aiming to elucidate potential differences in oocyte yield and follicular puncture outcomes between the two protocols across consecutive cycles.

This manuscript presents a thorough investigation into the effects of the GNRHant and OPP protocols on oocyte count relative to the punctured follicle number in consecutive IVF cycles. By critically examining and comparing these two widely utilized protocols, we aim to contribute valuable insights that can inform clinical decision-making, refine ART practices, and ultimately enhance the overall success rates of assisted reproduction.

Materials and Methods

Study Design

This study utilized a retrospective comparative design, examining data sourced from patient records at the Acıbadem Hospital IVF Unit for fertility treatment. The research adhered to ethical guidelines and received approval from the Acıbadem University Ethical Committee, with the assigned (approval number: 2022-04/137 no: 10.03.2023). The study adhered to ethical standards outlined in the Declaration of Helsinki. Patient confidentiality and privacy were strictly maintained throughout the study.

Study Participants

The study included a cohort of women undergoing consecutive IVF cycles at Acıbadem Hospital IVF Unit. Inclusion criteria comprised women aged 20-45 years, with a diagnosis of infertility and undergoing IVF treatment using the GNRHant for the first cycle and OPP protocol for the consecutive COH.

Treatment Protocols

GNRHant protocol

On the third day of the menstrual cycle, controlled ovarian stimulation was initiated by administering daily doses of r-FSH (300 IU follitropin alpha, Gonal-F, Serono, Geneva, Switzerland, or 16 mcg follitropin delta, Rekovelle®, Ferring, Saint-Prex, Switzerland). Upon visualizing at least one follicle ≥14 mm, patients received a subcutaneous injection of 0.25 mg GnRH antagonist (GnRHa, Cetrotide®; Merck KGaA, Darmstadt, Germany). When three or more follicles reached a mean diameter of ≥17 mm, and adequate serum estradiol levels were observed, administration of r-FSH and GnRH antagonist

were discontinued. Final follicular maturation was triggered by subcutaneous administration of recombinant human chorionic gonadotropin (r-hCG, Ovidrel®, Merck KGaA, Darmstadt, Germany). In cases where a patient was at risk of developing ovarian hyperstimulation syndrome (OHSS), a GnRH agonist (0.2 mg triptorelin acetate, Gonapeptyl daily®, Ferring GmbH, Kiel, Germany) was administered subcutaneously, instead of r-hCG. Oocyte retrieval was performed 37 hours after r-hCG or GnRH analog administration through transvaginal follicular aspiration guided by ultrasound, during which the patient underwent sedation. Metaphase II oocytes were selected for intracytoplasmic sperm injection (ICSI).

OPP Protocol

On the third day of the menstrual cycle, controlled ovarian stimulation was initiated by administering daily doses of r-FSH (300 IU follitropin alpha, Gonal-F, Serono, Geneva, Switzerland, or 16 mcg follitropin delta, Rekovelle®, Ferring, Saint-Prex, Switzerland). The r-FSH dosage was adjusted based on follicular development, monitored through ultrasound scans.

In the oral progesterone-primed group, starting from the third day of the cycle, patients received oral doses of medroxyprogesterone acetate (10 mg/day, Tarlusal®, Deva, Türkiye) until the trigger day. Oocyte retrieval was performed 37 hours after r-hCG or GnRH analog administration through transvaginal follicular aspiration guided by ultrasound, during which the patient underwent sedation. Metaphase II oocytes were selected for ICSI.

Data Collection and Analysis

Relevant clinical and demographic data, including age, body mass index (BMI), and baseline hormonal levels, were extracted from patient records. The primary outcomes, namely oocyte count and punctured follicle number, were analyzed using appropriate statistical methods (e.g., t-tests, chi-square tests). Subgroup analyses were conducted to explore potential variations in outcomes across different age groups and other relevant factors.

Limitations

Potential limitations, such as the retrospective nature of the study and the influence of confounding variables, were acknowledged.By employing these rigorous methodologies, the study aimed to provide robust insights into the comparative effects of the GNRHant and OPP protocols on oocyte count and punctured follicle numbers across consecutive IVF cycles.

Results

The study was conducted at Acıbadem Hospital between June 2019 and July 2023, involving a total of 49 women whose ages ranged from 20 to 45 years, with a mean age of 35.76±5.21.

The number of cycles among the participants ranged from 2 to 5, with a mean of 2.51±0.74. Among the participants, 20.4% (n=10) were found to have low ovarian reserve as the cause of infertility, while 16.3% (n=8) had genetic anomalies, 8.2% (n=4) had advanced maternal age, 22.4% (n=11) had male factor infertility, and 32.7% (n=16) were diagnosed with unexplained infertility.

The BMI measurements ranged from 16.2 to 37.9 kg/m^2, with a mean of 23.94±4.07 kg/m^2.

Concerning pregnancy, 67.3% (n=33) did not conceive, 24.5% (n=12) conceived once, and 8.1% (n=4) conceived two or more times.

Concerning the antral follicle count (AFC) on day 3 (D3), participants on GNRHant had a range of 2 to 32, with a mean of 10.61±6.34, while they had a range of 2 to 30 on the OPP protocol, with a mean of 10.22±5.94. No statistically significant difference was observed in AFC measurements between both protocols for the same patient cohort (p>0.05). The expected number of oocytes on GNRHant protocol ranged from 1 to 26, with a mean of 7.46±5.43, while on OPP, it ranged from 1 to 24, with a mean of 8.23±5.70. No statistically significant difference was found in expected oocyte count between the two protocols (p>0.05). The number of oocytes retrieved for GNRHant protocol ranged from 1 to 26, with a mean of 7.18±5.81, and for OPP, it ranged from 1 to 31, with a mean of 7.88±6.49 without clinical significance (p>0.05). The oocyte/AFC ratio on GNRHant protocol ranged from 0.25 to 2, with a mean of 0.94±0.36. On OPP, it ranged from 0.25 to 1.57, with a mean of 0.90±0.28, without any clinical significance, although there was a slight increase observed on GNRHant protocol compared to OPP (p>0.05). (Figure 1) Regarding endometrial thickness, GNRHant protocol had a range of 6.2 to 16.3, with a mean of 11.16±2.81, while OPP had a range of 5 to 16.6, with a mean of 9.48±9.30. GNRHant had a statistically

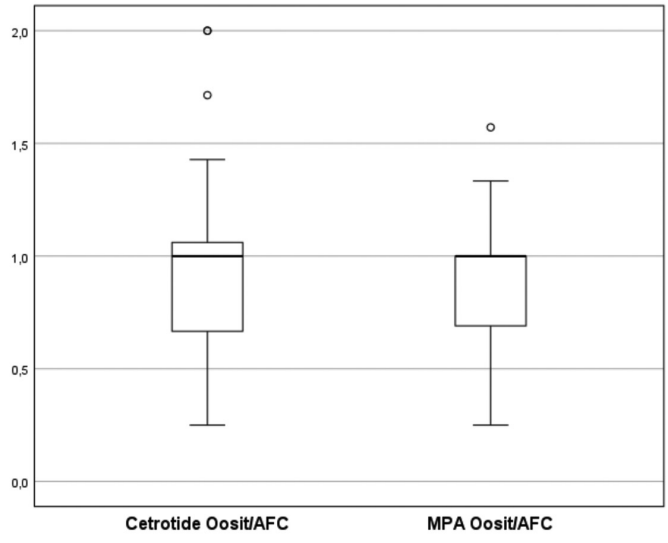

Figure 1. Distribution of retrieved occyte over the expected oocyte count for GNRHant and OPP protocols

GNRHant: Gonadotropin releasing hormone antagonist, OPP: Oral progestin-primed, AFC: Antral follicle count

significant thicker endometrium compared to OPP, with a mean difference of 1.78±2.29 mm (p=0.001; p<0.01) (Figure 2). On the trigger day, Estradiol (E2) levels for GNRHant ranged from 98 to 7499 pg/mL, with a mean of 1578.83±1347.91 pg/mL, while for OPP, the levels ranged from 297 to 8977 pg/mL, with a mean of 1555.07±1487.34 pg/mL. No statistically significant difference was found in trigger day E2 levels between both protocols (p>0.05). The number of Day 3 embryos obtained from GNRHant ranged from 0 to 14, with a mean of 3.14±3.39, and for OPP, it ranged from 0 to 19, with a mean of 4.27±3.75. GNRHant had a statistically significantly lower number of Day 3 embryos compared to OPP, with a mean difference of 1.31±3.03 (p=0.003; p<0.01) (Table 1). The number of Day 5 embryos obtained from GNRHant users ranged from 0 to 6, with a mean of 0.66±1.58, and for OPP, it ranged from 0 to 8, with a mean of 1.23±2.25. No statistically significant difference was found in the number of Day 5 embryos obtained between both protocols (p>0.05) (Table 2).

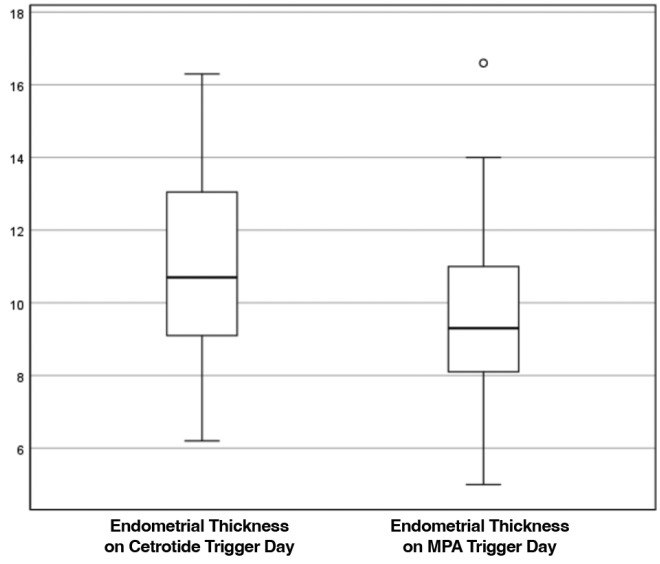

Figure 2. Distribution of endomerial thickness on the trigger day of GNRHant and OPP protocols

GNRHant: Gonadotropin releasing hormone antagonist, OPP: Oral progestin-primed, MPA: Medroxyprogesterone acetate

Table 1. Descriptive characterictics

Age	Mean ± SD Median (min-max)	36.76±5.21 36 (20-45)
BMI	Mean ± SD Median (min-max)	23.94±4.07 22.8 (16.2-37.9)
Number of IVF cycles	Mean ± SD Median (min-max)	2.51±0.74 2 (2-5)
Cause of inftiliy	Diminished ovarian reserve Genetic cause Advanced maternal age Male infertility Unexplained	10 (20.4) 8 (16.3) 4 (8.2) 11 (22.4) 16 (32.7)

IVF: In vitro fertilization, BMI: Body mass index, SD: Standard deviation

Statistical Analysis

SPSS 27 (Statistical Package for the Social Sciences) software was used for statistical analysis. Descriptive statistical methods (mean, standard deviation, median, frequency, percentage, minimum, maximum) were used for data evaluation. Normal distribution of quantitative data was tested using the Kolmogorov-Smirnov test, Shapiro-Wilk test, and graphical analysis. The dependent samples t-test was used for intra-group comparisons of quantitative variables showing normal distribution. The Wilcoxon signed-ranks test was used for intra-group comparisons of quantitative variables not exhibiting normal distribution. Statistical significance was accepted as p<0.05.

The results presented here provide a comprehensive overview of the outcomes associated with the GNRHant and OPP protocols in consecutive IVF cycles. The observed differences/similarities in oocyte yield, punctured follicle numbers, and age-specific responses contribute valuable insights for clinicians and researchers in the field of assisted reproductive technologies.

Discussion

The present study aimed to compare the effects of GNRHant protocol and the OPP protocol on oocyte count and the number of punctured follicles over two consecutive IVF cycles. Our findings revealed notable differences between the two protocols in terms of oocyte yield and ovarian response; shedding light on their distinct mechanisms of action and potential clinical implications.

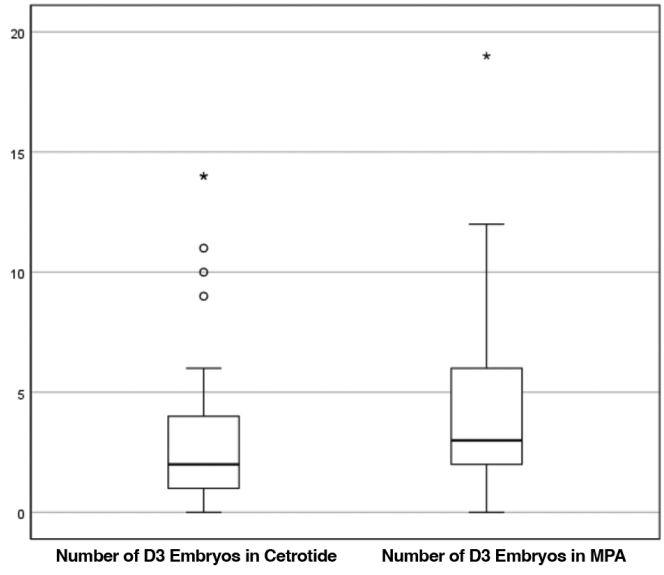

Figure 3. Distribution of D3 embryo number of GNRHant and OPP protocols

GNRHant: Gonadotropin releasing hormone antagonist, OPP: Oral progestin-primed, MPA: Medroxyprogesterone acetate

Table 2. GNRHant ve OPP evaluation

		GNRHant	OPP	Difference	test value; p
D3 AFC	Mean ± SD Median (min-max)	10.61±6.3 9 (2-32)	10.22±5.94 9 (2-30)	0.39±3.22 0 (-9-7)	t= 0.843 [a]**0.403**
Expected ooycte count	Mean ± SD Median (min-max)	7.46±5.43 6 (1-26)	8.23±5.70 7 (1-24)	-0.54±3.46 0 (-10-7)	Z=-0.895 [b]**0.371**
Retrieved oocyte count	Mean ± SD Median (min-max)	7.18±5.81 6 (1-26)	7.88±6.49 6.5 (1-31)	-0.58±3.76 0 (-11-8)	Z=-1.097 [b]**0.273**
Oocyte/AFC	Mean ± SD Median (min-max)	0.94±0.36 1 (0.25-2.0)	0.90±0.28 1 (0.25-1.57)	0.04±0.41 0 (-0.5-1.67)	Z=-0.257 [b]**0.798**
Endometrial thickness	Mean ± SD Median (min-max)	11.16±2.81 10.7 (6.2-16.3)	9.48±9.30 2.3 (5-16.6)	1.78±2.29 1.45 (-2.4-7.8)	t=5.151 [a]**0.001****
Trigger day E2	Mean ± SD Median (min-max)	1578.83±1347.91 1289.5 (98-7499)	1555.07±148.34 1272 (297-8977)	-37.34±734.50 -13 (-1662-1252)	Z=-0.168 [b]**0:866**
Number of D3 embryo	Mean ± SD Median (min-max)	3.14±3.39 2 (0-14)	4.27±3.75 3 (0-19)	-1.31±3.03 -1 (-8-7)	Z=-2.953 [b]**0.003****
Number of D5 embryo	Mean ± SD Median (min-max)	0.66±1.58 0 (0-6)	1.23±2.25 0 (0-8)	-0.50±1.98 0 (-7-6)	Z=-1.743 [b]**0.081**

[a]: Paired samples test, [b]: Wicoxon signed ranks test **: p<0.01, D3: Day 3, AFC: Antral follicle count, GNRHant: Gonadotropin releasing hormone antagonist, OPP: Oral progestin-primed, SD: Standard deviation

Moreover, while our study focused on oocyte count and punctured follicle numbers as key outcome measures, future research should investigate the impact of these protocols on live birth rates, pregnancy outcomes, and long-term maternal and neonatal health. A comprehensive understanding of the clinical implications of different ovarian stimulation protocols is essential for optimizing ART success rates and improving patient outcomes.

The study cohort consisted of 49 women with a diverse range of infertility etiologies, including low ovarian reserve, genetic anomalies, advanced maternal age, male factor infertility, and unexplained infertility. Notably, the mean age of participants was 35.76 years, reflecting a typical demographic profile for IVF treatment. The use of diverse patient populations enhances the generalizability of the study findings and underscores the relevance of the results in clinical practice.

In comparing the GNRHant and OPP protocols, the study observed no significant differences in the number of oocytes retrieved, expected oocyte count, or punctured follicle numbers between the two protocols. These findings align with previous studies suggesting comparable ovarian response and oocyte yield between GNRHant and progestin-primed protocols[4]. However, it's important to note, that while the oocyte count may not significantly differ between protocols, the nuances in ovarian response, as indicated by punctured follicle numbers, offer additional insights into the efficacy of ovarian stimulation regimens.

In the randomised controlled trial of Jabarpour et.al recently showed that viable embryo count is higher in OPP group compared to GNRHant group which is aligning with the outcome our study underscoring the significantly decrease in obtained day 3 embryos of the patients when they have undergone GNRHant protocole compared OPP[5].

Generally, rates of live births, ongoing pregnancies, clinical pregnancies, and pregnancy loss per embryo transfer are found to be similar between PPOS and GnRH analogue cycles. However, in certain analyses, particularly when examining patients with polycystic ovarian syndrome, PPOS may show a significantly higher clinical pregnancy rate per embryo transfer [Yildiz et al.[6]; Ata et al.[7]; Ata and Kalafat[8]].

This comparative study provides valuable insights into the differential effects of the GNRHant and OPP protocols on oocyte count and punctured follicle numbers in consecutive IVF cycles. The findings underscore the importance of protocol selection based on individual patient characteristics and treatment goals. By addressing these key points, our study contributes to the growing body of knowledge surrounding ovarian stimulation protocols, guiding clinicians in optimizing treatment strategies and ultimately improving ART success rates.

Conclusion

In conclusion, our comparative study meticulously examined the effects of GNRHant and OPP protocols on oocyte count and punctured follicle numbers, and their embryologic outcomes across consecutive IVF cycles. Even the slight discrepancies among the protocols emphasize the protocol-specific impact on the quantity of retrieved oocytes, underscoring the importance of tailored protocol selection in optimizing IVF outcomes. Further prospective investigations and randomized controlled trials are warranted to validate these findings, refine treatment

algorithms, and ultimately improve the success rates and safety profiles of IVF procedures. As we advance in understanding the intricacies of ovarian stimulation, the path to optimizing fertility treatments becomes clearer, offering successful outcomes with tailored solutions to individuals seeking assisted reproduction.

Ethics

Ethics Committee Approval: The research adhered to ethical guidelines and received approval from the Acıbadem University Ethical Committee, with the assigned (approval number: 2022-04/137 no: 10.03.2023). The study adhered to ethical standards outlined in the Declaration of Helsinki.
Informed Consent: Retrospective study.

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Footnotes

Authorship Contributions

Surgical and Medical Practices: Ö.K., B.T., A.Y., Concept: N.A., Design: N.A., Data Collection or Processing: Ö.K., Analysis or Interpretation: N.A., Literature Search: N.A., Writing: N.A., B.T.
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The association of the FOXE1 polyalanine tract length with the occurrence of premature ovarian insufficiency in the Greek population: A pilot, case-control study

FOXE1 polialanin yolu uzunluğunun Yunan toplumunda prematür over yetmezliği oluşumuyla ilişkisi: Pilot, olgu-kontrol çalışması

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Abstract

Objective: To investigate the relationship between the *FOXE1* gene polyalanine tract length and premature ovarian insufficiency (POI) in the Greek population.

Materials and Methods: Peripheral blood was collected from 28 women with POI and 29 healthy controls. DNA was extracted and the gene was amplified using the polymerase chain reaction (PCR) technique. The PCR product was sequenced and the number of alanine tracts and the genotypes was recorded. Statistical analysis examined differences in allele and genotype frequencies between the groups.

Results: The patients' group mean age was 31.68 years with a mean age of POI diagnosis of 25.18 years. Five alleles (8, 12, 14, 16, 17 comprising alanine residues) and seven genotypes (14/14, 14/16, 16/16, 14/17, 16/17, 8/16, 12/14) were identified. The 8-alanine allele was exclusive to patients, while the 12-alanine allele appeared only in controls. The most common genotype in the study group was 14/16 (64.29%), whereas the most common genotype in the control group was 14/14 (41.4%). No differences of statistical significance were observed in the prevalences of the allele with 14 (p=0.590) and 16 (p=0.594) residues or the genotype prevalences between the two groups (p=0.066).

Conclusion: Our preliminary findings suggest no correlation between FOXE1 polyalanine tract length and POI, but given the study's small sample size, they should be interpreted with caution. Further research is deemed necessary.

Keywords: Premature ovarian insufficiency, POI, FOXE1, polyalanine tract, genetics

PRECIS: We investigated the relationship between the *FOXE1* gene polyalanine tract length and premature ovarian insufficiency in Greek women, but no significant association was found.

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Öz

Amaç: Bu çalışmanın amacı *FOXE1* gen polialanin yolu uzunluğu ile Yunan toplumunda prematür over yetmezliği (POY) arasındaki ilişkiyi araştırmaktır.

Gereç ve Yöntemler: POY olan 28 kadından ve 29 sağlıklı kontrolden periferik kan örnekleri toplandı. DNA çıkarıldı ve gen, polimeraz zincir reaksiyonu (PZR) tekniği kullanılarak çoğaltıldı. PZR ürünü dizilendi ve alanin yolu sayısı ve genotipler kaydedildi. İstatistiksel analiz, gruplar arasındaki alel ve genotip sıklıklarındaki farklılıkları inceledi.

Bulgular: Hasta grubunun ortalama yaşı 31,68 yıl, POY tanısının ortalama yaşı ise 25,18 yıldı. Beş alel (alanin kalıntıları içeren 8, 12, 14, 16, 17) ve yedi genotip (14/14, 14/16, 16/16, 14/17, 16/17, 8/16, 12/14) tanımlandı. Sekiz-alanin aleli sadece hastalara özgüyken, 12-alanin aleli sadece kontrollerde görüldü. Çalışma grubunda en yaygın genotip 14/16 (%64,29) iken, kontrol grubunda en yaygın genotip 14/14 (%41,4) idi. On dört (p=0,590) ve 16 (p=0,594) kalıntılı alelin yaygınlıklarında veya iki grup arasındaki genotip yaygınlıklarında istatistiksel olarak anlamlı bir fark gözlenmedi (p=0,066).

Sonuç: Ön bulgularımız FOXE1 polialanin yolu uzunluğu ile POY arasında bir korelasyon olmadığını göstermektedir, ancak çalışmanın örneklem büyüklüğünün küçük olması göz önüne alındığında, bunlar dikkatli bir şekilde yorumlanmalıdır. Daha fazla araştırmanın gerekli olduğu düşünülmektedir.

Anahtar Kelimeler: Prematüre over yetmezliği, POI, FOXE1, polialanin yolu, genetik

Introduction

Premature ovarian insufficiency (POI) is defined as the loss of ovarian function before the age of 40 and impacts around 1% of the female population. The causes of POI vary, including chromosomal anomalies, gene variants, infectious and iatrogenic causes, and environmental factors. In 2016, the European Society of Human Reproduction and Embryology (ESHRE) provided diagnostic and management guidelines for POI, outlining specific criteria for its identification. The diagnostic work-up of women with POI includes testing for chromosomal anomalies, including the performance of a karyotype, testing for pre-mutations of the *FMR1* gene, and testing for the presence of adrenocortical auto-antibodies[1].

Several genes have been proposed as the causative factors for the appearance of POI. Specific variants have been identified for some of these genes; however, their association with POI has not been clearly defined and needs further investigation[2]. Different gene variants have been associated with POI. Given their low prevalence, testing is not recommended unless indicated by specific traits or a strong family history[1].

The *FOXE1* gene (forkhead box E1) is located in the long arm of chromosome 9, at position 22 (9q22). It contains a single exon, which encodes a protein of 367 amino acids with a molecular weight of 42 kDa[3]. This protein is a transcriptional regulator and belongs to the Forkhead box protein family. It contains a well-preserved domain of 110 amino acids, the "forkhead" domain, characterised by a structure known as the winged-helix motif[4]. The protein contains a polyalanine tract, a characteristic also found in other transcriptional suppressor proteins[5,6]. The polyalanine tract may contain 11 to 19 alanine residues, but the variants with 14 and 16 residues are the most prevalent. It has been proposed that changes in the count of alanine residues lead to altered functionality of the FOXE1 protein as a transcription factor[7-9].

With regard to POI, Watkins et al.[6] were the first to study the association of the *FOXE1* gene and the POI occurrence. They found that the allele with 16 residues was more common, whereas the allele with 14 residues was less common among women with POI. For the rest of the alleles, no statistically significant correlations were found, probably owing to their rarity[6]. In a later study by Qin et al.[10], this finding was confirmed. Additionally, it was found that genotype 16/16 was significantly more common, whereas genotype 14/14 was significantly less common in the study group than in the control group. Finally, in a smaller study, the roles of two different genes, FOXE1 and BMP15, in POI were studied. It was found that FOXE1 gene variants having a number of alanine residues other than 14 or 16 were significantly more common in patients with POI[11].

The study aimed to explore the possible role of the *FOXE1* gene in the occurrence of POI in the Greek population, and more specifically, to examine whether the length of the alanine tract of the *FOXE1* gene is associated with the condition. Our original hypothesis was that different *FOXE1* gene variants may contribute to a predisposition for POI.

Materials and Methods

Study Design

This was a pilot, case-control study, conducted from January 2018 until December 2021 at Alexandra General Hospital, Athens, Greece. Local Scientific Committee (an Institutional Review Board) approval was obtained (approval number: 972, date: 07.12.2018 - National and Kapodistrian University Ethics Committee). The study protocol complied with the Declaration of Helsinki and a signed informed consent was obtained from all the participants before their enrollment in the study.

The primary outcome of this study was the detection of the FOXE1 variants in women with POI and controls.

Participants

POI patients and controls were recruited for this study at the outpatient clinic of Alexandra General Hospital, Athens, Greece. Affected women were assessed for other causes of POI using the diagnostic workup suggested by ESHRE. More specifically, the diagnostic criteria were oligo/amenorrhea for at least 4 months and an elevated FSH level greater than 25 IU/l on two occasions, more than 4 weeks apart. The diagnostic work-up included testing for chromosomal anomalies through karyotype analysis, testing for pre-mutations of the *FMR1* gene, and testing for the presence of adrenocortical auto-antibodies. Patients with abnormal karyotype, positive 21-hydroxylase

autoantibodies, or pre-mutations of the *FMR1* gene were excluded. Additionally, women who had iatrogenic menopause (bilateral oophorectomy, chemotherapy, radiation therapy) were also excluded. All cases were matched with controls at 1:1 ratio.

The control group comprised healthy women of the general population, who had delivered at least once in the past, had entered menopause after the age of 45, had no serious medical conditions, and had presented for a routine gynaecological examination.

DNA Extraction and Detection of FOXE1 Polymorphism

For the purposes of the study, we collected peripheral blood samples from women with idiopathic POI and healthy controls. DNA was isolated using the PureLink Genomic DNA kit (Invitrogen). The polymerase chain reaction (PCR) amplification technique was performed to detect the FOXE1 polymorphism. All primers were designed by Eurofins Genomics and their sequences were as follows: FOXE1F, 5'GCGGAGGACATGTTCGAGA3' and FOXE1R, 5'CGCGGGGTAGTAGACTGGAG3'. The PCR protocol included 10X PCR Buffer minus Mg2+, 0.5 μL 10 mM dNTP mixture, 0.5 μL Primer Sense mix, 0.5 μL Primer Antisense mix, 2 μL Template DNA, 0.2 μL Taq DNA polymerase (OneTaq DNA polymerase kit, New England Biolabs), and 16.3 μL distilled water.

The PCR conditions were as follows: 95 °C for 15 min, 95 °C for 1 min, 53 °C for 1 min, 72 °C for 1 min for 29 cycles, and a final elongation step at 72 °C for 10 min. Subsequently, agarose electrophoresis was applied and the 249bp, PCR product was visualized under UV.

Sanger sequencing was applied in an ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems™) to determine FOXE1 polymorphisms in all samples. BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems™) was used in the sequencing reactions.

Statistical Analysis

Statistical analysis was performed using the SPSS 26.0 Software (SPSS Inc., Chicago, IL). Continuous variables were summarized using mean values with standard deviations and median with interquartile range. We used the two-sided Fisher's exact test and chi-square test to compare allele polymorphisms between the study group and the controls.

In this study, we did not perform a formal power analysis prior to data collection. While we acknowledge that power analysis is a crucial step in designing studies to ensure an adequate sample size to detect effects, the rarity of the condition studied posed significant challenges in recruiting a large sample size within a feasible timeframe.

Results

We recruited in total 28 women with idiopathic POI and 29 healthy controls. No statistically significant differences in the population characteristics were found between the two groups, apart from age (31.68±12.86 *vs.* 55.37±5.7, p<0.001). This was intentional, as the control group was composed of women with documented menopause after the age of 45.

The study group's mean age was 31.68 years, and the mean age at the onset of POI was 25.18 years. The women's average height was 1.64 meters, their average weight was 63.2 kilograms, and their average body mass index was 23.41. Nine women (32.14%) had a family history of POI. One woman had a history of melanoma, another was diagnosed with neutropenia, a third had a history of right ovarian torsion, a fourth had celiac disease, and another had short stature.

We detected 5 different alleles, with 8, 12, 14, 16, and 17 alanine residues. Seven genotypes were identified: 14/14, 14/16, 16/16, 14/17, 16/17, 8/16, and 12/14. The allele with 8 alanine residues appeared only in one patient, while the 12-residue allele appeared only in controls. The genotypes 8/16 and 14/17 were observed only in the patients' group, while the genotypes 12/14 and 16/17 were noted only in the control group. The most prevalent genotype in the study group was 14/16 (64.29%), whereas the 14/14 genotype was the most common in controls (41.4%). There were no statistically significant differences in the prevalence of the recorded genotypes between the two groups (p=0.066) (Table 1). Moreover, the individual frequencies of the alleles with 14 and 16 residues among patients and controls did not differ significantly (p=0.590 and 0.594, respectively). These results are summarized in Table 2.

Discussion

Main Findings and Comparison with Existing Literature

Although different causes of POI have been identified, in most cases, there is no causal factor, and hence these cases are termed idiopathic. Genetic studies have revealed several gene variants that predispose their carriers to developing POI. Such an example is the *FMR1* gene, for which the presence of premutations is investigated in all cases of primary ovarian insufficiency. Likewise, other potential gene variations could

Table 1. The various genotypes that were observed in the study and the control group

Genotype	Study group		Control group		p-value
	n	%	n	%	
8/16	1	3.6	0	0	-
12/14	0	0	1	3.4	-
14/14	6	21.4	12	41.4	-
14/16	18	64.3	10	34.5	-
14/17	1	3.6	0	0	-
16/16	2	7.1	5	17.2	-
16/17	0	0	1	3.4	-
Total	28	100.00	29	100.00	0.066

Table 2. Individual frequencies of alleles 14 and 16 in the study and the control group

Allele	Study group (n=56)	Control group (n=58)	p-value
	n (%)	n (%)	
14	31 (55.5)	35 (60.3)	0.59
16	23 (41.1)	21 (36.2)	0.596

be included in the diagnostic work-up of POI, depending on their prevalence and clinical significance. *FOXE1* gene variants have previously been associated with the occurrence of POI; however, the exact association and relative risk are still unclear. Repeated alanine residues have been found in several genes, where the length of the polyalanine tract is highly conserved, likely separating functional protein domains. Alterations in polyalanine length have been associated with various conditions[6,12], as seen in the *FOXL2* gene, which shares structural similarities with FOXE1, including a polyalanine tract and a well-preserved "forkhead" domain. In FOXL2, increases in polyalanine length are responsible for 25-30% of mutations leading to Blepharophimosis (BPES) type II[13,14] while decreases in length have been linked to a case of POI without BPES[15]. This evidence suggests that FOXE1 polyalanine tract length could similarly play a role in POI, although this remains uncertain.

This study examined the possible association between variations in the number of alanine residues in the *FOXE1* gene's polyalanine tract and the occurrence of POI among women from Greece. We found that the alleles with 14 and 16 residues are the most common among both patients and controls. The genotype 14/16 was the most prevalent in the study group, whereas the genotype 14/14 was the most prevalent in the control group. No statistically significant differences were noted in the prevalence of the detected genotypes or the prevalence of the most common alleles (14 and 16 residues) between the two populations.

FOXE1 is a transcription factor with multiple functions[4]. During fetal development, it is expressed in the growing thyroid, the pituitary gland, and the branchial arches, while in adulthood, it is expressed in the thyroid gland, the epidermis, the hair follicles, and the pre-pubertal testis[5,16,17]. Mutations of the gene can cause the Bamforth-Lazarus syndrome, which is characterized by congenital hypothyroidism, thyroid dysgenesis, cleft palate, spiky hair, bifid epiglottis, ocular hypertelorism, and choanal atresia[18,19]. It seems to be associated with the occurrence of various cancers, such as thyroid, colon, skin, breast, and liver cancer[20-24]. It is likely a major transcription factor with more functions than those described here.

Previous publications have proposed that variations in the *FOXE1* gene's polyalanine tract length are associated with the appearance of POI. Watkins et al.[6] found that the allele with 16 residues was significantly more common, while the allele with 14 residues was significantly less common among women with POI. Qin et al.[10] came to the same conclusion and they also found that genotype 16/16 was significantly more prevalent, whereas genotype 14/14 was significantly less prevalent in the study group than in the control group. Settas et al.[11] also found that alleles with an altered number of alanine residues (other than 14 or 16) were significantly more frequent among patients with POI.

Interestingly, the prevalence of the different genotypes among the European and the Chinese populations differed significantly, as described by the studies of Watkins et al.[6] and Qin et al.[10]. Based on the study by Watkins et al.[6], genotype 14/14 was found in 27.2% of the patients with POI and 46.5% of the control group, respectively. In the study by Qin et al.[10], the prevalence of the same genotype was 81.2% for the study and 96.4% for the control group. Additionally, the frequency of the allele with 16 residues was significantly lower in the Chinese population than in the European population, indicating a high heterogeneity between these two populations, probably owing to their geographic distance[6,10].

In our study, there were no statistically significant differences in the prevalence of the observed genotypes between the study and the control group. Additionally, no correlation was found between the detected alleles and the presence of POI. These results seem to contradict the findings of both Watkins et al.[6] and Qin et al.[10] and raise concerns as to whether the *FOXE1* gene is causally related to POI.

Study Limitations

The lack of a formal power analysis and the small sample size are two main limitations of our study. Given the rare nature of the condition under investigation and the relatively small Greek population, it was difficult to recruit a larger sample size within a feasible timeframe. Therefore, our results should be interpreted with caution. It is unclear whether the null results observed are indeed due to the absence of genetic effects or simply due to insufficient power to detect such effects. This limits the interpretability of our findings.

Implications

Our preliminary findings suggest no correlation between *FOXE1* genes and POI. Further research with larger sample sizes and appropriate power calculations is deemed necessary to confirm these findings and to draw more definitive conclusions about the genetic effects under study. Until then, *FOXE1* gene detection could not be supported as a diagnostic tool for POI. Furthermore, future studies could use whole genome sequencing to provide a more comprehensive analysis of the genetic factors involved in POI.

Conclusion

The *FOXE1* gene encodes a transcriptional factor with multiple functions. It has been associated with various conditions such as Bamforth-Lazarus syndrome, cleft palate, thyroid dysgenesis, and various forms of malignancy. Even though changes in the

polyalanine tract of the gene have been associated with the occurrence of POI, its role in the pathogenesis of POI remains unclear. Our preliminary findings seem to contradict the findings of previous studies, but they should be interpreted with caution, given the study's small sample size. Further research on the *FOXE1* gene or an extended genetic panel is necessary to elucidate the causes of POI.

Ethics

Ethics Committee Approval: Local Scientific Committee (an Institutional Review Board) approval was obtained (approval number: 972, date: 07.12.2018 - National and Kapodistrian University Ethics Committee).
Informed Consent: A signed informed consent was obtained from all the participants before their enrollment in the study.

Footnotes

Authorship Contributions

Concept: A.K., L.M., Design: A.K., P.D., L.M., Data Collection or Processing: A.K., S.I., Analysis or Interpretation: A.K., M.P., D.M., Writing: A.K., M.P., S.I., D.M., P.D., L.M.
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Association study of *interleukin-10* and *P53* polymorphisms and their influence on Iranian women with recurrent abortion

İnterlökin-10 ve P53 polimorfizmlerinin ilişki çalışması ve bunların tekrarlayan düşük yapan İranlı kadınlar üzerindeki etkileri

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Abstract

Objective: Recurrent spontaneous abortion (RSA), characterized by multiple miscarriages without a known cause, includes both genetic and non-genetic factors. In this research, we studied the association between two polymorphisms of the *interleukin (IL)-10* and *P53* genes and RSA for the first time in the southwest of Iran.

Materials and Methods: This was a case-control study involving 62 patients with a history of at least two RSA of unknown etiology, as well as 66 healthy individuals. Clinical factors were analyzed. Genomic DNA was extracted from whole blood. Genotyping was performed using amplification refractory mutation system-polymerase chain technique to investigate two single nucleotide polymorphisms (SNPs) of *P53* and *IL-10* genes. Gene-gene interactions were analyzed by logistic regression. Statistical analysis was performed using a significance level of $p<0.05$.

Results: Allelic and genotypic frequencies as well as dominant, recessive and over dominant models for two SNPs, rs1042522 and rs1800871, were investigated. No significant association with RSA ($p>0.05$) was found. The combination of the homozygote CC for the polymorphism rs1042522 in the P53 gene and the homozygote CC for the polymorphism rs1800871 in the homozygote CC for the polymorphism rs1800871 in the *IL-10* gene was associated with an increased risk of spontaneous abortion ($p=0.01$). Meanwhile, the phenotypic frequency of individuals with a history of consanguineous marriage was statistically significant between the case and control groups ($p=0.003$).

Conclusion: Limited studies have been conducted on the association between these two polymorphisms and RSA, and conflicting results have been obtained. Further investigation with a larger sample size may confirm results. Genetic research, such as this, helps understand genetic factors associated with the risk of RSA.

Keywords: Polymorphism, infertility, abortion, spontaneous

PRECIS: In this research, the association of *interleukin-10* and *P53* genes polymorphisms with recurrent spontaneous abortion was studied.

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Öz

Amaç: Bilinen bir nedeni olmayan birden fazla düşükle karakterize tekrarlayan spontan düşükler (RSA), hem genetik hem de genetik olmayan faktörler ile ilişkilidir. Bu araştırmada, ilk kez İran'ın güneybatısında *interlökin (IL)-10* ve *P53* genlerinin iki polimorfizmi ile RSA arasındaki ilişkiyi inceledik.

Gereç ve Yöntemler: Bu çalışma, en az iki bilinmeyen etiyolojiye sahip RSA öyküsü olan 62 hastayı ve 66 sağlıklı grubu içeren bir olgu kontrol çalışmasıydı. Klinik faktörler analiz edildi. Tam kandan genomik DNA çıkarıldı. *P53* ve *IL-10* genlerinin iki tek nükleotid polimorfizmini (SNP) araştırmak için amplifikasyona dirençli mutasyon sistemi-polimeraz zincir reaksiyonu tekniği kullanılarak genotipleme yapıldı. Gen-gen etkileşimleri lojistik regresyonla analiz edildi. İstatistiksel analiz p<0,05 dikkate alınarak yapıldı.

Bulgular: İki SNP olan rs1042522 ve rs1800871 için allelik ve genotipik frekanslar ile dominant, resesif ve aşırı dominant modeller araştırıldı. RSA ile anlamlı bir ilişki bulunamadı (p>0,05). Polimorfizm rs1042522 *P53* geni için homozigot CC ve polimorfizm rs1800871 *IL-10* geni için homozigot CC'ye sahip olanların genotip kombinasyonu spontan düşük riskini artırmıştır (p=0,01). Akraba evliliği öyküsü olan bireylerin fenotipik sıklığı olgu ve kontrol grupları arasında istatistiksel açıdan anlamlı olarak farklıydı (p=0,003).

Sonuç: Bu iki polimorfizm ile RSA arasındaki ilişki üzerine sınırlı sayıda çalışma yürütülmüş ve çelişkili sonuçlar elde edilmiştir. Daha büyük bir örneklem büyüklüğü ile yapılacak daha fazla araştırma bu çalışmanın sonuçlarını doğrulayabilir. Bu gibi genetik araştırmalar, RSA riskiyle ilişkili genetik faktörleri anlamaya yardımcı olmaktadır.

Anahtar Kelimeler: Polimorfizm, kısırlık, kürtaj, spontan

Introduction

Miscarriage, clinically termed spontaneous abortion, represents one of the prevailing complications in pregnancy, defined as the natural termination and expulsion of a fetus before it can independently sustain life. The term "miscarriage" commonly encompasses all forms of pregnancy loss occurring prior to the 20th week of gestation[1]. Recurrent spontaneous abortion (RSA) occurs when spontaneous abortion occurs more than twice during the initial 20 weeks of pregnancy, posing a significant challenge as it affects up to 20% of known pregnancies[2]. There are approximately one to two percent of women of childbearing suffering from RSA, which describes women who suffer from three or more continuous miscarriages in a row[2]. Predominantly observed between 8 and 12 weeks of gestation, with reduced prevalence in instances where etiology diverges, RSA substantially impacts pregnancy outcomes, warranting exploration into its underlying genetic determinants[3]. Furthermore, the prevalence of RSA approximates one in every 300 births, underscoring its clinical significance[3]. Notably, in individuals with a history of RSA, the likelihood of subsequent miscarriage increases progressively, with probabilities of 24%, 30%, and 40-50% after two, three, and four miscarriages, respectively[3]. RSA, from a medical standpoint, may also precipitate infertility owing to successive pregnancy losses[3].

The identifiable causes of RSA primarily include chromosomal abnormalities, anatomical irregularities, and hormonal imbalances (such as progesterone, estrogen, diabetes, thyroid disorders)[4]. However, over half of RSA cases (>50%) are attributed to deficiencies in blood coagulation proteins[5]. This deficiency in the blood clotting system is often associated with defects in coagulation inhibitors or proteins involved in the fibrinolytic pathway, resulting in an increased risk of blood clot formation[5]. Limited literature reports instances where deficiencies in clotting factors serve as the underlying cause of RSA. The most common deficiencies implicated include factor XIII, factor XII, and fibrinogen, which includes afibrinogenemia and dysfibrinogenemia[6].

RSA is a multifactorial disease, and different genes affect the occurrence of this disease. Genetic involvement in pregnancy complications is substantiated by candidate gene-based association studies, wherein specific polymorphic variants of genes are scrutinized for their potential roles in pregnancy[7]. In this way, several polymorphisms are associated with adverse pregnancy outcomes such as RSA[8,9].

Interleukin (IL)-10 orchestrates intricate interactions with various factors and cell types pivotal to pregnancy[10]. A successful gestation relies on maintaining equilibrium between immune responses mediated by Th1 and Th2 cells[11]. Notably, fetal survival correlates with a prevailing Th2 immune response, while a Th1-dominant response is linked to pregnancy failure[12]. IL-10, acting as a crucial regulator of Th2 immune responses, exerts potent anti-inflammatory effects by suppressing proinflammatory cytokine synthesis, thereby fostering a Th2 cytokine milieu that downregulates Th1 cytokine expression[13]. Enhanced maternal IL-10 production is associated with successful pregnancies, whereas diminished levels are implicated in recurrent fetal loss, potentially predisposing to immune system compromise and placental vascular insufficiency during pregnancy[13].

The regulation of IL-10 production is genetically determined and controlled at the transcriptional level, likely involving regulatory sequences within its promoter region[14]. Several studies suggested that certain IL-10 polymorphisms may heighten the risk of spontaneous abortion or confer protective effects against it[15]. Notably, several single nucleotide polymorphisms within the *IL-10* gene promoter region, such as -1082(A/G) (rs1800896), -819 (C/T) (rs1800871), and -592 (C/A) (rs1800872), have been implicated in increased rates of spontaneous abortions among some populations[16]. However, the precise role of *IL-10* gene polymorphisms in RSA remains a subject of debate.

On the other hand, the *P53* gene, known for its pleiotropic effects, plays a crucial role in vasculogenesis and cell apoptosis, essential processes for successful trophoblast cell invasion[17].

Recent investigations have revealed elevated apoptosis levels in chorionic villi and decidua, alongside heightened *P53* gene expression in placental villi among patients with recurrent unexplained spontaneous abortions[18]. These findings suggest that *P53* gene-induced apoptosis may contribute to RSA, underscoring its potential role as a pregnancy mediator with estrogen and progesterone activities. Various reports have linked several polymorphisms of the *P53* gene with RSA.

In a multivariate analysis, the P53 p.Pro72Arg (rs1042522) polymorphism was investigated, revealing an association with RSA and increased risk. The *P53* gene induces the expression of leukemia inhibitory factor (LIF); it is appropriate to consider the association of *P53* gene polymorphisms in patients who experience RSA and infertility, compared to fertile control groups[19]. A proline-rich domain at codon 72 is associated with reduced LIF expression, lower apoptosis rates, and G1 cell cycle arrest compared with arginine at codon 72[20]. These findings suggest that this polymorphism in the *P53* gene may serve as a risk factor for spontaneous abortion. In this study, for the first time, we aimed to investigate the potential association between genetic polymorphisms in the *IL-10* gene (rs1800871) and the *P53* gene (rs1042522) with RSA of unknown etiology in the population of Southwestern Iran.

Materials and Methods

This case-control study targeted 62 women with a primary diagnosis of RSA as the patient group and 66 women with no history of abortion as the control group. Firstly, informed consent forms were obtained from all patients and healthy individuals participating in the study. The study protocol was approved by the Ethics Committee of Islamic Azad University North Tehran Branch (approval no: IR.IAU.TNB.REC.1401.065, date: 13.12.2022). Blood samples were collected from infertility treatment clinics for women in Ahvaz city, referred by gynecologists. The samples were then transferred to the Genetics Department Laboratory of Jundishapur University of Medical Sciences, Ahvaz, following strict adherence to cold chain protocols.

Inclusion and Exclusion Criteria for Patients with RSA and A Healthy Control Group

In this study, women experiencing RSA, characterized by a history of at least two spontaneous abortions before the 20th week of pregnancy, were selected as the patient cohort. Medical records and examination results of all enrolled subjects were thoroughly reviewed for evaluation of chromosomal, anatomical, and pathological abnormalities, as well as common coagulation factors (c.C667T, c.A1298C), MTHFR, FACTOR II (c. G20210A), FACTOR V (c.G1691A), PAI 1 (4G/5G). This was done to ensure normalcy. Additionally, structured questionnaires were administered to gather pertinent information, including age, ethnicity, parity, history of abortion or infertility in the family, and consanguinity. Exclusion criteria encompassed the presence of chromosomal abnormalities in children and parents, anatomical uterine and ovarian disorders, antiphospholipid syndrome, and mutations in coagulation factors. A cohort of women free from a history of miscarriage, infertility, hormonal and anatomical abnormalities, and possessing at least two healthy children was chosen as the control group. Similar to the approach used for the patient cohort, a comprehensive questionnaire was administered to gather demographic and clinical data.

Blood DNA Extraction and Genotyping

Genomic DNA was extracted from anticoagulated peripheral blood samples using a DNA blood extraction kit (Sina Clon Company, Iran), and stored at -20 °C. All DNA extraction procedures were meticulously carried out in a Biosafety Level 2 laboratory. Genotyping of rs1800871 (c.-C819T) in IL-10 and rs1042522 (p.Arg72Pro) in P53 was performed using the amplification refractory mutation system-polymerase chain reaction (PCR) method. Designed primers were used for PCR amplification, with details provided in Table 1[21,22]. PCR reactions were conducted in a thermal cycler, and the resulting amplicons were analyzed via 1% agarose gel electrophoresis. Subsequently, cycles of denaturation, annealing, and extension were carried out. The reaction mixture for both polymorphisms underwent an initial denaturation step at 94 °C for 4 minutes, followed by 30 cycles of denaturation at 94 °C for 30 seconds, annealing and extension at 72 °C for 30 seconds for both single nucleotide polymorphisms (SNPs). Concerning the rs1800871 polymorphism, the annealing step was set at 60 °C for 30 seconds, and for the rs1042522 polymorphism, the annealing step was set at 64 °C for 30 seconds. Both protocols concluded with a final extension step: 72 °C for 2 minutes for both SNPs. Then, 10% of the samples were evaluated to genotype both polymorphisms by the Sanger sequencing method.

Statistical Analysis

In this research, the Statistical Package for the Social Sciences, version 26.0 (Chicago, Illinois, USA), and the χ^2 test were used for data analyses. Continuous variables were expressed in the form of mean ± standard deviation. The Kolmogorov-Smirnov test was applied to check whether the data were normally distributed. Normally distributed and abnormally distributed data between two groups were calculated by t-test and Mann-Whitney U test, respectively.

In order to verify any genotyping error, Hardy-Weinberg equilibrium (HWE) was calculated using the chi-squared test. The odds ratio (OR) and 95% confidence intervals (CIs) women with miscarriage compared to fertile women were calculated among four frequent genetic inheritance models, including the allelic model, dominant, recessive, and overdominant model. The p-value less than 0.05 was considered significant. At the end of the work to verify the specificity of the primers used and the accuracy of genotyping, Sanger sequencing was performed using the PCR primer and Big Dye Terminators (model 3130 Genetic Analyzer, Applied Biosystems Foster City CA USA).

Then, Sanger sequence analysis was conducted by Chromas 2.6.6 software.

Results

Demographic Characteristics of the Studied Subjects

Sampling for this study was conducted exclusively among case and control groups in Southwest Iran. The patient cohort with RSA, for whom the cause remained undiagnosed despite specialist consultation and genetic/pathological investigations, requires further study. The average age of the patient group was 30.69±4.76 years, with an average history of 3.18 miscarriages. On the other hand, the average age of the healthy control group was 32.73±5.31 years. Key demographic information, including average age, smoking habits, consanguinity, and history of abortions and infertility, is summarized in Table 2. A statistically significant disparity in the rate of consanguineous marriages among parents was observed between the patient and control groups (p=0.003), highlighting a potential contributing factor to RSA. Furthermore, no significant difference was noted in the average age between patients and healthy controls (p=0.1), and smoking habits were comparable across both groups (p=0.6). The t-test was employed for quantitative variables such as age, whereas the χ^2 test, was utilized to assess smoking habits as smoking habits are qualitative.

Association Study of rs1800871 and rs1042522 Polymorphisms with Recurrent Spontaneous Abortion

Statistical analysis of genotypic and allelic distributions for both rs1800871 and rs1042522 SNPs, is presented in Table 3. No significant associations were observed in genotypic frequencies between control subjects and patients in these two polymorphisms. Herein, we presented different models, including allelic, dominant, recessive, and over dominant models in Tables 4 and 5. Besides, we compared heterozygote with normal homozygote genotypes in the case and control groups for both SNPs. No significant differences between case and control groups, when using different models for both studied polymorphisms in *P53* and *IL-10* genes (Tables 4 and 5). Furthermore, OR calculations for the CT and TT genotypes using rs1800871 polymorphism, as well as the CG and GG genotypes for rs1042522 polymorphism did not demonstrate significant associations with RSA (p>0.05) (Tables 4 and 5). The HWE equilibrium analysis confirmed the balance of the rs1800871 and rs1042522 SNPs within the study population.

Combined Genotype Distribution for rs1800871 and rs1042522 Polymorphism

The combined genotype distribution of rs1800871 and rs1042522 SNPs was analyzed in both RSA cases and control groups. Among the comparisons that have been made, the CC + CC genotype combination was significantly more frequent in patients with RSA compared to the control group in this study (16.1% vs. 3.0%, OR: 0.163, 95% CI: 0.034-0.755, p=0.011). However, other genotype combinations, including GG + CC, CC + CT, CC + TT, CG + CC, CG + CT, CG + TT, GG + CT, and GG + TT, did not demonstrate significant differences between the two groups (Table 6). Overall, these findings suggest a potential association between specific genotype combinations of rs1800871 and rs1042522 SNPs and susceptibility to recurrent abortion, providing insights into the genetic factors underlying this condition.

Table 1. The primers used to survey rs1042522 and rs1800871 polymorphisms[22-41]

Gene name	Polymorphim	Primer type	Primer sequence (5' to 3')
IL-10	Rs1800871	R*	AGGATGTGTTCCAGGCTCCT
IL-10 N*	Rs1800871	F*	CCCTTGTACAGGTGATGATGTAAC
IL-10 M*	Rs1800871	F	ACCCTTGTACAGGTGATGTAAT
P53 N	Rs1042522	F	TCCCCCTTGCCGTCCCAA
P53 N	Rs1042522	R	CTGGTGCAGGGGCCACGC
P53 M	Rs1042522	F	GCCAGAGGCTGCTCCCCC
P53 M*	Rs1042522	R	CGTGCAAGTCACAGACTT

*R: Reverse, N: Normal, *F: Forward, M: Mutant, IL: Interleukin

Table 2. Clinical and demographic characteristics of the studied subjects

Characteristics	Patients (n=62)	Controls (n=66)	p-value
Age (mean)	30.69±4.76	32.73±5.31	0.104
Consanguineous marriage (Percentage %)	69.2% (n=27)	30.8% (n=12)	**0.003**
History of abortion and infertility (Percentage %)	25.92% (n=14)	-	-
Tobacco consumption (Percentage %)	3.7% (n=2)	5.6% (n=3)	0.647

n: Number

Table 3. Frequency distribution of rs1042522 and rs1800871 polymorphisms in all cases and controls

	Genotypes	Case	Control	Total	χ^2	p-value
P53	CC	35.5% (n=22)	27.3% (n=18)	31.3% (n=40)	1.499	0.473
	CG	54.8% (n=34)	57.6% (n=38)	56.3% (n=72)		
	GG	9.7% (n=6)	15.2% (n=10)	12.5% (n=16)		
	Total	100.0% (n=62)	100.0% (n=66)	100.0% (n=128)		
IL-10	CC	32.3% (n=20)	31.8% (n=21)	32.0% (n=41)	0.18	0.91
	CT	54.8% (n=34)	57.6% (n=38)	56.3% (n=72)		
	TT	12.9% (n=8)	10.6% (n=7)	11.7% (n=15)		
	Total	100.0% (n=62)	100.0% (n=66)	100.0% (n=128)		

IL: Interleukin

Table 4. Association study and different genetic models analysis of polymorphism rs1800871 between recurrent spontaneous abortion and control groups

Genotype/allele	Type	Patient	Control	Odds ratio	(95% confidence interval)		p-value
		n (%)	n (%)		Lower	Upper	
CC	Genotype	19 (35.2%)	19 (35.2%)	-	-	-	-
CT	Genotype	31 (57.4%)	30 (55.6%)	1.033	0.46	2.323	0.937
TT	Genotype	4 (7.4%)	5 (9.3%)	0.8	0.186	3.446	0.255
TT vs CC +CT recessive	Genetic model	50 (92.5%)	49 (90.7%)	0.874	0.199	3.093	0.727
CT +TT vs CC dominant	Genetic model	35 (64.8%)	35 (64.8%)	0.968	0.43	2.179	0.938
CT vs TT+CC over dominant	Genetic model	23 (42.5%)	24 (44.4%)	1.078	0.504	2.308	0.846
C	Allele	69 (63.9%)	68 (63.0%)	-	-	-	-
T	Allele	39 (36.1%)	40 (37.0%)	0.961	0.552	1.672	0.888

Discussion

To date, the simultaneous association of the SNPs rs1042522 and rs1800871 with RSA has not been investigated on a global scale. In this research, we studied the association of two polymorphisms in *P53* and *IL-10* genes with RSA, in the southwest of Iran for the first time. One of our main findings indicated that the genotypes frequency of P53 rs1042522 and IL-10 rs1800871 SNPs didn't show significant differences between RSA and control groups (p>0.05). Meanwhile, the comparison of alleles, recessive, dominant, and over dominant models did not demonstrate significant association between patients and healthy controls.

Previous research has analyzed the genotype and alleles of these polymorphisms individually, yielding results that either align with or contradict our findings. These findings demonstrated a complex association between *P53* and *IL-10* gene polymorphisms and RSA, which may vary across different populations. Concerning the rs1042522 polymorphism, similar results lacking an association between this polymorphism and RSA have been reported in previous studies. For instance, Yoon et al.[23] in 2015 in Korea studied 594 individuals (294 patients

Table 5. Association study and different genetic models analysis of polymorphism rs1042522 between recurrent spontaneous abortion and control groups

Polymorphism	Type	Patients	Control	(95% confidence interval)		Odds ratio	p-value
		n (%)	n (%)	Lower	Upper		
CC	Genotype	23 (42.6%)	15 (27.8%)	-	-	-	-
CG	Genotype	25 (46.3%)	31 (57.4%)	0.228	1.215	0.526	0.131
GG	Genotype	6 (11.1%)	8 (14.8%)	0.141	1.694	0.489	0.255
CG+GG vs CC dominant	Genetic model	31 (57.4%)	39 (72.2%)	0.232	1.158	0.518	0.107
GG vs CC+CG recessive	Genetic model	48 (88.9%)	46 (85.2%)	0.231	2.232	0.719	0.567
CG vs GG+CC over dominant	Genetic model	29 (53.7%)	23 (42.6%)	0.299	1.367	0.64	0.248
C	Allele	71 (65.7%)	61 (56.5%)	-	-	-	-
G	Allele	37 (34.3%)	47 (43.5%)	0.39	1.172	0.676	0.163

Table 6. Combined genotype distribution for rs1800871 and rs1042522 polymorphisms in recurrent spontaneous abortion and control group

p.Arg 72 pro + c.- C819T	Case (n=62)	Control (n=66)	Odds ratio	p-value
CC+CC	16.1% (n=10)	3.0% (n=2)	0.163 (0.034-0.755)	0.011
CC+CT	14.5% (n=9)	19.7% (n=13)	0.692 (0.273-1.757)	0.437
CC+TT	4.8% (n=3)	4.5% (n=3)	1.068 (0.207-5.500)	0.937
CG+CC	14.5% (n=9)	22.7% (n=15)	0.577 (0.232-1.4360)	0.234
CG+CT	32.3% (n=20)	30.3% (n=20)	1.095 (0.519-2.313)	0.812
CG+TT	8.1% (n=5)	4.5% (n=3)	1.842 (0.421-8.056)	0.411
GG+CC	1.6% (n=1)	6.1% (n=4)	0.254 (0.028-2.339)	0.194
GG+CT	8.1% (n=5)	7.6% (n=5)	1.070 (0.294-3.892)	0.918
GG+TT	0.0% (n=0)	1.5% (n=1)	1.015 (0.985-1.046)	0.331

and 300 healthy individuals) and revealed no significant association (p=0.3) between the rs1042522 polymorphism and RSA. On the other hand, Allafan et al.[25] in 2015 in Iran, Mashhad city (in the northeast of Iran), investigated 120 individuals (80 patients and 40 healthy individuals) and reported no association between the rs1042522 polymorphism and repeated in vitro fertilization (IVF) failure (p>0.05). In 2011, Wiwanitkit[26] studied a population in India a population including 302 patients, 302 patients, 57 controls, and 70 IVF failure cases. They reported no significant association between rs1042522 polymorphism and RSA or recurrent implantation failure (p>0.05).

Conversely, contrasting results have been reported in some studies for the rs1042522 polymorphism. For instance, in one of the cities in the center of Iran, Firouzabadi et al.[27] conducted a study in 2009, involving 62 individuals (41 patients and 21 healthy individuals), which revealed that the rs1042522 polymorphism may be a contributing factor in recurrent miscarriage and implantation failure (p=0.038). In 2013, a study in Spain by Lldeo et al.[28] demonstrated a significant association (p<0.05) between the rs1042522 polymorphism and RSA in a population, which included 98 patients and 83 healthy individuals. Similarly, a study in Spain in 2018 by Turienzo et al.[29] reported a significant association between the rs1042522 polymorphism and RSA in a non-selective population comprising 89 patients and 89 healthy individuals (p<0.05). Another study on RSA, conducted in Greece in 2022 by Dedousi et al.[30], involving 206 individuals (100 patients and 106 controls), confirmed a significant association between the studied polymorphism and RSA (p=0.002).

Concerning association studies of the rs1800871 polymorphism in IL-10 with RSA, various studies have demonstrated contradictory results. For instance, similarly to our results, in 2013, in India, Parveen et al.[31] found no significant association between RSA and rs1800871 polymorphism in a population of 298 individuals (134 patients and 164 healthy individuals) (p=0.3). Similarly, in 2014 in Bahrain, Qaddourah et al.[32] demonstrated no significant association between RSA and the rs1800871 polymorphism [-819(C/T)] in an Arab population comprising 296 patients and 305 healthy individuals (p=0.3).

Conversely, some studies have confirmed the association of the rs1800871 polymorphism with RSA. In Iran, in 2014, Bahadori et al.[33] investigated the association between rs1800871 polymorphism and RSA in a population of 196 individuals, comprising 191 patients and 95 healthy controls, in Tehran, and the results of their research were reported to be significant (p=0.006). In another study in 2014 in Ukraine, Zastavna et al.[34] demonstrated a significant association between rs1800871 polymorphism and RSA in a population of 100 patients and 73 healthy individuals (p<0.001). Similarly, in Romania in the same year, Bohiltea and Radoi[35] found a significant association between the rs1800871 polymorphism and RSA in a population of 69 patients and 64 healthy individuals (p=0.02). In 2015, Liu et al.[36] showed a significant association between the rs1800871 polymorphism and RSA in a Chinese Asian population comprising 284 patients and 284 healthy individuals. Additionally, in 2017, in the Indian Asian population, Vidyadhari et al.[23] showed a significant association between the rs1800871 polymorphism and RSA in a population of 180 individuals (100 healthy and 80 patients) (p<0.001).

Regarding all contradictory results in different studies, the non-significance observed in our study's findings may be attributed to ethnic differences and possibly the need for a larger sample size to investigate these polymorphisms further. Despite the high allelic frequency of these two studied polymorphisms, studying more samples across various countries and ethnicities is necessary to obtain a more accurate understanding of its association with RSA. Such studies are crucial for identifying factors involved for women with a history of RSA in different populations.

The second main result of this study was that individuals with the CC homozygous genotype for rs1042522 and rs1800871 polymorphisms were at an increased risk of spontaneous abortion (p=0.01) (Table 6). This is the first study that demonstrates the combined genotype distribution of these two polymorphisms, with RSA, which might provide novel insights into their combined influence on RSA. However, further studies need to be performed to confirm this effect.

The last main findings in our study were from investigating several phenotypic characteristics such as consanguineous marriage and the age of women in the target and control groups. According to the results obtained from this study, consanguineous marriage is likely to be associated with RSA (p=0.003), although so far most studies have been mainly cross-sectional and selected in specific regions and ethnic groups. The highest rate of consanguineous marriage can be observed in Middle Eastern countries. the prevalence of consanguineous marriage in Iran is about 38.6%, Due to socio-cultural factors. There is controversy as to whether there is any correlation between RSA and consanguinity[36]. Rad[38] determined that RSA occurs more frequently among related couples than those unrelated in India. An Iranian study conducted in 2010 found RSA to be more prevalent in the consanguineous group as compared to the non-consanguineous group. However, Saad and Jauniaux[39] found in 2002 that consanguinity did not correlate with RSA in Qatar. They concluded that this finding could be explained by the fact that autosomal recessive alleles are uncommon in Qatar. It could also be explained by the lack of association between consanguinity and RSA. In 2011, Gowri et al.[40] reported that consanguinity appears not to have any significant impact on the etiology of RSA and has no association with it in Oman. Further studies will be necessary to draw a conclusion.

Conclusion

As a result, research on rs1042522 and rs1800871 SNP and RSA is limited in Iran. Previous studies have *linked IL-10* gene rs1800871 and *P53* gene rs1042522 polymorphisms with RSA with different results. Research in this area is necessary to enhance understanding of the genetic factors of RSA and to identify possible risk factors for it.

Ethics

Ethics Committee Approval: The study protocol was approved by the Ethics Committee of Islamic Azad University North Tehran Branch (approval no: IR.IAU.TNB.REC.1401.065, date: 13.12.2022).
Informed Consent: Informed consent was obtained from the patients.

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Footnotes

Authorship Contributions

Concept: P.G., Design: P.G., Data Collection or Processing: N.R.B., K.S., F.S., Analysis or Interpretation: K.S., F.S., P.G., Literature Search: N.R.Z., P.K., Writing: N.R.Z.
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Evaluation of prenatal and postnatal outcomes of fetuses with intrauterine cardiac anomalies: Tertiary center experience

İntrauterin dönemde kardiyak anomali tanısı alan fetusların prenatal ve postnatal sonuçlarının değerlendirilmesi: Tersiyer merkez deneyimi

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Abstract

Objective: Fetal cardiac anomalies are among the leading causes of infant mortality due to congenital anomalies. The prenatal diagnosis of congenital heart diseases allows for the acquisition of prognostic information before birth and provides insights into treatment options either before or after delivery. This study aims to observe the correlation between the prenatal and postnatal diagnoses of fetuses with cardiac anomalies detected in our perinatology clinic. The goal, by tracking postnatal outcomes and identifying risk factors, is to assist in selecting the most appropriate approach, prioritizing maternal and fetal health.

Materials and Methods: The records of 188 fetuses diagnosed during the prenatal period by the Perinatology Department of Obstetrics and Gynecology at Çukurova University Faculty of Medicine, delivered and admitted to the Çukurova University Neonatal Intensive Care Unit, and undergoing fetal echocardiography by the Pediatric Cardiology Clinic between January 2016 and December 2021, were retrospectively evaluated. Postnatal transthoracic echocardiography results of the infants were also reviewed.

Results: Our study was conducted with 188 pregnant women. The most frequently detected cardiac anomalies in the fetuses were conotruncal anomalies, followed by right heart anomalies. The concordance between prenatal and postnatal findings was 88.8%, with a sensitivity of 96.55% and a specificity of 100%. Among the live-born infants with congenital heart disease, significant differences were observed between the group that survived the neonatal period and those who did not, in terms of parental consanguinity, gestational age at birth, birth weight, APGAR scores, and the rate of chromosomal anomaly assessment.

Conclusion: Our study emphasized several risk factors. A high concordance was found between our prenatal and postnatal echocardiography findings. In conclusion, we believe that increasing awareness and making screening a routine practice are essential to contributing to healthier future generations. This can be achieved by reducing perinatal mortality and morbidity through appropriate management and equipment, thereby optimizing the well-being of affected individuals in society.

Keywords: Congenital heart diseases, fetal anomaly, prenatal diagnosis

PRECIS: Fetal heart anomalies are the leading cause of infant mortality due to congenital anomalies. Prenatal diagnoses provide information about prognosis and help to determine treatment options. This study aims to evaluate the concordance between prenatal diagnoses and postnatal outcomes, to identify risk factors and to suggest the most appropriate management strategies.

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Öz

Amaç: Fetal kardiyak anomaliler, doğumsal anomalilere bağlı bebek ölümlerinin önde gelen nedenleri arasındadır. Konjenital kalp hastalıklarının prenatal tanısı, doğumdan önce prognostik bilgi edinilmesini sağlar ve doğumdan önce veya sonra tedavi seçenekleri hakkında fikir verir. Bu çalışmanın amacı, perinatoloji kliniğimizde tespit edilen kardiyak anomalili fetüslerin prenatal ve postnatal tanıları arasındaki korelasyonu gözlemlemektir. Amaç, doğum sonrası sonuçları izleyerek ve risk faktörlerini belirleyerek, anne ve fetüs sağlığına öncelik veren en uygun yaklaşımın seçilmesine yardımcı olmaktır.

Gereç ve Yöntemler: Ocak 2016-Aralık 2021 tarihleri arasında Çukurova Üniversitesi Tıp Fakültesi Kadın Hastalıkları ve Doğum Perinatoloji Bölümü tarafından prenatal dönemde tanı alan, doğum yapan ve Çukurova Üniversitesi Yenidoğan Yoğun Bakım Ünitesi'ne kabul edilen ve Çocuk Kardiyoloji Kliniği tarafından fetal ekokardiyografi yapılan 188 fetüsün kayıtları retrospektif olarak değerlendirildi. Bebeklerin doğum sonrası transtorasik ekokardiyografi sonuçları da gözden geçirildi.

Bulgular: Çalışmamız 188 gebe kadın ile gerçekleştirilmiştir. Fetüslerde en sık saptanan kardiyak anomaliler konotrunkal anomalilerdi, bunu sağ kalp anomalileri izledi. Prenatal ve postnatal bulgular arasındaki uyum %88,8, duyarlılık %96,55 ve özgüllük %100 idi. Konjenital kalp hastalığı olan canlı doğan bebekler arasında, yenidoğan dönemini atlatan ve atlatamayan grup arasında ebeveyn akrabalığı, doğumdaki gebelik yaşı, doğum ağırlığı, APGAR skorları ve kromozomal anomali değerlendirme oranı açısından anlamlı farklılıklar gözlenmiştir.

Sonuç: Çalışmamızda çeşitli risk faktörleri vurgulanmıştır. Prenatal ve postnatal ekokardiyografi bulgularımız arasında yüksek uyum saptandı. Sonuç olarak, farkındalığı artırmanın ve taramayı rutin bir uygulama haline getirmenin gelecek nesillerin daha sağlıklı olmasına katkıda bulunmak için gerekli olduğuna inanıyoruz. Bu, uygun yönetim ve ekipman yoluyla perinatal mortalite ve morbiditeyi azaltarak ve böylece toplumdaki etkilenen bireylerin refahını optimize ederek başarılabilir.

Anahtar Kelimeler: Konjenital kalp hastalıkları, fetal anomali, prenatal tanı

Introduction

Congenital heart diseases (CHD) refer to structural or functional abnormalities of the heart and large vessels associated with the heart that occur during the intrauterine period. CHD is the most common congenital anomaly[1], with an incidence of approximately 11 per 1,000 live births[2,3]. Approximately 40% of cardiac anomalies are diagnosed within the first year of life[4], suggesting that the actual prevalence of CHD may be higher. Recent studies have shown a high degree of heritability both independently and in association with other cardiovascular anomalies, especially left ventricular outflow tract obstructive disorders[5]. In 1 out of every 100 children with cardiac anomalies, there is an accompanying genetic or chromosomal anomaly, such as Down syndrome. Besides genetic factors, the risk of cardiac anomalies in the current pregnancy is increased by factors such as excessive alcohol consumption during pregnancy, maternal medication use, viral infections like rubella or measles, particularly during the organogenesis period, and a family history of cardiac anomalies in the mother or siblings[6]. In some complex or non-viable anomalies, termination may be considered based on the family's preference. Due to the risks of perinatal mortality and morbidity in patients with congenital cardiac anomalies, it is essential to properly manage these patients and identify high-risk groups for appropriate analysis. Prenatal evaluation plays a crucial role in this analysis and planning. CHD is the most lethal malformation in fetuses born with congenital anomalies. Therefore, early identification of the anomaly through prenatal diagnosis facilitates planned referral of the patient to the appropriate healthcare facility and reduces perinatal mortality and morbidity rates through proper management.

This study aims to contribute to the literature by examining the correlation between prenatal and postnatal diagnoses in fetuses with cardiac anomalies detected in our perinatology clinic during the prenatal period, identifying risk factors, and determining the most appropriate approaches through careful monitoring of high-risk groups.

Materials and Methods

In our study, we retrospectively reviewed the records of 188 fetuses diagnosed with cardiac anomalies during the prenatal period through detailed ultrasound examinations. These fetuses were delivered and monitored in the Neonatal Intensive Care Unit of Çukuorva University Faculty of Medicine, Department of Obstetrics and Gynecology and Perinatology, between January 2016 and December 2021. These records include cases where the fetuses were found to have cardiac anomalies and were delivered and survived.

Ethical approval was obtained from Çukurova University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee (decision no: 78, date: 05.11.2021). Data were collected on maternal age, parity, gestational age at the time of delivery or termination, presence of chromosomal abnormalities, and performance of an invasive procedure. The presence of consanguinity, extra cardiac anomalies in the fetus, and family history were assessed. History of cardiac anomalies, obstetric history, and maternal drug use during pregnancy were also questioned. Echocardiographic results in the prenatal and postnatal period were evaluated. Additionally, newborns' history of surgery during the neonatal period, postnatal morbidity, and mortality outcomes was thoroughly evaluated.

The mode of delivery and the clinical findings of the patients in both the prenatal and postnatal periods were analyzed. Fetal hearts were evaluated during the prenatal period by high-risk pregnancy specialists in our clinic using a Voluson E6 ultrasound machine. In both the prenatal and postnatal periods, echocardiographic evaluations were performed using two-dimensional, pulsed-wave Doppler, and color Doppler

techniques with the Sonos 7500 (3-8 MHz convex probe) and Epiq 7 (2-9 MHz convex probe) machines. Two-dimensional and color Doppler imaging was used to assess the four-chamber view of the heart, the origins of the great vessels, the tracheal view, and views of the aortic and ductal arches. Examinations included the location of the heart and abdomen, systemic and pulmonary venous return, atrioventricular and ventriculoarterial connections, inter-atrial and inter-ventricular valves, heart chambers, and the ductal and aortic arches.

Statistical Analysis

Fetal echocardiography (ECHO) was performed by two pediatric cardiologists between 2016 and 2021. For patients with suspected pathological findings, repeated echocardiographic follow-ups were performed. Congenital cardiac anomalies were classified into seven subgroups, as presented in Table 1. Isolated hyperechogenic cardiac focus was not considered a cardiac anomaly.

Results

The maternal age range varied between 16 and 46 years, with a mean age of 29.46±6.3 years. Consanguinity was observed in 38 (20.2%) patients, and only 4 (2.1%) of the total number of patients, the parental consanguinity status was unknown. The gestational age at which mothers first presented and underwent fetal ECHO ranged from 12 and to 42 weeks, with a mean gestational age of 27.57±7.1 weeks. A history of multigravidity was present in 142 mothers, and 19 of these patients had previously given birth to a baby with a fetal cardiac anomaly. Among the 188 patients, 85.5% (n=159) led to live births. Of these, 59.7% were delivered by cesarean section, and 40.3% were delivered vaginally.

Termination was performed in 29 patients, of which 4 underwent feticide procedures prior to the procedure. In 2 patients, termination was performed due to intrauterine fetal death. A total of 38 patients accepted invasive procedures. Amniocentesis was the most commonly performed invasive procedure, applied to 22 patients. Genetic testing was conducted to further investigate and detail the genetic profiles of the samples obtained through invasive procedures. The most frequent result from chromosomal analysis was a normal genetic profile; however, Trisomy 21 and DiGeorge Syndrome with a 22q11 deletion, which presents with clinical findings, were observed among the associated anomalies (Table 2).

Maternal comorbidities and the potential role of certain medications in fetal development were also included in our analysis. No additional medical conditions were observed in 121 mothers within the study population. The most prominent factors observed were, due to a poor obstetric history, use of low molecular weight heparin, and the presence of diabetes mellitus in the mothers (Table 2).

Other system anomalies may accompany cardiac anomalies in these fetuses. In line with our study, anomalies of the genitourinary system and central nervous system are more prominently noted (Table 3).

The findings obtained from the ECHO performed by our perinatology team during the intrauterine period showed an agreement of 88.8% with the examinations conducted by our pediatric cardiologists in the neonatal intensive care unit, and a partial agreement of 11.3%. Among the 188 patients,

Table 1. Congenital cardiac anomalies

Code in statistics	Cardiac anomaly group	Subgroupings of CHD
0	CHD not observed	
1	Conotruncal malformations	Tetralogy of Fallot, TGA, truncus arteriosus, double outlet right ventricle
2	Malformations of the right heart	Ebstein anomaly tricuspid atresia/dysplasia Pulmonary atresia, stenosis
3	Malformations of the left heart	Hypoplastic left heart, coarctation of the aorta and interrupted aortic arch, aortic stenosis, double-entry left ventricle
4	Abnormal placement	Situs inversus, heterotaxy syndromes
5	Septal defects	ASD
		VSD
6	Myocardial and pericardial diseases	Hypertrophic CMP
		Dilated CMP
		Rhabdomyoma
		Pericardial teratoma
7	Abnormal cardiac Tachycardia/ bradiarrhythmia	Tachycardia/bradiarrhythmia

CHD: Congenital heart diseases, ASD: Atrial septal defect, VSD: Ventricular septal defect, CMP: Cardiomyopathy, TGA: Transposition of the great artery

12 exhibited discordance between antenatal and postnatal diagnosis, representing 6.5% of the total (Tables 4, 5).

The Kappa agreement values are interpreted with the following reference ranges, though the specific meanings are incomplete: <0 and 1. According to the analysis, a sensitivity of 96.55% and specificity of 100.0% were found in comparing the prenatal and postnatal diagnosis findings (p<0.001) (Figure 1).

Table 6 examines the differences between the parameters obtained from the 188 patients included in the study and the mortality outcomes. The rate of chromosomal anomalies was found to be significantly higher in patients who experienced mortality (p<0.001). The rate of maternal medication use was lower in patients who experienced mortality (p=0.039). Among the 38 patients who accepted invasive procedures, 12 underwent termination of pregnancy for the fetus. Chromosomal anomalies were detected in 2 of these 12 patients, one exhibiting trisomy 21 and the other triploidy. It was determined that 26.2% of the patients had their pregnancies terminated and subsequently experienced perinatal mortality (p<0.001). Additionally, the rate of cesarean deliveries was found to be higher in patients who experienced perinatal mortality (p=0.002). Cases of perinatal mortality had significantly lower values compared to live patients in terms of gestational age, 1-minute Apgar score, 5-minute Apgar score, duration of stay in the intensive care unit, and birth weight (p=0.009; p<0.001; p<0.001; p<0.001; p<0.001, respectively).

Table 2. Evaluation of the data of the cases

	Number (n)	Percent (%)
Invasive procedures	38	20.2
Chorionic villus sampling	11	28.9
Amniocentesis	22	57.9
Cordocentesis	1	2.6
Genetic analysis in the postnatal period	4	10.5
Chromosomal anomaly		
Not accepting the analysis	150	79.8
Accepting the analysis	38	20.2
Accepted chromosomal anomaly (n=38)		
Normal	28	73.7
DiGeorge syndrome	3	7.9
Triploidy	1	2.6
Jarcho-Levin syndrome	1	2.6
Trisomy 21	3	7.9
Trisomy 18	2	5.3
Maternal medication use	27	14.4
DM (insulin)	7	25.9
Thyroid (L-thyroxine or propylthiouracil)	7	25.9
Cardiovascular diseases (antihypertensives)	2	7.4
Epilepsy (antiepileptic medications)	4	14.8
Anticoagulants (low molecular weight heparins)	8	29.6
Others	2	7.4
Consanguinity		
No	146	77.7
Yes	42	22.3

Table 3. Associated anomalies

Associated anomalies	Number (n)	Percent (%)
	73	38.8
Gastrointestinal tract	7	9.6
Genitourinary system	10	13.7
Central nervous system	11	15.1
Skeletal system disorders	5	6.8
Craniopharyngeal anomalies	7	9.6
Other	33	45.2

Table 4. Evaluation of mortality, operation and medical treatment needs of the cases

	Number (n)	Percent (%)
Fetal mortalite		
Exitus	104	55.3
Alive	80	42.6
Feticide + medical termination	4	2.1
Need for emergency surgery		
No	129	68.6
Yes	59	31.4
Need for postnatal medical care		
yes	111	59.0
No	77	41.0
Termination	27	14.4
Termination	23	85.2
Feticide + medical termination	4	14.8

Table 5. Analysis of fit ratios

Compliance rates	Number (n)	Percent (%)
No	21	11.5
Yes	153	82.3
Partial compliance	12	6.5

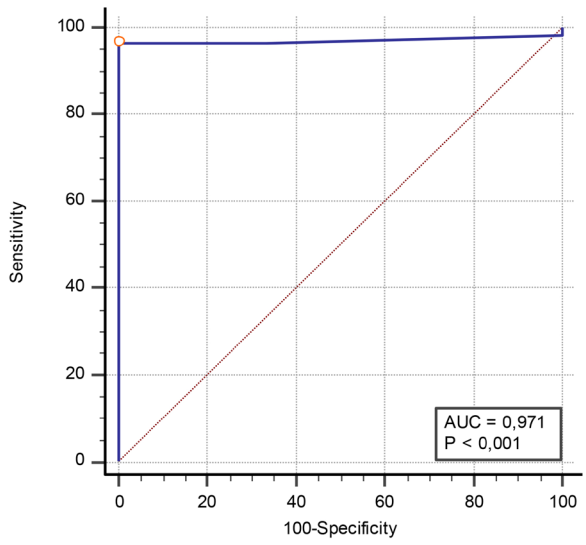

Figure 1. Diagnostic test performance between prenatal and postnatal diagnostic findings

AUC: Area under the curve

Table 7 discusses the striking differences in patients with perinatal mortality among the 188 patients included in the study are discussed. The rate of chromosomal anomaly was higher in patients who died (p<0.001). Maternal drug use rate was lower in patients who did not survive (p=0.039). Thirty-eight of our followed up pregnant women with fetuses with cardiac anomalies accepted the interventional procedure, and 12 of these pregnant women decided to terminate. In this group of 12 pregnant women with fetuses with cardiac anomalies whose pregnancies were terminated, chromosomal anomalies were detected in 2 fetuses. One of these patients had trisomy 21 and one had triploidy. In the group of patients with perinatal mortality, 26.2% of the pregnancies ended with termination (p<0.001). The rate of caesarean section was higher in cases with perinatal mortality (p=0.002). The time of delivery was earlier, and the 1st minute Apgar score, 5th minute Apgar score, duration of intensive care unit stay, and birth weight were lower in patients who developed perinatal mortality compared to living patients (p=0.009, p<0.001, p<0.001, p<0.001, and p<0.001, respectively).

Table 6. Differences between parameters and mortality findings for all patients (n=188)

	Perinatal mortality Yes (n=104)	Perinatal mortality No (n=84)	p†
	n (%)	n (%)	
Associated anomaly	43 (41.3)	30 (35.7)	0.431
Gastrointestinal system	4 (9.3)	3 (10)	0.921
Central nervous system anomalies	6 (14)	5 (16.7)	0.750
Genitourinary system anomalies	9 (20.9)	1 (3.3)	0.031*
Craniopharyngeal anomalies	5 (11.6)	2 (6.7)	0.479
Other anomalies	16 (37.2)	17 (56.7)	0.100
Skeletal system disorders	1 (2.3)	4 (13.3)	0.067
Invasive procedure	21 (20.2)	17 (20.2)	0.994
Chromosomal anomaly			
Not accepting the analysis	72 (69.2)	78 (92.9)	<0.001**
Accepting the analysis	32 (30.8)	6 (7.1)	
Accepted chromosomal anomaly analysis (n=38)			
Normal	28 (87.5)	-	<0.001**
DiGeorge syndrome	-	3 (50)	
Triploidy	1 (3.1)	-	
Jarcho-Levin syndrome	1 (3.1)	2 (33.3)	
Trisomy 21	2 (6.3)	-	
Trisomy 18	-	1 (16.7)	
Maternal medication use	10 (9.6)	17 (20.2)	0.039*
Termination	26 (25)	3 (3.6)	<0.001**

Table 6. Continued

	Perinatal mortality Yes (n=104)	Perinatal mortality No (n=84)	p†
	n (%)	n (%)	
Gravida			
Nullipar	30 (28.8)	17 (20.2)	0.175
Multipar	74 (71.2)	67 (79.8)	
Mode of delivery			
Vaginal	33 (30.8)	32 (38.1)	0.002**
Caesarean section	46 (43.0)	49 (58.3)	
Stillbirth	19 (17.8)	-	
Medical abortion	9 (8.4)	-	
Consanguinity	28 (26.9)	14 (16.7)	0.093
Maternal disease	19 (18.3)	25 (29.8)	0.064
Gender			
Male	64 (61.5)	55 (65.5)	0.578
Female	40 (38.5)	29 (34.5)	
Emergency surgery			
No	72 (69.2)	57 (67.9)	0.840
Yes	32 (30.8)	27 (32.1)	
Postnatal medical needs			
No	57 (54.8)	54 (64.3)	0.189
Yes	47 (45.2)	30 (35.7)	
	Mean ± SD	**Mean ± SD**	**p‡**
Diagnosis week	26.3±7.0	26.7±7.5	0.651
Birth week	34.4±7.1	37.3±4.9	**0.009****
1-minute APGAR score	4.51±3.1	6.79±2.0	**<0.001****
5-minute APGAR score	5.61±3.6	8.11±2.0	**<0.001****
Length of stay in the intensive care unit	11.0±18.2	18.6±19.9	**<0.001****
Weight (g)	2268.0±1145.2	2903.4±741.9	**<0.001****
Maternal age	28.6±6.3	30.2±6.3	0.053

*: p<0,05, **: p<0,001, †: Ki-kare, ‡: Mann-Whitney U, SD: Standard deviation

Discussion

Among congenital anomalies, congenital heart anomalies (CHA) account for the highest proportion of cases. These anomalies can either be life-threatening or impair the quality of life. Interventions may be necessary during the intrauterine or neonatal periods, and these affected fetuses may require emergency intervention[7]. Therefore, minimizing the loss of time provides us with a strategic approach and enables us to achieve success in reducing mortality and perinatal morbidity. The rapid advancement of technology, which has recently been reflected in our daily lives, has had positive contributions to the field of health. With these positive reflections of technology, especially in the last two decades, progress is being made in the field of perinatology to understand the intrauterine fetal period. Anatomical screening is recommended in pregnancy follow-up, examining the heart's anatomy, its position with the surrounding organs and large vessels, and the orientation and connections of the large vessels themselves. In general, a large number of patients are detected in routine screening.

Table 7. Differences between neonatal period parameters and mortality groups in pregnancies resulting in live birth (n=159)

	Perinatal mortality Yes (n=78)	Perinatal mortality No (n=81)	p†
	n (%)	n (%)	
Associated anomaly	33 (42.3)	28 (34.6)	0.316
Central nervous system anomalies	6 (18.2)	5 (17.9)	0.974
Genitourinary system anomalies	5 (15.2)	1 (3.6)	0.130
Craniopharyngeal anomalies	4 (12.1)	2 (7.1)	0.515
Other anomalies	12 (36.4)	16 (57.1)	0.105
Skeletal system disorders	1 (3.0)	4 (14.3)	0.110
Invasive procedure	12 (15.4)	15 (18.5)	0.599
Chromosomal anomaly			
Not accepting the analysis	51 (65.4)	77 (95.1)	<0.001**
Accepting the analysis	27 (34.6)	4 (4.9)	
Accepted chromosomal anomaly analysis (n=38)			
Normal	23 (85.2)	-	<0.001**
DiGeorge syndrome	-	3 (75)	
Triploidy	-	-	
Jarcho-Levin syndrome	1 (3.7)	-	
Trisomy 21	1 (3.7)	1 (25)	
Trisomy 18	2 (7.4)	-	
Maternal medication use	9 (11.5)	16 (19.8)	0.155
Termination	4 (5.1)	9 (11.1)	0.169
History of a child with cardiac anomaly			
Gravida	19 (24.4)	16 (19.8)	0.483
Nullipar	59 (75.6)	65 (80.2)	
Mode of delivery			
Vaginal	29 (37.2)	32 (39.5)	0.118
Caesarean section	44 (56.4)	49 (60.5)	
Stillbirth	5 (6.4)	-	
Consanguinity	24 (30.8)	13 (16)	0.028*
Maternal disease	16 (20.5)	24 (29.6)	0.185
Gender			
Male	49 (62.8)	53 (65.4)	0.731
Female	29 (37.2)	28 (34.6)	
Emergency surgery			
No	49 (62.8)	54 (66.7)	0.612
Yes	29 (37.2)	27 (33.3)	
Postnatal medical needs			
No	34 (43.6)	51 (63)	0.014*
Yes	44 (56.4)	30 (37)	

Table 7. Continued

	Perinatal mortality Yes (n=78)	Perinatal mortality No (n=81)	p†
	n (%)	n (%)	
Diagnosis week	28.0±7.1	27.1±7.3	0.469
Birth week	37.5±3.5	38.1±2.8	0.325
1-minute APGAR score	5.70±2.3	7.04±1.6	**<0.001****
5-minute APGAR score	7.09±2.4	8.41±1.3	**<0.001****
Length of stay in the intensive care unit	13.8±19.2	19.2±19.9	**0.005****
Weight (g)	2730.7±777.2	2963.6±638.3	0.085
Maternal age	28.8±6.4	30.2±6.4	0.148

*: p<0,05, **: p<0,001, †: Ki-kare, ‡: Mann-Whitney U, SD: Standard deviation

The proportion of live births with life-compatible CHD is approximately 0.8%[8]. If we consider the percentage of all pregnancies, this proportion becomes broader due to the inclusion of various pregnancy outcomes. This difference arises because some CHDs have severe clinical courses and can lead to fetal loss during the intrauterine period. Additionally, screenings conducted in perinatology often reveal that certain anomalies are incompatible with life before delivery and that the clinical presentation of the existing condition is aggressive. In such cases, families may be presented with the option of termination or they may seek care at our clinic due to intrauterine fetal death.

In terms of incidence, CHD occurs in about 8 per 1,000 live births, with its rate among stillbirths being 3-4%, and ranging from 10-25% in spontaneous abortions. Among premature neonates, the incidence is approximately 2%[4,9,10]. Our study does not reflect the prevalence of CHD in the population. This is because our study focuses on prenatal and neonatal periods, thus only considering anomalies detectable during the fetal stage.

The generally accepted ideal time for diagnosis is between 18 and 22 weeks of gestation. Fetal cardiac evaluation can even be performed during the first trimester using transvaginal or transabdominal ultrasound. In our study, the earliest diagnosis was made at 13 weeks-gestation, with the average gestational age for fetal transthoracic echocardiograms being 28±7.13 weeks. The earliest CHA we diagnosed was hypoplastic left heart syndrome, identified at 13 weeks of gestation. Our center's approach is to perform ECHO at 18-22 weeks gestation for assessing the risk of CHAs.

In our clinic's research, the most prominent anomalies observed were conotruncal anomalies, followed by right heart anomalies. A study indicated that septal defects were the most frequently observed; and among cyanotic heart diseases, the most common were conotruncal anomalies such as tetralogy of Fallot (TOF)[11]. Another study reported that septal anomalies were predominant[12]. In the study by Burger et al.[13], septal defects were also the most commonly observed isolated cardiac anomalies. In the work presented by Best et al.[14], among a total of 5070 patients with CHAs, the anomalies identified included ventricular septal defect (n=2182, percentage=43%), pulmonary stenosis (n=428, percentage=8.4%), atrial septal defect (n=422, percentage=8.3%), TOF (n=271, percentage=5.3%), atrioventricular septal defect (n=264, percentage=5.2%), and coarctation of the aorta (n=258, percentage=5%).

In our research, the sample size for definitive diagnosis was limited due to the low prevalence of pregnancies in our community. Invasive procedures were performed on 4% of patients. Among the samples we collected, amniocentesis was predominantly observed with 22q11 deletion microdeletion leading to the identification of DiGeorge syndrome.

In our study group, 37 patients accepted invasive procedures for a definitive diagnosis, with chromosomal anomalies detected in 9 patients (24%). In a study by Ko[15] in Korea, isolated CHAs and patients with extracardiac anomalies were examined in a cohort of 791 individuals, with amniocentesis performed on 182 patients. Chromosomal anomalies were identified in 21 patients (11.5%). Among this cohort of 791 patients, 627 (79.3%) had isolated CHAs, and live births were reported for 299 patients.

In the study conducted by Elshazali et al.[16], a total of 141 infants were evaluated. It was found that CHAs were more common in male fetuses. A total of 11.3% were dysmorphic (n=16) (including Down syndrome, Noonan syndrome, and others). Additionally, 9% (n=9) had previously mentioned exclusion criteria, that positively contributed to birth weight. A very small percentage of families had a positive history of CHAs (0.7%). The average birth weight of the samples was 2.59 kg, with 31.9% having low or very low birth weight. All cases had low birth weight; 50% were reported to have very low birth

weight. In the study by Levin et al.[17], among 37 infants with CHD, 21 were of appropriate weight for gestational age, while 16 were small for gestational age.

In a study conducted by Lopes et al.[18] in Brazil involving 52 infants with CHD, the mortality risk among newborns with CHD was found to be twofold higher than that among low-birth-weight premature infants, particularly for newborns with CHD who have an Apgar score of less than 7 during the first minute of life. The presence of certain comorbidities, in addition to CHD, was associated with mortality outcomes, increasing the risk nearly threefold. The average length of stay in the neonatal unit was observed to be 75 days, with 25% of patients failing to reach this duration. In our study, the weights of the newborns varied. There was a subgroup of patients with cardiac anomalies who did not reach term, with the highest weight recorded at 3885 g and the lowest at 685 g, resulting in an average weight of 2985 g. In cases where mortality occurred, the gestational age, 1-minute Apgar score, 5-minute Apgar score, duration of stay in the intensive care unit, and birth weight of the patients were all found to be significantly lower than those who survived ($p=0.009$; $p<0.001$; $p<0.001$; $p<0.001$; $p<0.001$, respectively). The presence of a low Apgar score at one minute suggests that certain cardiac defects may be active during the intrauterine period, potentially compromising blood flow and thereby affecting perinatal morbidity and mortality by preventing adequate nutrient and oxygen supply to the fetus. This underscores the importance of adequate prenatal diagnosis and monitoring.

In studies where the subject did not have CHD, the risk of having a sibling with CHD was observed to be between 2% and 6%. The likelihood of detecting CHD in a fetus increases with the number of affected siblings[19]. In our study, the proportion of patients with a family history of CHD was 10.1%. We believe another reason for the elevated rate and increase in incidence may be that the institution is a tertiary center, and therefore the patient population consists of high-risk cases in our country compared to Western societies.

Women with diabetes prior to pregnancy have been observed to have approximately four times the likelihood of experiencing a pregnancy affected by CHD compared to those without diabetes[20]. Researchers indicate that about 8% of the CHDs occurring each year may arise from poorly controlled diabetes before and in the early stages of pregnancy[21]. In our study, there was no observed increase in fetal CHD risk among pregnancies with gestational diabetes, included in the maternal diabetes group. The lack of increased fetal CHD rates in infants of mothers with pre-existing diabetes may be attributed to the inclusion of gestational diabetes in the same group, and the small sample size as a limitation.

Our study demonstrated an agreement rate of 88.8%, with 11.2% discordance. The sensitivity of prenatal and postnatal diagnoses was found to be 96.55%, with 100% specificity. In a study conducted by Ozkutlu et al.[22] in 2022, the sensitivity for comparing prenatal and postnatal diagnoses of cardiac anomalies was reported to be 93%, with 100% specificity. Another study conducted by Özbarlas et al.[23] at our center calculated the sensitivity of ECHO examinations to be 96%, with 99% specificity. Considering the classification used by Silva et al.[24], 77.6% of cases showed congruence between prenatal and postnatal CHD diagnoses. In the study by Chakraborty et al.[25], the agreement rate for complex CHDs between prenatal and postnatal diagnoses was reported to be over 80%. The results of our study align with the literature and highlight the importance of prenatal ECHO.

Study Limitations

The primary limitation of our study is its retrospective design, which has led to difficulties in accessing certain data. The patients who underwent fetal echocardiographic examination were not consistently monitored, and there were gaps in the prenatal follow-up schedules at our hospital after the optimal diagnosis week during the third trimester. Additionally, a significant portion of patients was referred from external centers outside the city. Furthermore, socioeconomic factors and religious beliefs resulted in the inability to perform invasive procedures for definitive diagnosis in many cases, which constitutes a limitation of our study.

Conclusion

The prevalence of fetal heart anomalies is significantly higher among live births, and their distribution varies. The correlation observed in a substantial proportion of fetuses diagnosed with CHD, approached with a preliminary diagnosis of heart anomaly during the neonatal period, underscores the importance of managing patient stratification accurately. This must be done within the healthcare system by ensuring appropriate conditions before birth. This is crucial for effectively managing higher-level care and necessary preparations during deliveries. By implementing suitable management and facilities, we can reduce perinatal mortality and perinatal morbidity, thereby contributing to the optimal well-being of individuals in our society and promoting healthy generations. Therefore, it is essential to integrate screenings into routine practice and enhance awareness to educate the community.

Ethics

Ethics Committee Approval: Ethical approval was obtained from Çukurova University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee (decision no: 78, date: 05.11.2021).

Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: G.M.Y., S.C.D., S.A., İ.C.E., M.S., Concept: G.M.Y., S.C.D., S.A., İ.C.E., M.S., Design: G.M.Y., S.C.D., S.A., İ.C.E., M.S., Data Collection or Processing:

G.M.Y., S.C.D., S.A., İ.C.E., M.S., Analysis or Interpretation: G.M.Y., S.C.D., S.A., İ.C.E., M.S., Literature Search: G.M.Y., S.C.D., S.A., İ.C.E., M.S., Writing: G.M.Y., S.C.D., S.A., İ.C.E., M.S.

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Which urinary incontinence inquiry form should be used in women with urinary incontinence?

İdrar kaçırma sorunu olan kadınlarda hangi idrar kaçırma sorgu formu kullanılmalıdır?

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Abstract

Objective: To determine urinary incontinence (UI) inquiry forms to be used in the follow-up of incontinence according to UI type.

Materials and Methods: This prospective cohort study was conducted at the University of Health Science Hospital between 2020 and 2022. A total of 449 patients referred for urodynamic evaluation for UI were included herein, and clinical results regarding UI types were collected and reviewed. The validated urogenital distress inventory 6 (UDI-6), incontinence impact questionnaire (IIQ-7), and incontinence quality of life (I-QOL) questionnaires were completed by all patients. The demographic data of the patients, total questionnaire scores, and urodynamic results were compared between the groups according to UI type.

Results: Forty-nine percent of the participants were in the menopausal period, and 41% required regular ped use. A total of 52.1% of patients experienced 5 years of UI. Stress incontinence was reported in 4.2% of patients, urge incontinence in 10%, stress-predominant mixed UI in 59.2%, and urge-predominant mixed UI in 24.7%. The mean ± standard deviation values were 59.62±20.62 for the UDI-6, 54.72±24.84 for the IIQ-7, 62.41±23.52 for the total I-QOL, 21.85±8.55 for the I-QOL limitation of behaviors subscale, 27.99±10.86 for the I-QOL psychological influence subscale, and 12.64±5.72 for the I-QOL social isolation subscale. A statistically significant difference was assessed between the urodynamics results and the UDI-6, IIQ-7, total I-QOL, I-QOL limitation of behaviors subscale, I-QOL psychological influence subscale, and I-QOL social isolation subscale scores ($p<0.001$ for all variables).

Conclusion: In patients diagnosed with UI, when each of the 3 questionnaires for UI diagnosis was compared, the best inquiry questionnaire for the prediction of mixed-type UI was the UDI-6.

Keywords: Urinary incontinence, urogenital distress inventory 6, incontinence impact questionnaire, incontinence quality of life, urodynamic results

Öz

Amaç: Bu çalışmanın amacı idrar kaçırma tiplerine göre inkontinans takibinde kullanılacak idrar kaçırma sorgulama formlarını belirlemektir.

Gereç ve Yöntemler: Bu, 2020-2022 yılları arasında Sağlık bilimleri Üniversite Hastanesi'nde yürütülen prospektif bir kohort çalışmasıdır. Üriner inkontinans (Üİ) için ürodinamik değerlendirme için sevk edilen toplam 449 hasta çalışmaya dahil edildi ve Üİ tiplerine ilişkin klinik sonuçlar toplandı ve incelendi. Tüm hastalar tarafından geçerliliği kanıtlanmış ürogenital sıkıntı envanteri 6 (UDI-6), inkontinans etki anketi (IIQ-7) ve inkontinans yaşam kalitesi (I-QOL) anketleri dolduruldu. Hastaların demografik verileri, anketlerin toplam puanları ve ürodinamik sonuçları, idrar kaçırma tiplerine göre gruplar arasında karşılaştırıldı.

PRECIS: We compared the Urogenital Distress Inventory 6, Incontinence Impact Questionnaire, and Incontinence Quality of Life questionnaires in women with urinary incontinence.

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Bulgular: Katılımcıların %49,7'si menopoz dönemindeydi ve %41'i düzenli ped kullanımına ihtiyaç duyuyordu. Toplamda %52,1'i 5 yıl idrar kaçırma sorunu yaşamıştır. Hastaların %4,2'sinde stres inkontinansı, %10'unda sıkışma inkontinansı, %59,2'sinde stres ağırlıklı karma idrar kaçırma ve %24,7'sinde sıkışma ağırlıklı karma idrar kaçırma bildirilmiştir. Ortalama ± standart sapma değerleri UDI-6 için 59,62±20,62, IIQ-7 için 54,72±24,84, toplam I-QOL için 62,41±23,52, I-QOL davranış sınırlamaları alt ölçeği için 21,85±8,55, I-QOL psikolojik etki alt ölçeği için 27,99±10,86 ve I-QOL sosyal izolasyon alt ölçeği için 12,64±5,72'dir. Ürodinamik sonuçlar ile UDI-6, IIQ-7, toplam I-QOL, I-QOL davranışların sınırlandırılması alt ölçeği, I-QOL psikolojik etki alt ölçeği ve I-QOL sosyal izolasyon alt ölçeği puanları arasında istatistiksel olarak anlamlı bir fark değerlendirildi (tüm değişkenler için p<0,001).

Sonuç: Üİ tanısı konulan hastalarda, Üİ tanısı için 3 anketin her biri karşılaştırıldığında, karma tip Üİ'yi tahmin etmek için en iyi sorgulama anketi UDI-6 idi.

Anahtar Kelimeler: İdrar kaçırma, ürogenital sıkıntı envanteri 6, inkontinans etki anketi, inkontinans yaşam kalitesi, ürodinamik sonuçlar

Introduction

Urinary incontinence (UI) is defined by the International Continence Society (ICS) as the involuntary leakage of urine that leads to social and hygienic problems, significantly affecting the patient's quality of life (QOL)[1]. In the ICS's 2002 standardization report described UI as any involuntary leakage of urine[2]. The prevalence of UI ranges from 10% to 40% worldwide[3], with studies in Türkiye reporting prevalence rates between 16.4% and 68.8%[4].

UI contributes to increased social dysfunction in women[5]. Those experiencing more severe symptoms of UI often report a greater impact on their physical activities, social, travel, and emotional well-being[6]. The lower urinary tract (LUT) consists of the urinary bladder and urethra, which allow for conscious, controlled, and coordinated urine expulsion while storing urine at low pressure.

Urodynamics involves measuring the physiological parameters of the LUT to evaluate its function and dysfunction. Clinicians may perform urodynamics non-invasively or invasively. The standard urodynamic test combines both assessment methods, including non-invasive evaluations of urinary bladder emptying and invasive assessments of storage and emptying functions[7]. Typically, standard urodynamic tests begin with non-invasive uroflowmetry, followed by invasive cystometry and pressure-flow studies. Additional tests, such as simultaneous electromyography (EMG) of pelvic floor muscles and urethral pressure profiles, may provide further clinical insights[8].

The current guidelines do not recommend routine urodynamic investigations in patients with incontinence. However, such investigations are indicated for patients with: 1) discordance between complaints and symptoms; 2) plans for surgery; 3) therapy-resistant overactive bladder; 4) a history of unsuccessful incontinence surgery; 5) obstructive voiding symptoms; 6) a history of neurological disease; and 7) increased post-void residual volume (PVR)[9-11].

The purpose of UI inquiry forms is to select appropriate treatment methods and evaluate therapy results rather than to obtain a direct diagnosis[2]. Given that selecting the appropriate inquiry forms according to the UI type yields more accurate results post-treatment, this study aimed to compare UI tests before treatment, such as the urogenital distress inventory 6 (UDI-6), incontinence impact questionnaire (IIQ-7), and the incontinence quality of life (I-QOL) inquiry forms, evaluating their efficiencies and observing changes.

Materials and Methods

Study Population and Data Collection

This cross-sectional study enrolled patients who underwent urodynamic and clinical evaluation at the Urogynecology Outpatient Clinic of a University of Health Sciences Training and Research Hospital between July 2020 and July 2022. Ethical approval was obtained from the University of Health Science Türkiye, Zeynep Kamil Women and Children Diseases Training and Research Hospital Clinical Research Ethics Committee (decision no: 138, date: 08.07.2020). Participants were evaluated by a urogynecologist to ensure that they met the following inclusion criteria: 1) Female patients aged over 18 years; 2) patients with sufficient literacy to complete the questionnaires; and 3) patients who provided informed consent. Exclusion criteria: 1) pelvic organ prolapse stage 3; 2) pregnancy; 3) urinary tract infections; 4) current drug therapy for UI; 5) neurological diseases; and 6) neoplastic diagnosis or risk. The study's methodology and objectives were explained to all eligible patients before they signed the informed consent form. After obtaining written consent, participants completed a questionnaire that recorded sociodemographic (age, educational level, marital status, profession, smoking) and physical characteristics (body mass index, height, weight, menstrual and status parity).

Questionnaires

Turkish versions of the I-QOL scale, UDI-6, and IIQ-7 were administered. Urogynecological examinations and urodynamic tests were performed to assess the UI type.

Incontinence Quality of Life Scale Scores

Developed by Wagner et al.[12] in 1996 to assess the QOL of patients with UI in the USA, the I-QOL was revised by Patrick et al.[13] in the same year, reducing the number of questions to 22 by removing six, based on psychometric evaluations for European versions. In Türkiye, Öztaç Özerdoğan and Kızılkaya[14] conducted validity and reliability studies on the Turkish adaptation of the I-QOL. The Turkish I-QOL demonstrated strong internal consistency (Cronbach's α=0.96) and very strong test-retest reliability (Spearman's rho=0.97).

All I-QOL items were evaluated using a five-point Likert-type response scale (1: very much, 2: pretty much, 3: moderate, 4: a little, 5: not at all). The total final score was converted into a scale value from 0 to 100 for clarity, with higher

scores indicating a better QOL. The I-QOL consists of three subdimensions: limitation of behaviors (items 1, 2, 3, 4, 10, 11, 13, 20), psychosocial influence (items 5, 6, 7, 9, 15, 16, 17, 21, 22), and social isolation (items 8, 12, 14, 18, 19). Higher scores indicate better QOL than lower scores[12-14].

Urogenital Distress Inventory-6

The UDI-6 is a scale used to determine symptoms related to stress-related UI, bladder outlet obstruction, and detrusor overactivity. The UDI-6 short form consists of six questions that are scored on a scale from 0 to 3. Higher scores indicate a greater impact on QOL. The Turkish adaptation's validity and reliability were established by Cam et al.[15], with strong internal consistency (Cronbach's α=0.74) and very strong test-retest reliability (Spearman's rho=0.99).

Incontinence Influence Questionnaire Form

The IIQ-7 comprises seven questions that are assessed using a four-point Likert-type scale. Scores from the IIQ-7 and UDI-6 are evaluated from 0 to 100, where "0" indicates no bother at all, and "100" indicates significant bother. Higher scores imply poorer QOL[15]. The Turkish IIQ-7 also demonstrated strong internal consistency (Cronbach's α=0.87) and very strong test-retest reliability (Spearman's rho=0.99).

After completing each of the three questionnaires, all participants underwent a urodynamic investigation performed by the principal researcher (CC), who was blind to each patient's questionnaire score. Urodynamic assessment primarily included filling cystometry and uroflowmetric studies.

Cystometric Method

Each participant with a negative urine culture was placed on the delivery table after emptying her bladder. After cleaning the external urethral meatus, urine from the PVR portion of the catheter was measured. To measure intra-abdominal pressure, an intra-abdominal pressure catheter was inserted into the rectum, and the distal part of the catheter was fixed to the thigh with tape. Room temperature saline was used as the filling fluid, and pressures were recorded using external pressure transducers. Intra-abdominal, intravesical, and calculated detrusor pressures were simultaneously displayed on a computer screen. The filling volume and EMG data were also recorded. Bladder filling typically began in the sitting position at a rate of 50-80 mL/min. The volumes at the first sensation of bladder filling and at the first, normal, and strong desire to void were recorded. At a bladder volume of 200 mL, each patient was instructed to perform the Valsalva maneuver. The Valsalva leak point pressure was recorded as the lowest intravesical pressure that resulted in incontinence during effort. The presence of provoked (e.g., cough, change in posture) or spontaneous involuntary contractions of the detrusor muscle indicated detrusor overactivity. The filling phase was completed when the participant could no longer hold the fluid or postpone voiding, and bladder compliance was recorded. The definitions set forth by the ICS were used to define lower urinary system dysfunction and related symptoms, findings, and urodynamic observations[16]. Urodynamic examination, clinical records, and urodynamic tests were performed to classify the UI type, with patients categorized into five groups: normal, stress incontinence, urge incontinence, stress-predominant mixed UI, and urge-predominant mixed UI.

Statistical Analysis

Continuous variables are expressed as mean ± standard deviation (SD) and/or median (minimum-maximum), whereas categorical data are presented as numbers and percentages. Normality analyses of continuous variables were conducted using the Kolmogorov-Smirnov test. One-Way ANOVA (post-hoc: Bonferroni) was applied to analyze variables that followed a normal distribution, while the Kruskal-Wallis test (post-hoc: Mann-Whitney U test with Bonferroni correction) was used for non-normally distributed variables. The linear relationship between scales was tested using Spearman's correlation analysis. Based on the results of urodynamics, the UDI-6, IIQ-7, and total I-QOL scores were utilized to predict incontinence levels (stress, urge, stress-predominant mixed UI, and urge-predominant mixed UI). The area under the curve (AUC) was calculated for subscale scores, and receiver operating characteristic (ROC) curve analysis was performed to determine cut-off values. Sensitivity, specificity, and positive and negative predictive values were calculated for significant breakpoints. A type I error of less than 5% was considered statistically significant. Statistical analyses were performed using IBM SPSS version 26.0 (IBM Corporation, Armonk, NY, USA).

Results

The mean ± SD values for age, average parity, and average urinary bladder capacity among the 449 patients were 48.9±9.3 years, 2.78±1.32, and 489.96±153.9 mL, respectively. Seventy-three percent of the participants had undergone normal vaginal delivery, and 49.7% were in the menopausal period. The sociodemographic health information of the participants is presented in Table 1. In the uroflow assessment, 83.3% of participants exhibited normal results, whereas 5.8% had inadequate volume, 3.3% showed obstruction, 7.1% had mild obstruction, and 0.4% had severe obstruction. Based on the clinical and urodynamic assessment results, 1.2% of patients were classified as normal, 4.2% as having stress UI, 10% as having urge UI, 59.2% as having stress-predominant mixed UI, and 24.7% as having urge-predominant mixed UI, as shown in Table 1.

The mean ± SD values of the UDI-6, IIQ-7, total I-QOL, I-QOL limitation of behaviors, I-QOL psychological influence, and I-QOL social isolation scales are presented in Table 2.

Table 1. Sociodemographic and clinical characteristics of the participants (n=449)

		Mean ± SD	Median (min-max)
Age (year)		48.88±9.33	48 (22-73)
Gravity		2.89±1.4	3 (0-9)
Parity		2.78±1.32	3 (0-9)
BMI (kg/m^2)		29.71±5.25	29 (16-48)
Bladder capacity (mL)		489.96±153.9	459 (188-1104)
		n	**%**
Delivery methods	Nulliparous	14	3.1
	Normal delivery	331	73.7
	Cesarean section	44	9.8
	Normal delivery + cesarean section	60	13.4
Menopausal status	Negative	226	50.3
	Positive	221	49.2
Educational status	Illiterate	40	8.9
	Primary school	235	52.3
	Secondary school	119	26.5
	High school	16	3.6
	University	38	8.5
Marital status	Married	382	85.1
	Single	15	3.3
	Divorced	52	11.6
Occupation	Employee	70	15.6
	Officer	13	2.9
	Retired	37	8.2
	Housewife	328	73.1
Perceived financial status	Low	54	12.0
	Medium	395	88.0
Smoking	Non-smoking	339	75.5
	Smoking	110	24.5
Incontinence frequency	No incontinence	4	0.9
	A few times a day	140	31.2
	A few times a week	84	18.7
	A few times a month	37	8.2
	Sufficient for regular ped use	184	41.0
Period of urinary incontinence	Absent	3	0.7
	1 month	12	2.7
	5 months	37	8.2
	1 year	163	36.3
	5 years	234	52.1

Table 1. Sociodemographic and clinical characteristics of the participants (n=449)

		Mean ± SD	Median (min-max)
Previous urogynecological surgery	Negative	422	94.0
	Operated	1	0.2
	Cystocele	1	0.2
	TVT or TOT	23	5.1
	Present unknown	2	0.4
Uroflow	Normal	374	83.3
	Insufficient volume	26	5.8
	Obstruction	15	3.3
	Mild obstruction	32	7.1
	Severe obstruction	2	0.4
Examination + clinical + result of the urodynamic assessment	Normal	8	1.2
	Stress incontinence	19	4.2
	Urge incontinence	45	10.0
	Stress-predominant mixed incontinence	266	59.2
	Urge-predominant mixed incontinence	111	24.7
Total		449	100.0

SD: Standard deviation, BMI: Body mass index, TVT: Tension-free vaginal tap, TOT: Transobturatory tape, Min: Minimum, Max: Maximum

Table 2. Scores for the UDI-6, IIQ-7, total I-QOL and subscales (n=449)

	Mean ± SD	Median (min-max)
% the UDI-6	59.62±20.62	61.1 (11.1-100.0)
% the IIQ-7	54.72±24.84	52.3 (0.0-100)
Total I-QOL	62.41±23.52	60 (22-110)
I-QOL limitations of behaviors	21.85±8.55	22 (8-40)
I-QOL psychological influences	27.99±10.86	28 (9-45)
I-QOL social isolation	12.64±5.72	12 (5-30)

UDI-6: Urogenital distress inventory 6, IIQ-7: Incontinence impact questionnaire, I-QOL: Incontinence quality of life, SD: Standard deviation, Min: Minimum, Max: Maximum

According to the clinical and urodynamic evaluation results, the UDI-6, IIQ-7 scale, total I-QOL scale, I-QOL behavioral limitation scale, I-QOL psychological impact scale, and I-QOL psychological impact scale scores were found to be statistically significant (p<0.001, p<0.001, p<0.001, p<0.001, p<0.001, p<0.001; respectively).

The UDI-6 scale scores were as follows: urge-predominant mixed UI [61.6 (22.2-100.0)] >stress-predominant mixed UI [61.1 (16.6-100.0)] >stress incontinence [44.4 (16.6-72.2)]=urge incontinence [44.4 (11.1-88.9)] >normal individuals [30.5 (16.6-77.7)] (p<0.001 for all variables).

The IIQ-7 scale scores were as follows: Urge-predominant mixed UI (62.46±22.50) >stress-predominant mixed UI (54.63±24.56) >urge incontinence (47.93±24.71) >stress incontinence (35.58±22.63) >normal individuals (33.92±28.39) (p<0.001 for all variables).

The total I-QOL scale scores were as follows: normal individuals (87.88±25.44) >stress incontinence (79.11±21.09) >urge incontinence (73.31±22.94) >stress-predominant mixed UI (60.98±23.13) urge-predominant mixed UI (56.75±21.39) (p<0.001 for all variables).

According to clinical and urodynamic assessment results, 1.2% of patients were normal, 4.2% had stress UI, 10% had urge UI, 59.2% had stress-predominant mixed UI, and 24.7% had urge-predominant mixed UI shown in Table 3.

The analysis between the scales reported a fairly strong positive and statistically significant correlation between the UDI-6 subscale score and the IIQ-7 subscale score (r=0.622, p<0.001; respectively).

It was determined that there was a negative, strong, and statistically significant correlation between the UDI-6 scale score and the I-QOL total scale score (r=-0.614, p<0.001; respectively).

The ROC curve analysis used to predict stress and urge UI did not confirm the clinical diagnosis prediction (p>0.05). We determined that the three scales we used to predict stress-predominant mixed UI predicted the clinical diagnosis to be statistically significant (p<0.05). The best cut-off values determined in the ROC analysis among the three scales and the calculated sensitivity, specificity, positive-predictive value, negative-predictive value, and AUC values are shown in Table 4 and Figure 1.

Table 3. Comparison of the UDI-6, IIQ-7, and total I-QOL scales and subscale scores

	Urodynamics evaluation					
	Normal (n=8)	Stress incontinence (n=19)	Urge incontinence (n=45)	Stress-predominant mixed incontinence (n=266)	Urge-predominant mixed incontinence (n=111)	p
%UDI-6 median (min-max)	[30.5 (16.6-77.7)][a]	[44.4 (16.6-72.2)][a]	[44.4 (11.1-88.9)][a]	[61.1 (16.6-100.0)][a]	[61.6 (22.2-100.0)][a]	<0.001**
%IIQ-7 mean ± SD	(33.92±28.39)	(35.58±22.63)[b]	(47.93±24.71)[b]	(54.63±24.56)[b]	(62.46±22.50)[b]	<0.001*
I-QOL mean ± SD	(87.88±25.44)[b]	(79.11±21.09)[b]	(73.31±22.94)[b]	(60.98±23.13)[b]	(56.75±21.39)[b]	<0.001*
I-QOL limitations of behaviors median (min-max)	[32 (12-39)][a]	[28 (11-40)][a]	[26 (9-39)][a]	[22 (8-40)]	[17 (8-40)][a]	<0.001**
I-QOL psychological influences median (min-max)	[43 (13-45)][a]	[37 (10-45)][a]	[34 (9-45)]	[27,5 (9-45)]	[26 (9-45)][a]	<0.001**
I-QOL social isolation median (min-max)	[20.5 (5-25)]	[17 (8-25)]	[17 (5-25)][a]	[11 (5-30)][a]	[11 (5-25)][a]	<0.001**

*: One-Way analysis of variance ([b]: Post-hoc: Bonferroni), **: Kruskal-Wallis Test ([a]: Post-hoc: ***Bonferroni-corrected*** Mann-Whitney U test), UDI-6: Urogenital distress inventory 6, IIQ-7: Incontinence impact questionnaire, I-QOL: Incontinence quality of life, SD: Standard deviation, Min: Minimum, Max: Maximum, SD: Standard deviation

Table 4. Cut-off values and ROC curve analysis results for the UDI-6, IIQ-7, and total I-QOL scales and subscale scores in the prediction of stress-predominant ***mixed*** urinary ***incontinence***

	Diagnostic test					ROC curve		p
	Cut-off	Sensitivity	Specificity	PPV	NPV	AUC	95% CI	
UDI-6	≥36.11	90.2	75.0	99.2	18.8	0.836	0.659-1.000	0.001**
IIQ-7	≥30.95	83.1	62.5	98.7	10.0	0.727	0.514-0.940	0.029**
Total I-QOL	≤79.5	74.8	87.5	99.5	9.5	0.804	0.609-0.998	0.003**
I-QOL limitations of behaviors	≤29.5	78.2	75.0	99.0	9.4	0.783	0.601-0.965	0.006**
I-QOL psychological influences	≤34.5	68.8	87.5	99.5	7.8	0.794	0.604-0.984	0.005**
I-QOL social isolation	≤15.5	72.9	87.5	99.5	8.9	0.793	0.588-0.997	0.005**

*: PPV, **: ROC curve analysis, NPV: Negative-predictive value, AUC: Area under the curve, CI: Confidence interval, UDI-6: Urogenital distress inventory 6, IIQ-7: Incontinence impact questionnaire, I-QOL: Incontinence quality of life, PPV: Positive-predictive value, ROC: Receiver operating characteristic

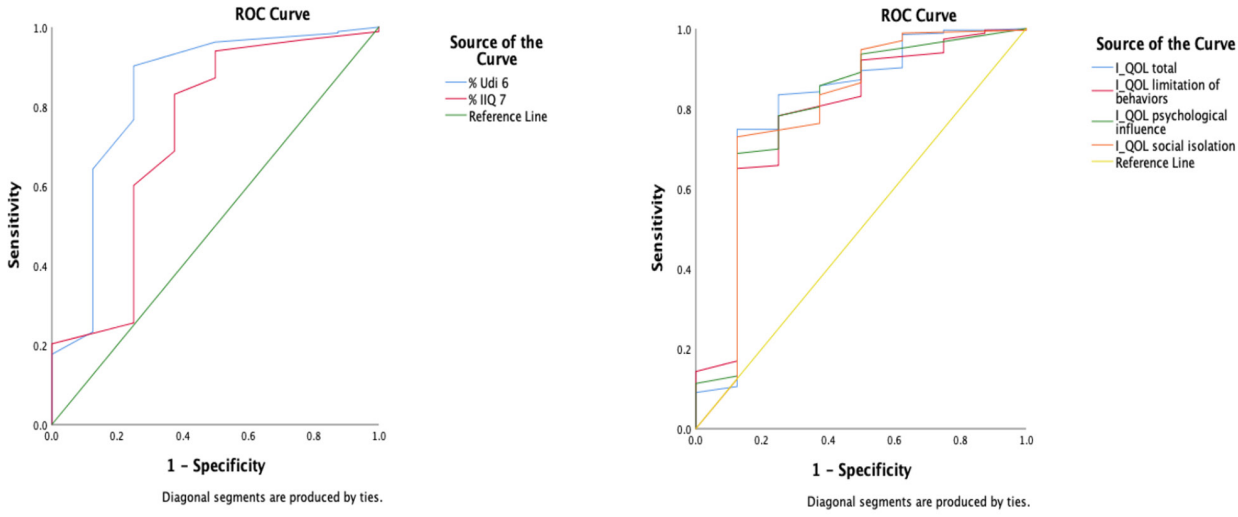

Figure 1. ROC curves of the UDI-6 and IIQ-7 scale scores for predicting stress-predominant mixed urinary incontinence (right) and the total I-QOL scale and subscale scores for predicting stress-predominant mixed urinary incontinence (left)

ROC: Receiver operating characteristic, UDI-6: Urogenital distress inventory 6, IIQ-7: Incontinence impact questionnaire, I-QOL: Incontinence quality of life

Discussion

The results of this study may help clinicians determine the type of symptomatic UI and guide further diagnosis and treatment. Each of the three scales used to predict the clinical diagnosis of stress-predominant mixed UI and urge-predominant mixed UI was effective. However, we found that the UDI-6 was the best scale for each clinical condition, and the other scales used to predict stress and urge incontinence could not reliably predict the clinical diagnosis (p>0.05). Our results comprise data from a large population, including a patient population that received a final diagnosis from more than one gynecologist.

By reviewing the literature on this topic, Wuytack et al.[17] defined the UI-specific and generic QOL outcome measurement tools used in women with UI and identified the most psychometrically robust tools to facilitate the selection of appropriate outcome criteria reported by patients to measure QOL in this population. Nineteen instruments used in studies performed in English-speaking populations were UI-specific, whereas four were generic. When reviewing instruments in other languages, nine urinary continence-specific instruments and three generic instruments were used across 19 different languages. Based on the evidence presented, we conclude that the I-QOL questionnaire is the most psychometrically robust specific tool for use in women with UI. The I-QOL is also the most widely-translated tool for other languages[17].

In the study by Skorupska et al.[18], the optimal cut-off score for distinguishing symptomatic from asymptomatic UI in women was a UDI-6 score of 33.33, with scores greater than 33.33 indicating higher distress caused by UI symptoms. Furthermore, a higher impact of UI on health-related QOL (HRQOL) was observed in women who scored 9 or more on the IIQ-7 questionnaire, indicating a perceived impairment in QOL. A UDI-6 score of 33.33 demonstrated 83.33% specificity and 97% sensitivity for determining higher distress caused by UI symptoms[18]. Our study population is not suitable for the cut-off value.

Bushnell et al.[19] employed standardized procedures, the I-QOL measure, and country-specific psychometric testing for validity, reliability, and responsiveness. Confirmatory factor analyses were conducted to evaluate the subscales of the I-QOL. The Incontinence-specific QOL measurement model consisted of three subscales. The summary and subscale scores were internally consistent across 15 versions (α values=0.91-0.96) and reproducible (intraclass correlation coefficients=0.72-0.97)[19].

Although the study by Ross et al.[20] frequently used multiple measures, better evidence is needed before deciding which single questionnaire should be considered the gold standard. Until such evidence is obtained, we recommend that researchers consider using the IIQ or I-QOL, with or without the UDI, as the first-choice method in their trials on incontinence treatments. This recommendation is based on an evaluation of the frequency of use, reliability, validity, sensitivity, and utility of these measurements. Consistent use of the IIQ or I-QOL, with or without the use of the UDI, will also encourage comparability between trials[20]. In our study, when all 3 questionnaires were compared, we found that the best questionnaire for predicting mixed-type UI was UDI-6.

In the study conducted by Öztaç Özerdoğan and Kızılkaya[14], women with stress UI had higher I-QOL scores than those with urge UI and mixed UI. This difference largely results from the

unpredictable nature of stress UI symptoms and related urine leakage. Similar results were found in our study.

Hannestad et al.[3] found that the prevalence of UI increased with age, with half of UI cases being stress-UI type, 11% being urge-UI type, and 36% being mixed-UI type[3].

In our study, clinical and urodynamic assessment results showed that 1.2% of patients were normal, 4.2% had stress-UI, 10% had urge UI, 59.2% had stress-predominant mixed UI, and 24.7% had urge-predominant mixed UI. The differing rates of UI types in our study can be attributed to the cross-sectional design, as the group underwent urodynamics for further assessment of UI. In the prediction of urge-predominant mixed UI, we determined that the three scales we used to predict urge-predominant mixed UI predicted the clinical diagnosis to be statistically significant ($p<0.05$). The best cut-off values determined in the ROC analysis among the three scales and the calculated sensitivity, specificity, positive-predictive value, negative-predictive value and area under the curve values are shown in Table 5 and Figure 2.

Table 5. Cut-off values and ROC curve analysis results for the UDI-6 and IIQ-7 scale scores for predicting urge-predominant *mixed* urinary *incontinence*

	Diagnostic test					ROC curve		p
	Cut-off	Sensitivity	Specificity	PPV	NPV	AUC	95% CI	
UDI-6	≥36.11	92.8	75.0	98.1	42.9	0.851	0.694-1.000	**0.001****
IIQ-7	≥30.95	90.1	62.5	97.1	31.3	0.785	0.580-0.991	**0.007****
Total I-QOL	≤79.5	82.9	87.5	98.9	26.9	0.833	0.633-1.000	**0.002****
I-QOL limitations of behaviors	≤29	89.2	75.0	98.0	33.3	0.832	0.658-1.000	**0.002****
I-QOL psychological influences	≤34.5	77.5	87.5	98.9	21.9	0.833	0.631-1.000	**0.002****
I-QOL social isolation	≤15.5	75.7	87.5	98.8	20.6	0.810	0.603-1.000	**0.004****

**: ROC curve analysis, NPV: Negative-predictive value, AUC: Area under the curve, CI: Confidence interval, UDI-6: Urogenital distress inventory 6, IIQ-7: Incontinence impact questionnaire, I-QOL: Incontinence quality of life, PPV: Positive-predictive value, ROC: Receiver operating characteristic

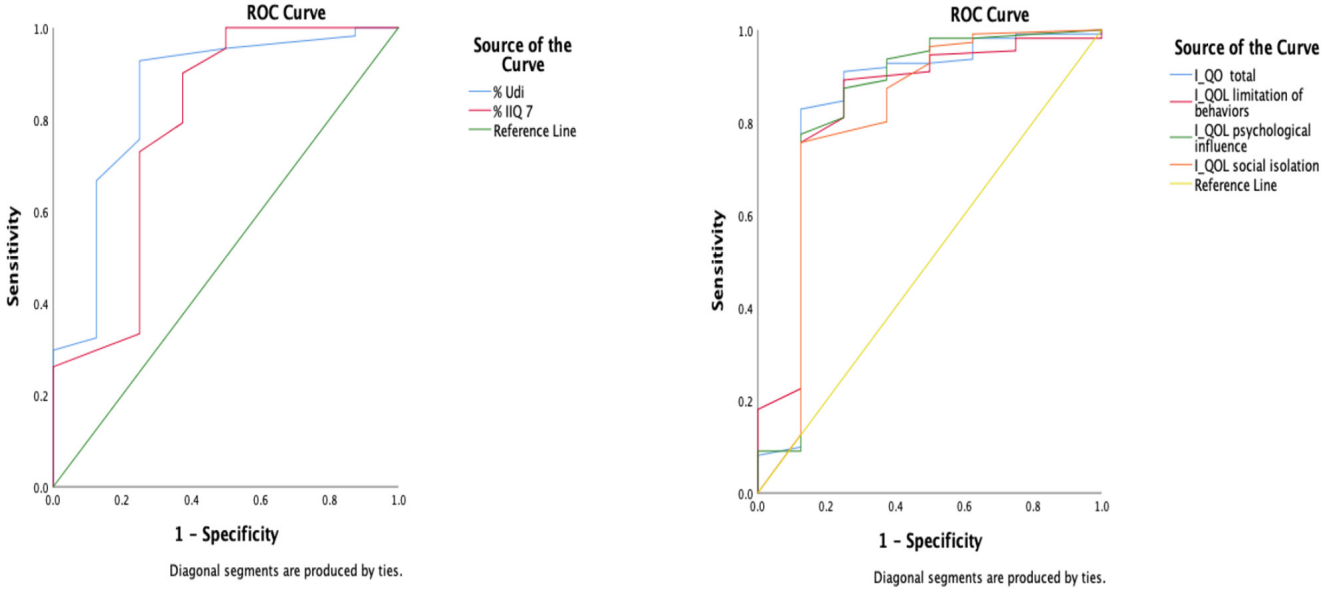

Figure 2. ROC curves of the UDI-6 and IIQ-7 scale scores for predicting urge-predominant mixed urinary incontinence (right), ROC curves of the total I-QOL scale, and subscale scores for predicting urge-predominant mixed urinary incontinence (left)

ROC: Receiver operating characteristic, UDI-6: Urogenital distress inventory 6, IIQ-7: Incontinence impact questionnaire, I-QOL: Incontinence quality of life

Study Limitations

The study limitations include the single-center design, exclusion of men, the referral nature of the study population, and the fact that 98.8% of cases were diagnosed with UI without a control group. Another potential concern is the absence of a true "gold standard" diagnostic test for UI. Since no such test exists for UI types, the validity of any diagnostic test for these types is contentious. Although urodynamic testing is not recommended for the basic clinical evaluation of UI in the current guidelines, it is frequently used in clinical practice to diagnose UI[21,22]. Given the absence of a control group for comparison and a "gold standard" diagnostic test in our study, further research is needed to verify these results in diverse populations.

Conclusion

Among the patients with the chief complaint of UI, we included those who underwent urodynamic evaluation for further diagnosis. All patients were classified according to the type of UI through urodynamic evaluation, with definitive diagnoses made by a urogynecologist. A total of 83.9% of patients presented with mixed UI.

Our study revealed that the UDI-6, IIQ-7, and I-QOL questionnaires showed high performance in predicting mixed urinary UI because they are easy to administer, inexpensive, and quick to complete. Although the UDI-6 questionnaire demonstrated the highest performance in predicting stress-predominant mixed UI and urge-predominant mixed UI, the IIQ-7 and I-QOL questionnaires also performed well in predicting mixed UI overall. For isolated stress UI and urge UI, none of the three questionnaires were predictive.

Ethics

Ethics Committee Approval: Ethical approval was obtained from the University of Health Science Türkiye, Zeynep Kamil Women and Children Diseases Traning and Research Hospital Clinical Research Ethics Committee (decision no: 138, date: 08.07.2020).

Informed Consent: Informed consent was obtained from the patients.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Ö.T., Ç.K., A.A., Concept: Ö.T., Design: Ö.T., Z.T., Data Collection or Processing: Ö.T., Ç.K., S.S.K., R.G.İ., Z.T, A.A., Ç.Y.V., A.T., Analysis or Interpretation: Ö.T., S.S.K., R.G.İ., Z.T, A.A., Literature Search: Ö.T., P.K., A.T., Writing: Ö.T., P.K., A.T.

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Review / Derleme

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Advancements in machine learning and biomarker integration for prenatal Down syndrome screening

Doğum öncesi Down sendromu taraması için makine öğrenimi ve biyobelirteç entegrasyonunda gelişmeler

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Abstract

The use of machine learning (ML) in biomarker analysis for predicting Down syndrome exemplifies an innovative strategy that enhances diagnostic accuracy and enables early detection. Recent studies demonstrate the effectiveness of ML algorithms in identifying genetic variations and expression patterns associated with Down syndrome by comparing genomic data from affected individuals and their typically developing peers. This review examines how ML and biomarker analysis improve prenatal screening for Down syndrome. Advancements show that integrating maternal serum markers, nuchal translucency measurements, and ultrasonographic images with algorithms, such as random forests and deep learning convolutional neural networks, raises detection rates to above 85% while keeping false positive rates low. Moreover, non-invasive prenatal testing with soft ultrasound markers has increased diagnostic sensitivity and specificity, marking a significant shift in prenatal care. The review highlights the importance of implementing robust screening protocols that utilize ultrasound biomarkers, along with developing personalized screening tools through advanced statistical methods. It also explores the potential of combining genetic and epigenetic biomarkers with ML to further improve diagnostic accuracy and understanding of Down syndrome pathophysiology. The findings stress the need for ongoing research to optimize algorithms, validate their effectiveness across diverse populations, and incorporate these cutting-edge approaches into routine clinical practice. Ultimately, blending advanced imaging techniques with ML shows promise for enhancing prenatal care outcomes and aiding informed decision-making for expectant parents.

Keywords: Machine learning, Down syndrome, trisomy 21, biomarkers, prenatal screening, genetic profiling, epigenetics

Öz

Makine öğreniminin (MÖ) biyobelirteç analizinde kullanımı, Down sendromunun tanı doğruluğunu artıran ve erken tespiti mümkün kılan yenilikçi bir stratejidir. Son araştırmalar, etkilenen bireylerin ve tipik akranlarının genomik verilerini karşılaştırarak, Down sendromu ile ilişkili genetik varyasyonları

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belirlemede MÖ algoritmalarının etkinliğini göstermektedir. Bu inceleme, MÖ ve biyobelirteç analizinin doğum öncesi taramayı nasıl geliştirdiğine odaklanmaktadır. Gelişmeler, maternal serum belirteçleri, ense kalınlığı ölçümleri ve ultrason görüntülerinin rastgele ormanlar ve derin öğrenme evrişimli sinir ağları gibi modern algoritmalarla entegrasyonunun, yanlış pozitif oranlarını düşük tutarak tespit oranlarını %85'in üzerine çıkardığını göstermektedir. Ayrıca, yumuşak ultrason belirteçleri ile yapılan invaziv olmayan doğum öncesi test, tanı duyarlılığını ve özgüllüğünü artırarak doğum öncesi bakımda önemli bir değişim sağlamaktadır. İnceleme, gelişmiş istatistiksel yöntemlerle kişiselleştirilmiş tarama araçları geliştirilmesinin yanı sıra, ultrason biyobelirteçlerini kullanan sağlam tarama protokollerinin uygulanmasının önemini vurgulamaktadır. Genetik ve epigenetik biyobelirteçlerin MÖ ile birleştirilmesi, Down sendromu patofizyolojisinin tanısal doğruluğunu artırma potansiyelini de araştırmaktadır. Sonuçlar, algoritmaların optimize edilmesi, farklı popülasyonlarda etkinliğin doğrulanması ve bu yaklaşımların klinik uygulamalara dahil edilmesi için devam eden araştırmalara duyulan ihtiyacı vurgulamaktadır. Sonuç olarak, gelişmiş görüntüleme tekniklerinin MÖ ile birleştirilmesi, doğum öncesi bakımın sonuçlarını iyileştirerek anne adaylarının bilinçli karar almalarına yardımcı olma potansiyeli taşımaktadır.

Anahtar Kelimeler: Makine öğrenimi, Down sendromu, trizomi 21, biyobelirteçler, doğum öncesi tarama, genetik profilleme, epigenetik

Introduction

The integration of artificial intelligence in medicine is transforming diagnostics and personalized treatment plans by facilitating accurate and efficient disease identification through advanced machine learning (ML)[1,2]. The use of ML in biomarker analysis for predicting Down syndrome exemplifies this innovative approach, enhancing diagnostic accuracy and enabling early detection. Recent studies demonstrate the effectiveness of ML algorithms in identifying genetic variations and expression patterns associated with Down syndrome by comparing genomic data from affected individuals to typically developing counterparts[3-5]. In prenatal diagnosis, ML techniques have significantly improved early Down syndrome assessment through the analysis of fetal ultrasound images, with deep learning (DL) architectures surpassing traditional methods in recognizing phenotypic traits indicative of Down syndrome[6,7]. Additionally, integrating various biomarkers from maternal serum screening has strengthened predictive models, enabling comprehensive risk assessments during pregnancy. Facial recognition technology, powered by DL, has emerged as a non-invasive diagnostic tool to identify subtle phenotypic traits characteristic of Down syndrome, showcasing ML's capability to detect complex patterns beyond human recognition[8,9]. The reliability of these ML models depends heavily on robust datasets that accurately reflect the target population, as shifts in datasets can greatly impact prediction effectiveness[10-12]. Incorporating multi-omics data, including genomic, transcriptomic, and proteomic information, further enhances the identification of relevant biomarkers, leading to more accurate and holistic diagnostic approaches for Down syndrome[13,14]. The advantages of this integration include improved detection rates, personalized care through extensive data analysis, and cost-effectiveness by reducing the need for invasive procedures. However, challenges such as data imbalance, the necessity for comprehensive datasets, and ethical concerns regarding privacy and consent must be addressed to fully integrate ML into routine clinical practice for Down syndrome screening[15,16].

This review summarizes current findings on integrating ML techniques with biomarker analysis to improve prenatal diagnostics for Down syndrome. It highlights the effectiveness of combining maternal serum markers, ultrasound imaging, and non-invasive prenatal testing (NIPT) with advanced algorithms such as random forests and DL. The review also explores the impact of genetic and epigenetic biomarkers, particularly transcriptomic and methylation profiling, on enhancing diagnostic accuracy for Down syndrome. Moreover, it evaluates personalized screening approaches, including tailored nomograms and predictive models, within clinical practice. By outlining recent advancements, the article aims to identify knowledge gaps, suggest future research directions, and emphasize the importance of incorporating these methodologies into routine prenatal care to enhance outcomes for expectant mothers and their infants.

Machine Learning Models

ML techniques are revolutionizing the prediction and screening of Down syndrome, particularly in prenatal settings, by enhancing detection rates while minimizing false positives[4,17]. Various studies have demonstrated the effectiveness of different algorithms, such as random forest models, which achieve an impressive 85.2% detection rate with only a 5% false positive rate, significantly outperforming traditional laboratory models[18]. Additionally, support vector machines (SVM) and advanced classification algorithms, alongside techniques like SMOTE-Tomek for data preprocessing, have maintained high detection rates[19]. DL methods, including Gaussian Processes for neuroimaging data analysis and convolutional neural networks for identifying genetic markers, have further pushed the boundaries of prediction accuracy[20]. Noteworthy applications in research highlight the role of artificial intelligence and ML in recognizing specific genetic variations, as seen in their work, and fostering improved early detection through models developed for different pregnancy trimesters, as shown by Leghari et al.[21] and He et al.[18]. Additionally, the efficacy of dense neural networks in ultrasound imaging, presented by Yousefpour Shahrivar et al.[3], showcases their superiority over traditional methods, while Qin et al.[22] highlight the potential for automated diagnosis through facial image analysis. The exploration of supervised learning algorithms, as emphasized by Feng et al.[23], also plays a crucial role in identifying biomarkers related to Down syndrome, while Li et al.[24] introduce a cascaded ML framework that addresses challenges of imbalanced data, offering a novel approach to enhancing prediction accuracy in Down syndrome cases.

He et al.[18] developed a ML model using random forest algorithms, to enhance Down syndrome prediction during second trimester antenatal screening, based on a retrospective analysis of data from 58.972 pregnant women, including 49 confirmed Down syndrome cases. The model achieved a Down syndrome detection rate of 66.7% with a 5% false positive rate in the initial dataset. When validated against an external dataset of 27.170 women, the detection rate improved to 85.2%, indicating its superiority over traditional lab risk models in China and its strong generalizability[18]. In another study, Xu et al.[25] explored the effectiveness of combining soft ultrasound markers (USM) with NIPT for diagnosing fetal chromosomal abnormalities, analyzing data from 856 high-risk pregnancies. Their findings showed that 15.07% of fetuses had one positive USM and 4.21% had two or more, with an overall chromosomal abnormality detection rate of 9.46%. Notably, multiple USMs correlated with a significantly higher incidence of abnormalities (36.11%) compared to those without USM (6.22%) and those with one positive USM (19.38%). The combination approach yielded diagnostic sensitivity, specificity, and accuracy of 96.72%, 98.45%, and 98.29%, respectively, highlighting its clinical value[25]. Zhang et al.[26] devised a DL model for trisomy 21 screening during the first trimester, utilizing nuchal ultrasonographic images from 822 participants across two Chinese hospitals. Their convolutional neural network achieved impressive areas under the curves (AUCs) of 0.98 and 0.95 for training and validation sets, surpassing traditional methods that yielded AUCs of 0.82 and 0.73. Moreover. Sun et al.[27] created an individualized nomogram for first-trimester screening of trisomy 21, utilizing fetal nuchal translucency (NT) thickness and various ultrasonographic facial markers. They analyzed 302 trisomy 21 cases and 322 euploid pregnancies, achieving AUC values of 0.983 in the training set and 0.979 in the validation set using the LASSO method, indicating strong predictive capability. In a study by Neocleous et al.[28], they sought to innovate non-invasive diagnostic procedures for aneuploidy using artificial neural networks trained on raw data from first trimester screenings of singleton pregnancies. With three datasets totaling 122.362 euploid and 967 aneuploid cases, the authors' models achieved a detection rate of 100% for trisomy 21, alongside detection rates exceeding 80% for other aneuploidies such as trisomies 13 and 18. This research showcases the potential of artificial neural networks to provide effective, non-invasive early screening tools that rival existing methodologies while addressing the social and financial burdens associated with prenatal testing.

Ultrasound Markers

The integration of ML techniques with ultrasound biomarkers offers a promising enhancement to the prediction and screening of Down syndrome during pregnancy. This innovative approach utilizes advanced computational methods to analyze complex data sets, improving detection rates and minimizing false positives[4,19]. Key USMs, such as NT, and additional indicators, such as the presence or absence of the nasal bone, significantly contribute to risk assessment, with NT alone detecting Down syndrome in 60% to 70% of cases at a 5% false positive rate[29]. By combining ultrasound data with maternal serum biomarkers, such as pregnancy-associated plasma protein A (PAPP-A) and free beta human chorionic gonadotropin (βhCG), ML algorithms, enhance detection rates to approximately 87% at the same false positive threshold[30]. Moreover, sophisticated predictive models incorporating multiple markers can reach detection rates of nearly 90%, offering improved accuracy over traditional methods[29,30]. This integration not only leads to enhanced screening precision but also reduces the need for invasive testing, such as amniocentesis, and allows for personalized screening strategies tailored to individual risk profiles, ultimately benefiting both mothers and infants.

Xu et al.[25] examined the effectiveness of combining soft USM with NIPT for diagnosing fetal chromosomal abnormalities using ML techniques. In a study involving 856 high-risk single pregnancies, NIPT was performed on 642 patients, all of whom also underwent amniocentesis and chromosomal karyotype analysis, to validate the diagnostic performance of USM, Down's syndrome screening, and NIPT. The results indicated that 15.07% of fetuses had one positive USM and 4.21% had two or more, resulting in an overall detection rate of 9.46% for chromosomal abnormalities. Importantly, multiple USMs correlated with a significantly higher incidence of abnormalities (36.11%) compared to no USMs (6.22%) and one positive USM (19.38%). The integration of USMs, Down's syndrome screening, and NIPT achieved high diagnostic sensitivity (96.72%), specificity (98.45%), and accuracy (98.29%), underscoring the value of this multimodal approach for improving the detection of fetal chromosomal anomalies[25].

In a separate study, Sun et al.[27] developed and validated a personalized nomogram for first-trimester screening of trisomy 21, using fetal NT thickness and various facial markers. Their retrospective case-control study involved analyzing two-dimensional midsagittal fetal profile images from 302 trisomy 21 cases and 322 euploid pregnancies, which were divided into training and validation sets. Using the least absolute shrinkage and selection operator (LASSO) method, they incorporated eight significant markers into a logistic regression model. The LASSO model demonstrated impressive area under the receiver-operating characteristic curve (AUC) values of 0.983 for the training set and 0.979 for the validation set, surpassing individual marker performance. Moreover, the nomogram showed strong discrimination capabilities, with C-indices of 0.983 and 0.981 for the training and validation sets, respectively. This study highlights the nomogram's potential as an effective tool for early trisomy 21 screening, providing a tailored risk assessment for expectant mothers[27].

Genetic and Epigenetic Biomarkers

The integration of ML techniques with genetic and epigenetic biomarkers represents a groundbreaking approach to enhancing

the prediction and diagnosis of Down syndrome[25,31]. This strategy utilizes advanced computational methods to analyze intricate biological data, which improves detection rates and offers insights into the condition's underlying mechanisms. At the genetic level, the presence of an extra copy of chromosome 21 is the primary cause of Down syndrome, resulting in an increased expression of key genes such as the amyloid precursor protein, which is linked to Alzheimer's disease pathology in individuals with Down syndrome as they age. Furthermore, genetic variants such as the ApoE ε4 allele are critical for assessing Alzheimer's risk, as they are associated with cognitive decline and amyloid accumulation. On the epigenetic front, modifications that drive neuroinflammation and specific proteomic changes in cerebrospinal fluid serve as potential biomarkers for cognitive decline and Alzheimer's onset[32,33]. ML applications, including predictive modeling using algorithms like SVM and neural networks, can analyze extensive datasets comprising genetic markers, epigenetic profiles, and clinical information to enhance detection rates significantly. Data mining techniques further reveal hidden correlations between biomarkers and patient outcomes, which can improve screening protocols[10]. Laufer et al.[34] utilized low-pass whole genome bisulfite sequencing (WGBS) to analyze DNA methylation profiles in neonatal dried blood spots (NDBS) related to Down syndrome. They highlighted that trisomy 21 leads to both genetic alterations and significant epigenetic changes, resulting in unique methylation patterns. Analyzing over 24 million CpG sites, the authors identified thousands of differentially methylated regions that differentiate Down syndrome from typical development and idiopathic developmental delay. Through ML refinement, they focused on 22 loci, primarily linked to genes vital for neurodevelopment, metabolism, and transcriptional regulation. Notably, the RUNX1 locus on chromosome 21 showed a ~28 kb hypermethylation region, emphasizing its role in the epigenomic dysregulation in Down syndrome. The study also explored the connection between differentially methylated regions (DMRs) and congenital heart disease in Down syndrome NDBS, advocating for the use of low-pass WGBS in epigenome investigations, and enhancing understanding of trisomy 21's early mechanistic pathways influencing epigenomic changes[34]. Volk et al.[31] investigated gene expression signatures as biomarkers for the prenatal diagnosis of trisomy 21. Noting the absence of a universal biomarker panel for high-risk pregnancies, they conducted a comprehensive transcriptome analysis to identify differentially expressed genes (DEGs) associated with Ts21. By profiling transcriptomic data from cultivated amniocyte samples of both Ts21 and normal euploid cases, they validated findings through reverse transcription polymerase chain reaction on a larger cohort and included gene expression omnibus repository datasets. Using a supervised ML algorithm, they assessed the classification performance of the Ts21 status, achieving significant results with an AUC of 0.97 for a multi-gene biomarker comprising nine gene expression profiles[31]. These findings reinforce the potential of transcriptomic alterations as diagnostic tools in prenatal settings, applicable to a wider range of genetic disorders stemming from cellular disturbances.

Integrating ML and Biomarkers Across Trimesters

Integrating ML techniques with genetic, epigenetic, and ultrasound biomarkers across trimesters represents a significant advancement in the screening and prediction of Down syndrome. This approach enhances detection rates while reducing false positives by analyzing complex datasets with advanced computational methods. In the first trimester, biomarkers such as NT and maternal serum markers, including PAPP-A and free βhCG, achieve detection rates around 85% when combined with maternal age. The second trimester benefits from additional markers like total hCG and inhibin-A, further improving screening performance[29,35,36]. Studies utilizing ML algorithms, such as SVM and classification trees, have developed predictive models that outperform traditional statistical methods. By integrating data from both trimesters, ML models can analyze a wider range of biomarkers, thus enhancing prediction accuracy and addressing dataset imbalances, particularly for scarce Down syndrome cases through techniques like synthetic minority over-sampling. Research conducted by He et al.[18] demonstrated that a random forest model improved second-trimester Down syndrome prediction, yielding an 85.2% detection rate in validation with external datasets. In the first trimester, Sun et al.[27] developed a personalized nomogram for trisomy 21 screening using fetal NT, and facial markers, attaining impressive AUC values of 0.983 and 0.979 for training and validation sets, respectively. Furthermore, Xu et al.[25] explored the efficacy of soft USM combined with NIPT, achieving notable sensitivity and specificity rates. Collectively, these studies highlight the promise of integrating ML techniques with biomarker data across trimesters to enhance Down syndrome screening and prediction. This approach ultimately improves detection rates up to 85% in the first trimester while minimizing false positives.

Discussion

Integrating ML with biomarker analysis for Down syndrome screening shows great promise in enhancing detection rates while minimizing false positives. The findings presented in Table 1 provide a comprehensive overview of the current studies exploring the integration of biomarkers and ML in the screening process for Down syndrome, highlighting both the advancements and the challenges faced in this evolving field. Recent studies by He et al.[18] and Zhang et al.[26] demonstrate the effectiveness of large datasets and advanced algorithms, such as random forests and DL convolutional neural networks, in improving prediction models. By combining maternal serum markers, NT measurements, and ultrasonographic images with sophisticated ML techniques, detection rates have exceeded 85%. The ability of these models to generalize across diverse

Table 1. Overview of studies on biomarkers and machine learning in Down syndrome screening

Authors	Methodology	Sample size	Detection rate	False positive rate	Notable findings
He et al.[18]	Machine learning model using random forest algorithms	58.972 pregnant women	66.7% (initial), 85.2% (validation)	5%	Model outperforms traditional methods; strong generalizability; potential for improved prenatal care.
Xu et al.[25]	Combination of ultrasound markers and non-invasive prenatal testing	856 high-risk single pregnancies	9.46% overall	-	High sensitivity (96.72%) and specificity (98.45%) for chromosomal abnormalities; emphasizes combined modalities.
Zhang et al.[26]	Deep learning model with convolutional neural network on nuchal ultrasonographic images	822 participants	AUC of 0.98 (training), 0.95 (validation)	-	Surpasses traditional screening based on nuchal translucency and maternal age; potential for universal screening.
Sun et al.[27]	LASSO method for developing a nomogram based on fetal NT thickness and facial markers	624 cases (302 trisomy 21, 322 euploid)	AUC of 0.983 (training), 0.979 (validation)	-	Strong discrimination ability; provides personalized risk assessment for trisomy 21.
Neocleous et al.[28]	Non-invasive screening for aneuploidy using artificial neural networks	123.329 cases (122.362 euploid, 967 aneuploid)	100% for Trisomy 21, >80% for others	Minimal	Effective non-invasive screening with financial considerations; optimal false positive and high detection rates.
Volk et al.[31]	Transcriptome analysis to identify biomarkers for Trisomy 21	10 Ts21 and 9 normal euploid samples, plus independent validation	AUC=0.97 to 1.00	-	Transcriptome analysis identifies gene profiles for prenatal Trisomy 21 diagnosis, achieving high classification performance (AUC up to 1.00).

AUC: Area under the curve, LASSO: Least absolute shrinkage and selection operator, NT: Nuchal translucency

populations, supported by external validations, reinforces a robust approach to personalized prenatal care. Additionally, integrating NIPT with soft USM has achieved remarkable diagnostic sensitivity, specificity, and accuracy, highlighting the potential of multi-modal strategies for early identification of fetal anomalies[25]. These advancements promise better clinical outcomes and suggest a shift toward individualized risk assessments for expectant mothers. Ongoing exploration of novel biomarkers and cutting-edge algorithms is expected to further enhance prenatal screening efficacy.

These findings emphasize the importance of ultrasound biomarkers and ML in improving prenatal diagnostics for conditions like Down syndrome. Results from Xu et al.[25] illustrate the effectiveness of combining soft USM with NIPT to boost detection rates of chromosomal abnormalities. Given the correlation between multiple unidentified subject matters and increased abnormality rates, establishing robust screening protocols that incorporate these biomarkers into clinical practice is essential. The high sensitivity (96.72%) and specificity (98.45%) of the combined diagnostic approach indicate a transformative shift in prenatal screening methodologies, providing reassurance to expectant parents and enabling informed decisions about further diagnostic interventions. Sun et al.'s[27] personalized nomogram highlights the value of tailored approaches in prenatal care, utilizing specific ultrasound parameters and advanced statistical techniques to create individualized screening tools. Strong AUC values and C-indices demonstrate its effectiveness in distinguishing between trisomy 21 and euploid pregnancies, aiding healthcare providers in delivering accurate risk assessments. Collectively, these studies suggest that combining ML with advanced imaging techniques enhances prenatal screening accuracy and aligns with the trend toward personalized medicine. Future research should focus on refining these algorithms, validating their effectiveness across diverse populations, and integrating them into routine clinical practice. Real-time data analytics and comprehensive training for healthcare professionals will be crucial for effective tool utilization, ultimately improving prenatal care outcomes for mothers and infants.

The integration of genetic and epigenetic biomarkers with advanced ML techniques represents a transformative approach to enhancing diagnostics for Down syndrome. Recent studies have clarified the complexity of Down syndrome through genetic alterations from trisomy 21 and significant epigenetic modifications affecting gene expression and regulation. Findings from Laufer et al.[34] work with low-pass WGBS highlight DNA methylation patterns as potential biomarkers for Down syndrome. The discovery of numerous differentially methylated regions associated with developmental and metabolic processes, particularly around neurodevelopment-critical genes, illustrates how epigenetic profiling can provide insights into the mechanisms underlying trisomy 21. The

significant hypermethylation of the RUNX1 locus points to a targeted approach for understanding epigenomic dysregulation in Down syndrome. Such DMRs not only help explain individual variations in disease presentation but also connect these alterations to comorbid conditions like congenital heart disease. This comprehensive understanding is crucial for developing targeted therapeutic interventions and personalized care strategies. Volk et al.[31] investigation of gene expression signatures in prenatal diagnostics further supports the potential of integrating transcriptomic analyses with ML. Their focus on DEGs among amniocyte samples demonstrates the feasibility of using comprehensive transcriptomic data to create predictive models for trisomy 21. The high AUC achieved with a multi-gene biomarker panel reinforces the viability of non-invasive prenatal screening. This combination of approaches marks a paradigm shift in diagnosing genetic disorders. By leveraging genetic and epigenetic markers alongside sophisticated computational methods, researchers have substantial potential to enhance diagnostic accuracy and timeliness. This integration not only facilitates early detection of Down syndrome but also deepens understanding of its pathophysiology, leading to improved management and outcomes for affected individuals. As researchers refine these methodologies and incorporate ML as a powerful analytical tool, the promise of accurate, early diagnosis of Down syndrome and related conditions becomes increasingly attainable, paving the way for enhanced clinical interventions and improved quality of life for individuals with Down syndrome.

The integration of ML techniques with genetic, epigenetic, and ultrasound biomarkers across trimesters marks a transformative step in the screening and prediction of Down syndrome, addressing previous challenges in detection rates and false positives. Advanced computational methods enable comprehensive analysis of complex datasets, enhancing the ability of predictive models to identify at-risk pregnancies. In the first trimester, established biomarkers such as NT and maternal serum markers achieve approximately 85%, detection rates, which are further improved in the second trimester with additional markers like total hCG and inhibin-A. ML algorithms, including SVM and random forest models, have shown superior performance compared to traditional statistical methods, demonstrating higher accuracy and efficiency in predictions[27]. Notably, innovative approaches, such as personalized nomograms and the incorporation of soft USM with NIPT, have yielded impressive AUC values and sensitivity rates. Synthesizing data from both trimesters not only enhances prediction accuracy but also addresses dataset imbalances, particularly for rarer cases of Down syndrome. As these studies illustrate, the fusion of ML with biomarker analysis holds significant promise for advancing clinical practices, ultimately aiming to optimize screening processes and improve outcomes for expectant parents.

Clinical Implications

The integration of biomarkers and ML for predicting Down syndrome has significant clinical implications, particularly for enhancing prenatal screening protocols. Advanced algorithms and large datasets enable healthcare providers to achieve higher detection rates of trisomy 21 while keeping false positive rates low, thereby reducing unnecessary anxiety and invasive procedures for expectant parents. Combining maternal serum markers, NT measurements, soft USM, and NIPT allows for a personalized risk assessment tailored to individual patients. This approach not only improves diagnostic accuracy but also aids in informed decision-making regarding further diagnostic interventions and management strategies. As these methods evolve and become standard in clinical practice, they promise to transform prenatal care, resulting in better outcomes for mothers and infants through earlier detection and intervention for conditions linked to Down syndrome. The focus on personalized risk assessments, along with the high sensitivity and specificity of these approaches, reassures parents and empowers healthcare professionals to provide more precise and effective prenatal care, facilitating early interventions and improved management of associated conditions.

Study Limitations

Despite promising advancements in integrating ML with biomarker analysis for predicting Down syndrome, several limitations must be acknowledged. A major challenge is the dependence on large datasets that may not represent diverse populations, potentially leading to biases in model performance and generalizability. Variability in biomarkers among different groups can affect efficacy, making validation in diverse demographics essential. The complexity of genetic and epigenetic factors in Down syndrome further complicates risk prediction, necessitating thorough training and validation of ML algorithms to prevent overfitting and maintain reliability in clinical settings. Additionally, healthcare providers may encounter barriers to adopting these technologies due to limited resources, inadequate training, or challenges in integrating them into current workflows. Finally, ethical issues related to data privacy, informed consent, and the implications of prenatal screening results must be carefully managed to ensure patients feel secure and informed throughout the screening process.

Conclusion

Integrating ML with biomarker analysis for Down syndrome screening marks a significant advancement in prenatal diagnostics, resulting in improved detection rates and fewer false positives. By merging maternal serum markers, ultrasound measurements, and advanced algorithms, studies indicate the potential for personalized risk assessments and better clinical outcomes. The addition of genetic and epigenetic biomarkers

enhances this approach, providing insights into trisomy 21 mechanisms and aiding in targeted interventions. The high sensitivity and specificity of these multi-modal strategies highlight their transformative effect on prenatal care, facilitating informed decision-making for expectant parents. As research continues to refine these methods and validate their effectiveness across diverse populations, the prospect of accurate and timely Down syndrome diagnosis becomes increasingly achievable, ultimately improving management and quality of life for affected individuals.

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Footnotes

Authorship Contributions

Surgical and Medical Practices: M.D., H.R., M.Y., S.A.D., R.B., S.A., F.J., A.M., A.S., A.Shi., K.A., M.M., H.N., Concept: M.D., H.R., S.A., M.M., Design: M.Y., F.J., H.N., Data Collection or Processing: S.A.D., Analysis or Interpretation: S.A., A.M., Literature Search: R.B., A.S., A.Shi., H.N., Writing: K.A., M.M., H.N.

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Review / Derleme

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Comparative analysis of laparoendoscopic single-site surgery and versus conventional laparoscopic surgery in adnexectomy: A systematic review and meta-analysis of surgical outcome

Adneksektomide laparoendoskopik tek-bölge cerrahisi ile konvansiyonel laparoskopik cerrahinin karşılaştırmalı analizi: Cerrahi sonuçların sistematik bir derlemesi ve meta-analizi

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Abstract

Although the removal of the adnexa technically removes more tissue, it may require less fine manipulation and dissection than cystectomy. Secondary to this, we sought to measure the effectiveness and safety of laparoendoscopic single-site surgery (LESS) versus conventional laparoscopy (CLS). We search six databases to find studies comparing LESS and CLS for ovarian lesions where removal of the entire ovary, with or without the fallopian tube, is necessary. Criteria used for study eligibility: both controlled trials and observational studies were included in this analysis. Study appraisal and synthesis methods: we used the Cochrane risk of bias assessment tool for the randomized clinical trials and the national heart, lung, and blood quality assessment tools for the observational studies. The statistical analysis was done using the review manager software. LESS showed a significantly longer operative time [mean difference (MD)=2.96 (-1.97, 7.90), p=0.24], but with moderate heterogeneity. Estimated blood loss was significantly lower for LESS [MD=-18.62 (-33.83, -3.42), p=0.02]. The length of patient hospital stay was comparable [MD=-0.02 (-0.50, 0.47), p=0.95]. Visual analog scale (VAS) pain scores at 24 hours [MD=0.23 (-0.09, 0.56), p=0.16] and 6 hours postoperatively [MD=0.15 (-0.04, 0.33), p=0.12] were similar. The LESS group required less postoperative analgesia [risk ratios (RR)=0.47 (0.32, 0.68), p=0.001]. The change in hemoglobin was comparable [MD=-0.11 (-0.26, 0.03), p=0.14]. Perioperative complications were higher in the LESS group [RR=2.236 (1.031, 4.851), p=0.04]. Compared with CLS, LESS required more operative time but resulted in significantly less blood loss and lower postoperative analgesic use. Hospital stays and VAS pain scores were similar. LESS had a higher incidence of perioperative complications, which questions the feasibility of its use in some situations.

Keywords: Adnexectomy, laparoendoscopic single-site surgery, conventional laparoscopic surgery, minimally invasive surgery, meta-analysis

Öz

Adnekslerin çıkarılması teknik olarak daha fazla doku çıkarsa da, kistektomiye göre daha az ince manipülasyon ve diseksiyon gerektirebilir. Bu durumu araştırmanın yanı sıra, bu yazıda, laparoendoskopik tek-bölge cerrahisinin (LTBC) konvansiyonel laparoskopiye (KL) göre etkinliğini ve güvenliğini ölçmeyi de amaçladık. Fallop tüpü çıkarılarak veya çıkarılmadan tüm yumurtalığın çıkarılmasının gerekli olduğu yumurtalık lezyonlarında LTBC ve KL'yi karşılaştıran çalışmaları bulmak için altı veritabanını taradık. Bu analize hem kontrollü çalışmalar hem de gözlemsel çalışmalar dahil edildi. Çalışma değerlendirme ve sentez yöntemleri: randomize kontrollü çalışmalar için Cochrane bias riski değerlendirme aracını ve gözlemsel çalışmalar için ulusal kalp, akciğer ve kan kalite değerlendirme araçlarını kullandık. İstatistiksel analiz Review Manager yazılımı kullanılarak yapıldı. LTBC, orta düzeyde heterojenlik ile anlamlı olarak daha uzun bir ameliyat süresi [ortalama fark (OF)=2,96 (-1,97, 7,90), p=0,24] ile ilişkili idi. Tahmini kan kaybı, LTBC'de anlamlı olarak

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daha az idi [OF=-18,62 (-33,83, -3,42), p=0,02]. Hastanede kalış süresi LTBC ve KL'de benzer idi [OF=-0,02 (-0,50, 0,47), p=0,95]. Postoperatif 24. saatteki [OF=0,23 (-0,09, 0,56), p=0,16] ve 6. saatteki [OF=0,15 (-0,04, 0,33), p=0,12] görsel analog ölçek (GAÖ) ağrı skorları benzerdi. LTBC grubu daha az postoperatif analjeziye ihtiyaç duydu [risk oranları (RO)=0,47 (0,32, 0,68), p=0,001]. Hemoglobin değişimi her iki grupta da benzerdi [OF=-0,11 (-0,26, 0,03), p=0,14]. Perioperatif komplikasyonlar LTBC grubunda daha yüksekti [RO=2,236 (1,031, 4,851), p=0,04]. KL ile karşılaştırıldığında, LTBC daha uzun ameliyat süresi ile ilişkili idi; ancak anlamlı olarak daha az kan kaybı ve daha az postoperatif analjezik kullanımıyla sonuçlandı. Hastanede kalış süreleri ve GAÖ ağrı skorları benzerdi. LTBC'nin daha yüksek bir perioperatif komplikasyon insidansı vardı, bu da bazı durumlarda uygulanabilirliğini sorgulatıyordu.

Anahtar Kelimeler: Adneksektomi, laparoendoskopik tek-bölge cerrahisi, konvansiyonel laparoskopik cerrahi, minimal invaziv cerrahi, meta-analiz

Introduction

Masses of the ovary and adnexa are frequently encountered pathologies. The best course of treatment for these masses can vary and is not always clear to the clinician[1]. Asymptomatic masses with a low probability of being malignant do not usually require surgical treatment. Masses that have the potential to be malignant, or are causing pain, can often be excised by laparoscopic techniques[2,3].

It is estimated that there are 350.000 adnexal surgeries carried out each year in the USA and that 65% of these are laparoscopic or robotic in nature[4,5]. While laparoscopic adnexal surgery in most cases is straightforward, in some patients with dense adhesions, obesity, prior pelvic surgery or endometriosis, surgery can be challenging[6].

In recent decades, improvements in medical technology and awareness of patients have pushed for the enhancement of minimally invasive surgical techniques. Laparoscopic surgery is preferred over open surgery because it causes less operative trauma, shorter operative time, less morbidity, faster recovery, and better cosmetic results[7-9].

Laparoendoscopic single-site surgery (LESS) is a relatively new technique within minimally invasive surgeries. LESS is performed via a single umbilical incision using specialized instrumentation. It has the potential benefits of minimizing abdominal scarring, decreasing the risk of trocar/port complications, and the potential for decreasing analgesic requirements[10]. Some studies have recently described LESS to be safe and effective for many gynecologic surgeries including adnexectomy, cystectomy, endometrioma excision, and hysterectomy[11,12].

In addition, as opposed to cystectomy which sometimes requires extensive dissection between the ovarian lesion and the ovary proper, removal of the entire adnexa is normally a more straightforward procedure that may lend itself more to minimally invasive techniques such as LESS.

As a result, our study aims to analyze the surgical outcomes and assess postoperative pain outcomes related to LESS and conventional laparoscopic surgery (CLS). We will limit this study to the treatment of benign ovarian lesions with oophorectomy or removal of the entire adnexal (ovary and fallopian tube).

Methods

We conducted our study based on preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines and recommendations[13].

Search Strategy and Information Sources

We developed a search strategy by combining the following keywords: ("laparoscopy" OR "laparoscopic surgery" OR "minimally invasive" AND "laparoendoscopic single-site surgery" OR "LESS" AND "conventional laparoscopic surgery" OR "CLS" AND "oophorectomy" OR "salpingo-oophorectomy" OR "salpingectomy" OR "adnexectomy", AND "benign ovarian lesions"). We searched six databases: Medline, PubMed, Cochrane Library, Web of Science, clinicaltrials.org, and SCOPUS.

Study Selection

The screening steps were performed by two independent authors. First, these authors screened the title and abstract of each paper. Following this, a full text screening was performed on the selected papers. A third author solved any potential conflict between the two authors. The articles ultimately included in our synthesis were selected according to these eligibility criteria:

- **Population:** Women diagnosed with benign ovarian cysts undergoing salpingo-oophorectomy with or without cystectomy. Patients who underwent cystectomy alone were excluded.
- **Intervention:** LESS.
- **Comparator:** CLS.
- **Outcomes:** Measures of operative outcomes (e.g., operative time, blood loss), postoperative pain, complications, and recovery metrics (e.g., hospital stay).
- **Study Design:** We included randomized clinical trials (RCTs), as well as observational studies.

Quality Assessment

To assess the quality of the included studies, we used the Cochrane risk of bias (ROB) assessment tool for RCTs. In addition, we used the national heart, lung, and blood quality assessment tools to assess the quality of the observational studies. Each study's ROB was categorized as low, high, or unclear[14].

Data Extraction

Data extraction was performed for three categories:

1. Demographic Information: This included baseline characteristics of the patients, such as age, body mass index (BMI), mass size, and previous abdominal surgery.

2. Outcomes: Data on operative time, blood loss, postoperative pain [measured by the visual analog scale, (VAS)], complication rates, and hospital stay duration.

3. Quality Assessment Data: Information from the quality assessment of each study.

Microsoft Excel was used to organize and manage the data collection process.

Statistical Analysis

In conducting the meta-analysis, review manager software and openmeta (Analyst)[15] were used. Both categorical and continuous variables were included in the analysis. The continuous data were presented and compared using the mean difference (MD) along with 95% confidence interval (CI), while the dichotomous data were compared using risk ratios (RR) and a 95% CI. For homogeneous data, a fixed-effects model was employed, while for heterogeneous data, a random-effects model was used. To evaluate the heterogeneity of the studies, the I^2 statistic and the chi-square tests were conducted, and the values of $p<0.1$ or $I^2>50\%$ were considered to indicate significant heterogeneity.

Results

Summary of the Included Studies

Ultimately, we included eleven studies in our analysis: three RCTs[6,16,17], one prospective comparative study[18], and seven retrospective studies[4,19-24]. All included studies compared the efficacy and safety measures of LESS and CLS for adnexectomy in the presence of benign ovarian lesions. The detailed results of our literature search are illustrated in the PRISMA flow chart (Figure 1). A total of 1.231 women were included in our analysis, 608 in the LESS group and 623 in the CLS group. The mean age of the included cases in the LESS group was 40.1±11.3 years, and the mean age in the CLS was 39.3±11.3 years. The mean BMI in the LESS group was 22.9±4.23, while in the CLS, it was 22.9±4.06. The mean mass size in centimeters was 5±2.8 in the LESS group and 6.1±4 in the CLS group. Tables 1-3 present the characteristics of the involved studies and the demographics of the women included.

The Results of the Quality Assessment

When looking at the results of the quality assessment, the average score was 10.5 on a scale with a maximum score of 14[4,18-24]. Table 4 can be referenced for a detailed description of all the factors included in the quality assessment. Regarding the randomized studies[6,16,17], all the included studies were properly randomized, although Hoyer-Sorensen et al.[17] and Shin et al.[16] lack sufficient blinding. Therefore, they were found to be at a high risk of both performance and detection bias. Another outlier study, Fagotti et al.[6] reported proper blinding of the physicians with a low risk of detection bias, as seen in Figure 2.

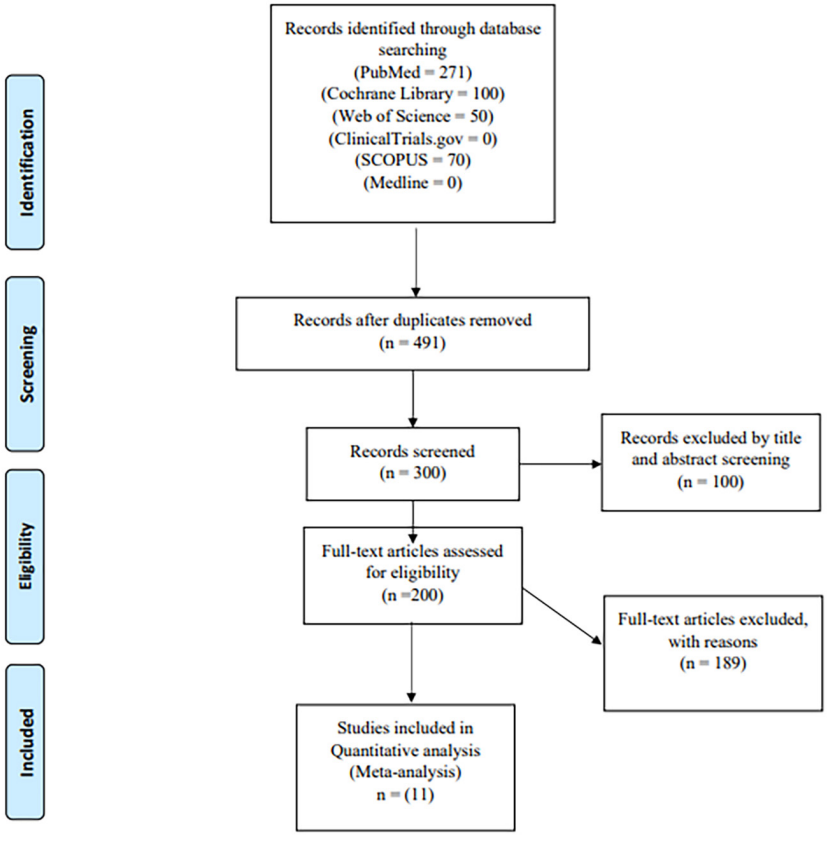

Figure 1. Prisma flow diagram of our literature search

Table 1. The inclusion criteria and study designs of the included studies

Study ID	Country	Inclusion criteria	Intervention	Study design	Sample size	
					LESS	CLS
Bedaiwy et al.[4] 2012	USA	Cases with a diagnosis of adnexal benign disease at an ultrasound examination and negative serum marker levels	Adnexectomy	Retrospective cohort	28	50
Fagotti et al.[6] 2011	Italy	Patients with unilateral adnexal disease requiring surgical evaluation, a normal CA-125, and a body mass index <35 who underwent adnexectomy via LESS or conventional operative laparoscopy were included	Cystectomy or salpingo-oophorectomy	RCT	30	30
Im et al.[18] 2011	Korea	Age less than 70 years, American Society of Anesthesiology Class 1 or 2. Cases that performed coexistence with other surgeries, such as uterine myomectomy were excluded	Cystectomy or salpingo-oophorectomy	Prospective comparative study.	18	15
Karasu et al.[23] 2017	Türkiye	Cases with benign adnexal mass.	Cystectomy or salpingo-oophorectomy	Retrospective cohort study	32	39
Kim et al.[24] 2012	Korea	Patients with unilateral adnexal disease requiring surgical evaluation, a normal CA-125, and a body mass index <35 who underwent adnexectomy via LESS or conventional operative laparoscopy were included	Cystectomy or salpingo-oophorectomy	Retrospective cohort study	94	94
Lee et al.[21] 2010	Korea	Age <70 years and an adnexal mass on ultrasonography or pelvic magnetic resonance imaging	Cystectomy or salpingo-oophorectomy	Retrospective case-control study	17	34
Lee et al.[20] 2014	Korea	Patients with benign adnexal tumors	Cystectomy or salpingo-oophorectomy	Retrospective cohort study	129	100
Shin et al.[16] 2019	Korea	An indication for adnexal surgery, no evidence of malignancy based on ultrasound or computed tomography, normal cervical cytology, and appropriate medical status for surgery (American Society of Anesthesiologists Physical Status classification 1 or 2)	Cystectomy or salpingo-oophorectomy with or without adhesiolysis or myomectomy	RCT	31	30
Hoyer-Sørensen et al.[17] 2012	Norway	Women greater than 18 years of age with presumed benign ovarian disease or a hereditary cancer risk, assessed as having an American Society of Anesthesiologists score of 1 or 2 and having an ovarian cyst of at least 6 cm	A salpingo-oophorectomy	RCT	20	20
Wang et al.[19] 2016	China	Patients who were diagnosed with the presence of adnexal masses on ultrasound without any severe complications	Cystectomy + oophorectomy	Retrospective case	99	104
Yim et al.[22] 2013	Korea	An adnexectomy was planned for cases of benign lesions in patients with adequate medical conditions for laparoscopic surgery. Patients who planned to have concurrent uterine surgery were not included	Adnexectomy. Patients receiving additional procedures were excluded	Retrospective case	110	107

RCT: Randomized clinical trial, LESS: Laparoendoscopic single-site surgery, CLS: Conventional laparoscopy

Table 2. Demographic and clinical characteristics of the included participants

Study ID	Age mean, SD/(IQR)		BMI mean, SD/(IQR)		Previous abdominal surgery n (%)		Mass size mean, SD/(IQR)	
	LESS	CLS	LESS	CLS	LESS	CLS	LESS	CLS
Bedaiwy et al.[4] 2012	42±8.6	44±9	26±8.2	27±8.5	8 (28.6%)	16 (32%)	5.5±2.5	6.7±83
Fagotti et al.[6] 2011	49.0 (20-73)	42.0 (15-73)	22.8 (17.6-37.0)	22.1 (18.2-30.0)	9 (30.0%)	10 (33.3%)	5.10 (1.4-8.3)	5 (2.0-9.0)
Im et al.[18] 2011	38.4 (21.1-67.4)	37.9 (26.7-60.2)	23.7 (20-44.6)	22.9 (19.4-29.6)	6 (33.3%)	6 (40%)		
Karasu et al.[23] 2017	31.1±8.35	29.9±7.96	24.8±3.69	23.4±2.83	12 (37.4%)	6 (15%)	7.92±1.41	7.48±1.89

Table 2. Continued

Study ID	Age mean, SD/(IQR)		BMI mean, SD/(IQR)		Previous abdominal surgery n (%)		Mass size mean, SD/(IQR)	
	LESS	CLS	LESS	CLS	LESS	CLS	LESS	CLS
Kim et al.[24] 2012	44.2±14.0	39.3±12.8	22.0 (15.6-37.0)	21.3 (17.0-34.1)	46 (48.9%)	29 (30.9%)	5.0 (2.0-25.0)	5.0 (2.0-9.0)
Lee et al.[21] 2010	44.7±12.1	39.9±10.1	22.8±3.2	23.3±3.5	12 (70.5%)	26 (76.5%)	5.6 (2.3-14.0)	6.2 (2.9-10.5)
Lee et al.[20] 2014	34 (16-70)	35 (22-65)	20.7 (16.8-39.0)	21.3 (16.8-29.7)	37 (28.7%)	19 (19.0%)	NR	NR
Shin et al.[16] 2019	36.5±14.5	39.9±15.8	21.1±3.0	22.3±3.0	8 (25%)	7 (23.3%)	6.9±4.6	6.8±3.2
Hoyer-Sørensen et al.[17] 2012	55.1±16.2	58.7±10.8	25.1±5.5	25.4±4.8	13±65	10±50	NR	NR
Wang et al.[19] 2016	32 (11, 58)	32 (15, 73)	22.1±3.2	22.2±3.1	17 (17.2%)	21 (20.2%)	4.9 (14.0, 226.0)	55.5 (5.0, 181.0)
Yim et al.[22] 2013	35.4±10.3	34.3 (10.8)	21.4 (2.82)	21.4 (2.7)	15 (13.6%)	21 (19.6%)	NR	NR

LESS: Laparoendoscopic single-site surgery, CLS: Conventional laparoscopy, SD: Standard deviation, IQR: Interquartile range, BMI: Body mass index

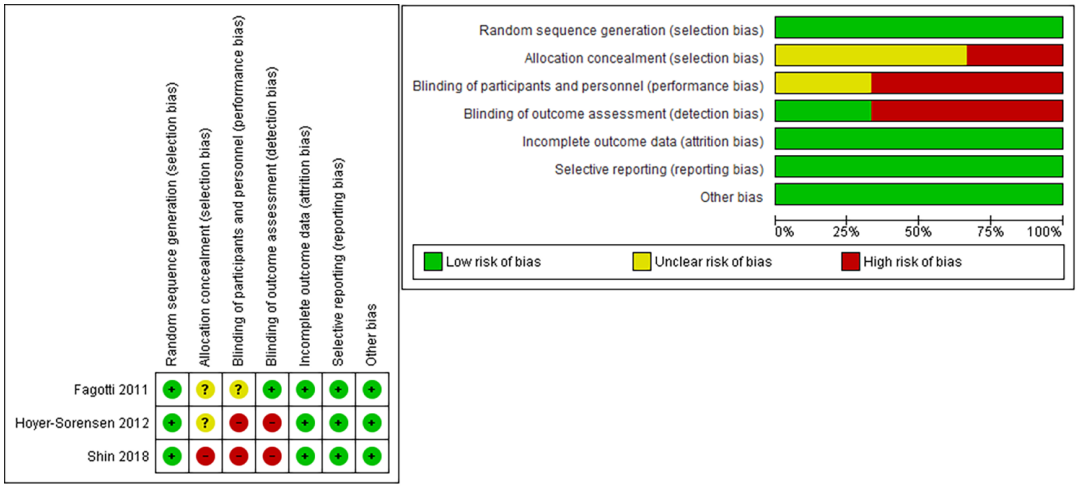

Figure 2. Details of the risk of bias assessment of the included randomized clinical trials

Analysis of Outcomes

1. Operative Time (Min)

Most of the studies included in our analysis reported the total operative time for both procedures[4,6,16,18-24]. Our analysis revealed that LESS was associated with a longer operative time than CLS [MD=2.96 (-1.97, 7.90), p=0.24], and a moderate amount of heterogeneity was observed (p=0.07); I^2=43. We managed the heterogeneity through a sensitivity analysis, resolving it by the exclusion of Lee et al.[20]. This resulted in [MD=3.52 (-1.01, 8.06), p=0.13] reduced heterogeneity (p=0.14, I^2=35%), as seen in Figure 3.

2. Estimated Blood Loss (EBL) (in mL)

Estimated blood loss during the surgery was measured by seven studies[4,6,18,19,21,22]. Our pooled analysis revealed that adnexectomy using LESS was associated with a statistically significant reduction in the EBL compared with conventional laparoscopy [MD=-18.62 (-33.83, -3.42), p=0.02]. The analysis showed significant heterogeneity (p=0.01, I^2=95%), which could not be addressed (as seen in Figure 4).

3. Length of Hospital Stay (in Days)

The mean hospital stay in the LESS group was 2.6 days, while in the CLS group it was 2.7 days. Our analysis showed that both operations had comparable hospital stay periods [MD=-0.02 (-0.50, 0.47), p=0.95] but significant heterogeneity was

Table 3. Summary of the histological type and the intervention performed

Study ID	Histological type								Intervention performed									
	Mature cystic teratoma		Mucinous cystadenoma		Serous cystadenoma		Others		Cystectomy/ enucleation		Salpingo-oophorectomy		Salpingectomy		oophorectomy		Others	
	LESS	CLS	LESS	CLS	LESS	CLS	LESS	CLS	LESS	CLS	LESS	CLS	LESS	CLS	LESS	CLS	LESS	CLS
Bedaiwy et al.[4] 2012	10 (36%)	18 (36%)	6 (21%)	8 (16%)	8 (28.5%)	12 (24%)	4 (14%)	12 (24%)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Fagotti et al.[6] 2011	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Im et al.[18] 2011	NR	NR	NR	NR	NR	NR	NR	NR	11 (61.1)	10 (66.7)	5 (27.8)	4 (26.7)	2 (11.1)	1 (6.7)	NR	NR	2 (11.1)	5 (33.3)
Karasu et al.[23] 2017	6 (18)	2 (5.1)	4 (12.5)	2 (5.1)	NR	NR	26 (81.25)	29 (90)	21	33	4	0	2	1	4	3	1	2
Kim et al.[24] 2012	15 (15.9)	37 (39.3)	NR	NR	NR	NR	79 (84)	53 (60.6)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Lee et al.[21] 2010	5 (29.3%)	15 (44.1)	NR	NR	3 (17.7)	2 (5.9)	2 (11.7)	6 (17.7)	12 (70.6)	20 (58.8)	NR	NR	NR	NR	5 (29.4)	14 (41.2)	NR	NR
Lee et al.[20] 2014	36 (27.9)	30 (30.0)	7 (5.4)	9 (9.0)	6 (4.7)	2 (2.0)	14 (10.9)	16 (16.0)	98 (76)	65 (65)	NR	NR	1 (0.7)	0	29 (22.4)	33	1 (0.7)	2 (2)
Shin et al.[16] 2019	6 (19)	8 (26)	2 (6)	3 (1)	4 (12)	7 (23)	19 (61)	12 (40)	25 (80)	21 (67)	5 (16)	8 (26)	1 (3)	1 (3)	NR	NR	12 (38)	7 (23)
Hoyer-Sørensen et al.[17] 2012	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	17 (85)	18 (90)	NR	NR	NR	NR	NR	NR
Wang et al.[19] 2016	27 (27.8)	19 (18.3)	14 (14.4)	24 (23.1)	NR	NR	41 (40.8)	57 (54.8)	53 (53.5)	49 (47.1)	6 (6.1)	6 (5.8)	NR	NR	13 (13.1)	8 (7.7)	2 (2.0)	2 (1.9)
Yim et al.[22] 2013	34 (30.9)	33 (30.8)	11 (10.0)	9 (8.4)	8 (7.3)	6 (5.6)	47 (42)	48 (43)	81 (73.6)	71 (67.0)	28 (25.5)	34 (32.1)	1 (0.9)	1 (0.9)	NR	NR	NR	NR

LESS: Laparoendoscopic single-site surgery, CLS: Conventional laparoscopy

Table 4. Quality assessment for the included observational studies

Study ID	Bedaiwy et al.[4] 2012	Im et al.[18] 2011	Karasu et al.[23] 2017	Kim et al.[24] 2012	Lee et al.[21] 2010	Lee et al.[20] 2014	Wang et al.[19] 2016	Yim et al.[22] 2013
1. Was the research question or objective in this paper clearly stated?	1	1	1	1	1	1	1	1
2. Was the study population clearly specified and defined?	1	1	1	1	1	1	1	1
3. Was the participation rate of eligible persons at least 50%?	1	1	1	1	0	1	1	1
4. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?	0	0	1	1	1	1	1	1
5. Was a sample size justification, power description, or variance and effect estimates	0	0	0	0	0	0	0	0
6. For the analyses in this paper, were the exposure (s) of interest measured prior to the outcome(s) being measured?	1	1	1	1	1	1	1	1
7. Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?	1	1	1	1	1	1	1	1
8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?	1	1	1	1	1	1	1	1
9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	1	1	1	1	1	1	1	1
10. Was the exposure(s) assessed more than once over time?	0	0	0	0	0	0	0	0
11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	1	1	1	1	1	1	1	1
12. Were the outcome assessors blinded to the exposure status of participants?	*	*	*	*	*	*	*	*
13. Was loss to follow-up after baseline 20% or less?	1	1	1	1	1	1	1	1
14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?	1	1	0	1	1	1	1	1
Total score (out of 14)	10/14	10/14	10/14	11/14	10/14	11/14	11/14	11/14

present (p=0.01, I²=92%), which we could not solve, as seen in Figure 5.

4. VAS Pain Score 24 hrs After Surgery

This outcome was reported by five studies[16-18,21,22]. There was no significant difference found between the two groups regarding the measured VAS score [MD=0.23 (-0.09, 0.56), (p=0.16)]. Our analysis of the data revealed considerable heterogeneity (p<0.005); I²=74%. The heterogeneity was solved by the exclusion of Shin et al.[16] [MD=0.41 (0.26, 0.56) p=0.01], I²=1%, as seen in Figure 6.

5. VAS Pain Score 6 hrs After Surgery

Both procedures were associated with similar pain scores six hours after surgery [MD=0.15 (-0.04, 0.33), (p=0.12)]. Our analysis of the data was homogeneous (p=0.12), I²=27%, as seen in Figure 7.

6. Analgesic Use

The incidence of requiring analgesia in the postoperative period was significantly lower in the LESS group than in the CLS group. RR=0.47 (0.32, 0.68), p=0.001. The pooled analysis was homogenous (p=023); I²=29%, as seen in Figure 8.

7. Change in Hemoglobin (HGB) Level

The outcome was reported by five studies[16,18,20-22]. Both groups were associated with comparable decreases in HGB with a homogenous analysis [MD=-0.11 (-0.26, 0.03) (p=0.14)], as seen in Figure 9.

8. Perioperative Complications

Six of the included studies evaluated the perioperative complications of both procedures. The incidence of perioperative complications was significantly higher in the LESS group than the CLS group [RR=2.236 (1.031, 4.851),

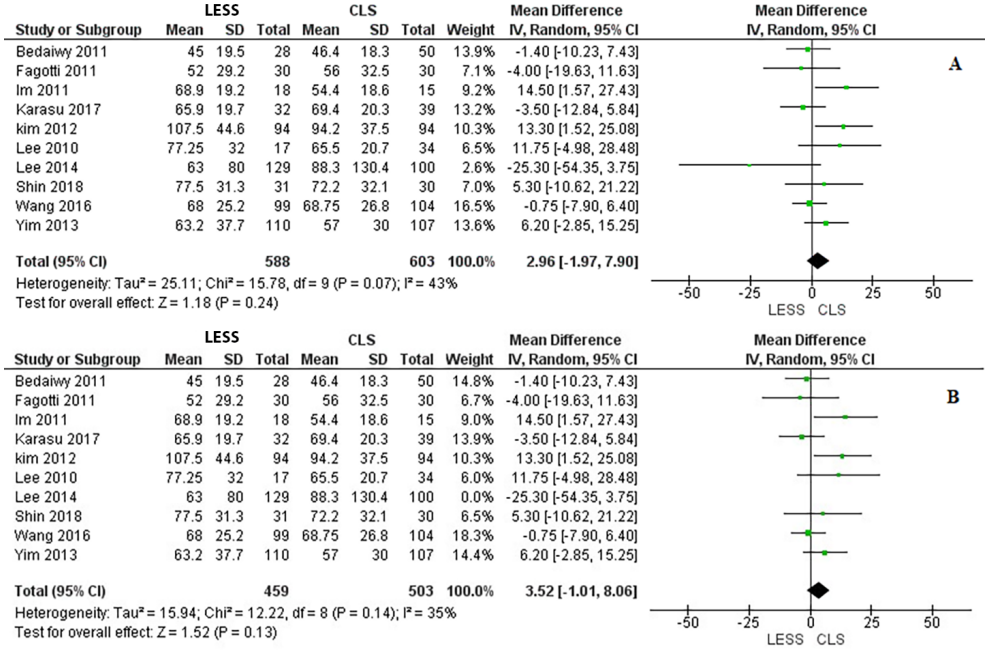

Figure 3. Meta-analysis of the total operative time

LESS: Laparoendoscopic single-site surgery, CLS: Conventional laparoscopy, CI: Confidence interval, SD: Standard deviation, IV: Inverse variance

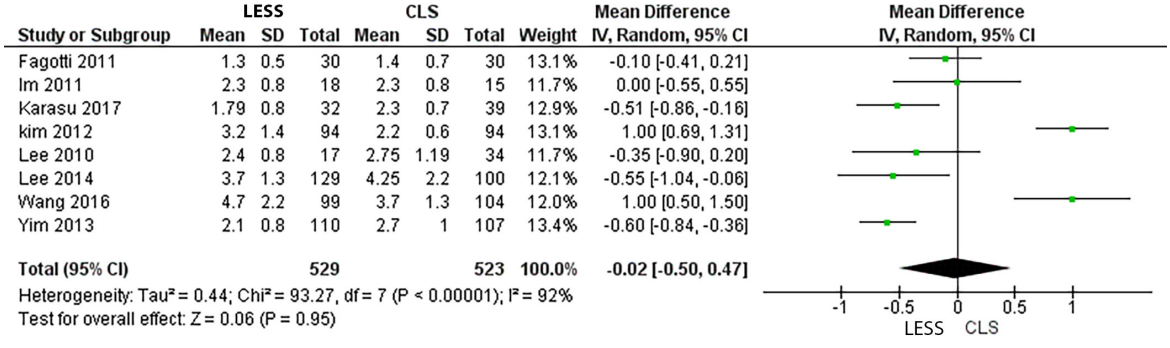

Figure 4. Meta-analysis of the length of hospital stay (in days)

LESS: Laparoendoscopic single-site surgery, CLS: Conventional laparoscopy, CI: Confidence interval, SD: Standard deviation, IV: Inverse variance

p=0.04]. The pooled analysis was homogeneous (p=0.9; I²=0%), as seen in Figure 10.

9. BMI and Previous Abdominal Surgery

We compared the BMI of the included cases, as well as the incidence of previous abdominal surgery, between the two procedures to determine if these factors could have affected the reliability of our analysis. We found that both BMI [MD=-0.07 (-0.48, 0.34), (p=0.74), I²=0%] and the history of previous abdominal surgeries [RR=1.16 (0.97, 1.38), (p=0.10), I²=0%] were nearly identical between the two groups, as seen in Figures 11 and 12.

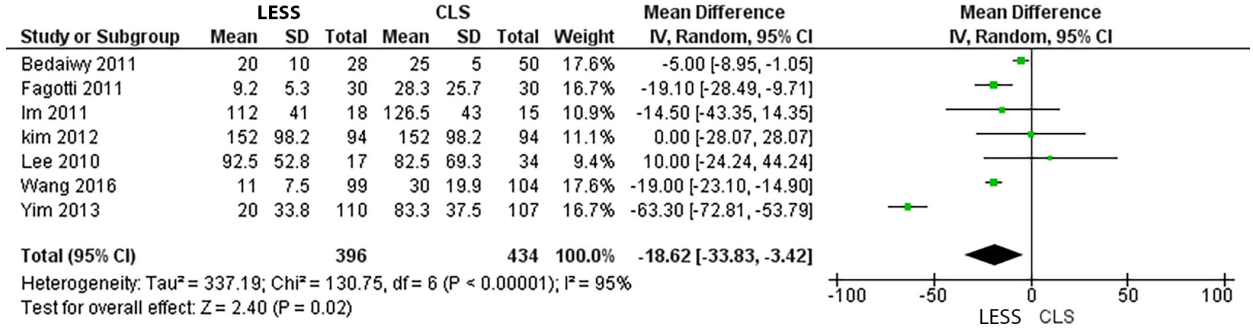

Figure 5. Meta-analysis of estimated blood loss

LESS: Laparoendoscopic single-site surgery, CLS: Conventional laparoscopy, CI: Confidence interval, SD: Standard deviation, IV: Inverse variance

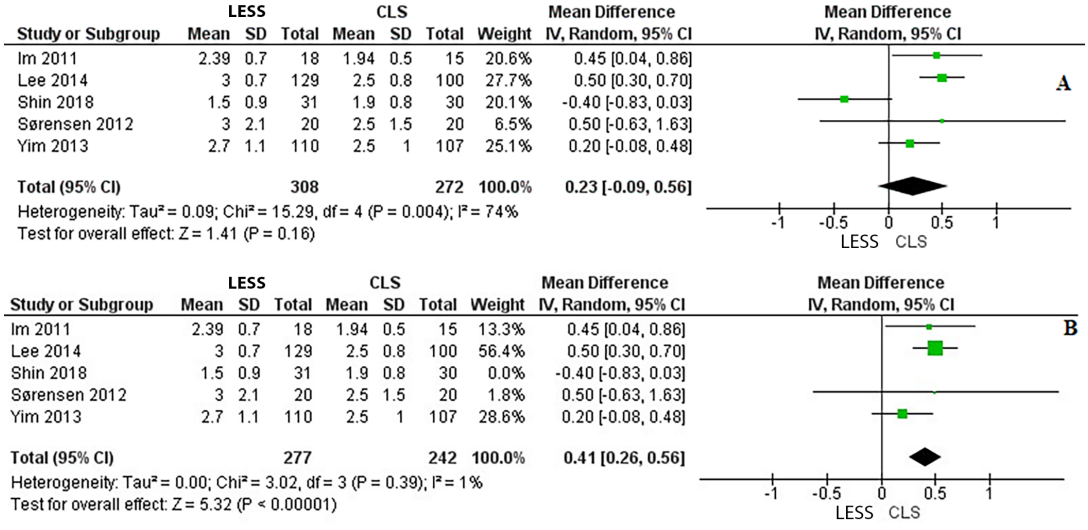

Figure 6. Meta-analysis of VAS pain scores at 24 hours after surgery

LESS: Laparoendoscopic single-site surgery, CLS: Conventional laparoscopy, CI: Confidence interval, SD: Standard deviation, VAS: Visual analog scale, IV: Inverse variance

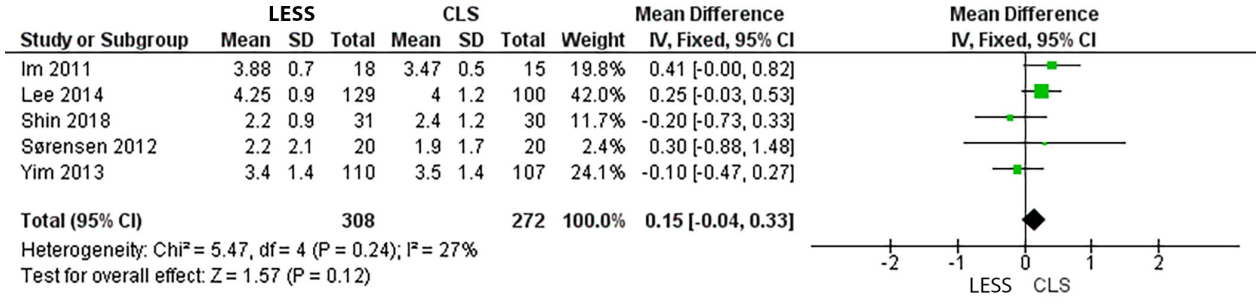

Figure 7. Meta-analysis of VAS pain scores at 6 hours after surgery

LESS: Laparoendoscopic single-site surgery, CLS: Conventional laparoscopy, CI: Confidence interval, SD: Standard deviation, IV: Inverse variance

Discussion

In our study comparing LESS and CLS in adnexectomy for benign adnexal disease, we focused on several efficacy and safety outcomes. As for the difference in operative time, LESS required slightly more time than CLS. The EBL was significantly reduced in the LESS compared with CLS. Hospital stays and VAS pain scores at 24 and 6 hours were similar between the two techniques. Analgesic use postoperatively was significantly lower in the LESS group. The change in HGB levels was comparable between the groups. However, the incidence of perioperative complications was significantly higher in the LESS group. This

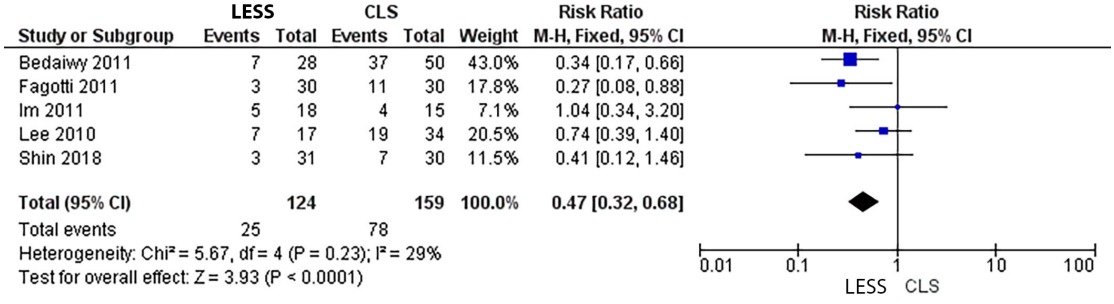

Figure 8. Meta-analysis of opioid analgesia usage in the postoperative period

LESS: Laparoendoscopic single-site surgery, CLS: Conventional laparoscopy, CI: Confidence interval, SD: Standard deviation

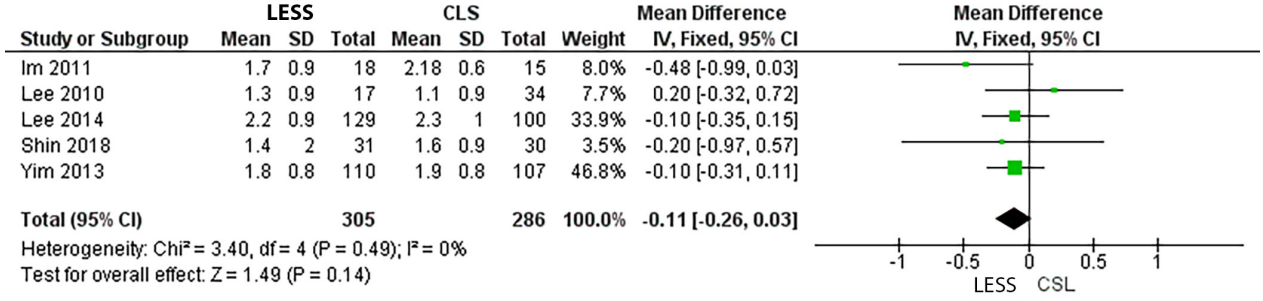

Figure 9. Meta-analysis of the change in hemoglobin postoperatively (in g/dL)

LESS: Laparoendoscopic single-site surgery, CLS: Conventional laparoscopy, CI: Confidence interval, SD: Standard deviation, IV: Inverse variance

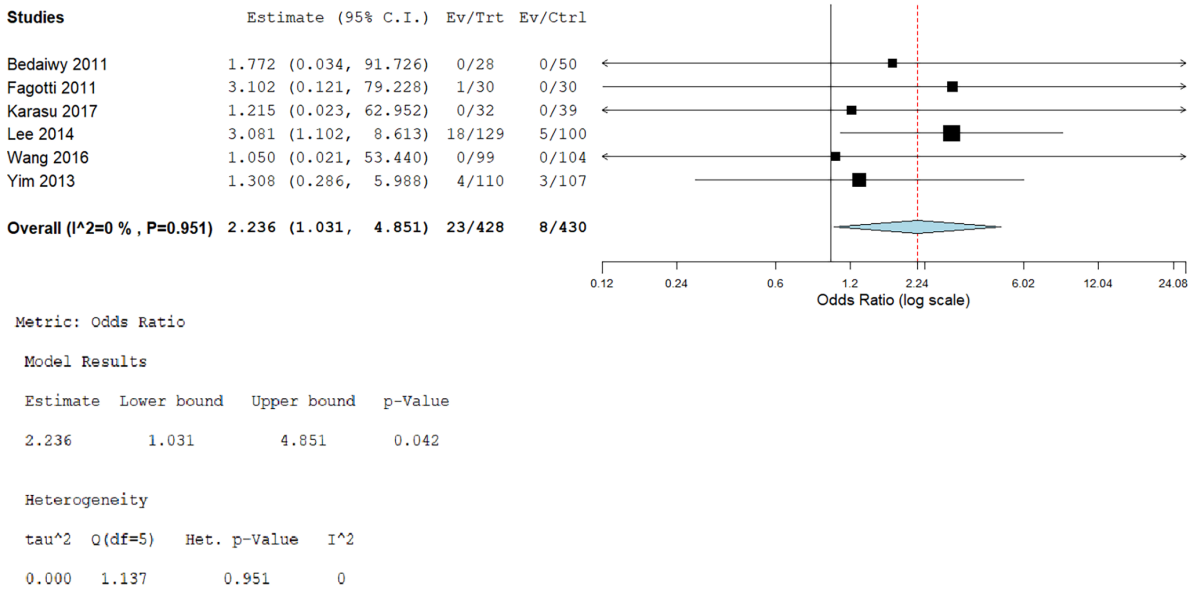

Figure 10. Meta-analysis of the perioperative complication rate

CI: Confidence interval

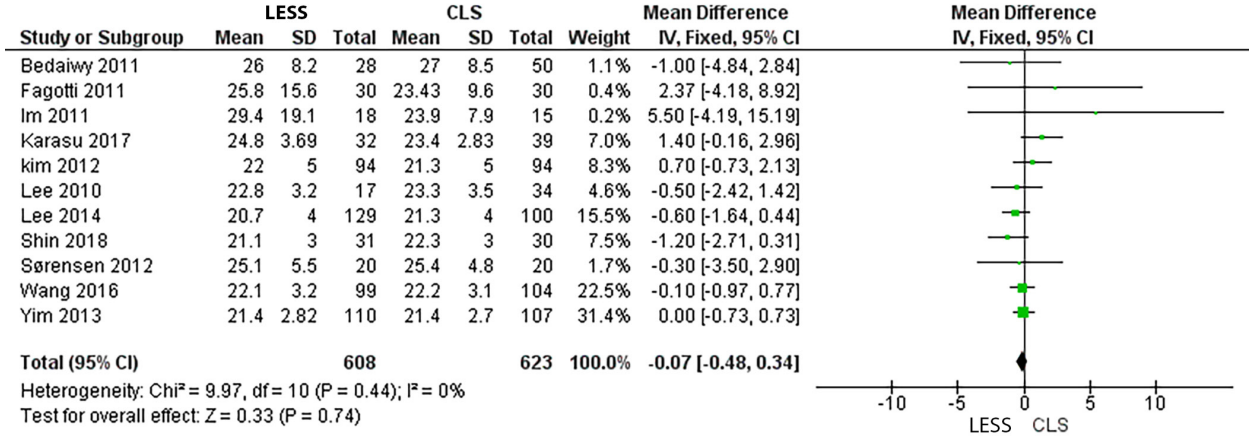

Figure 11. Meta-analysis of body mass index

LESS: Laparoendoscopic single-site surgery, CLS: Conventional laparoscopy, CI: Confidence interval, SD: Standard deviation, IV: Inverse variance

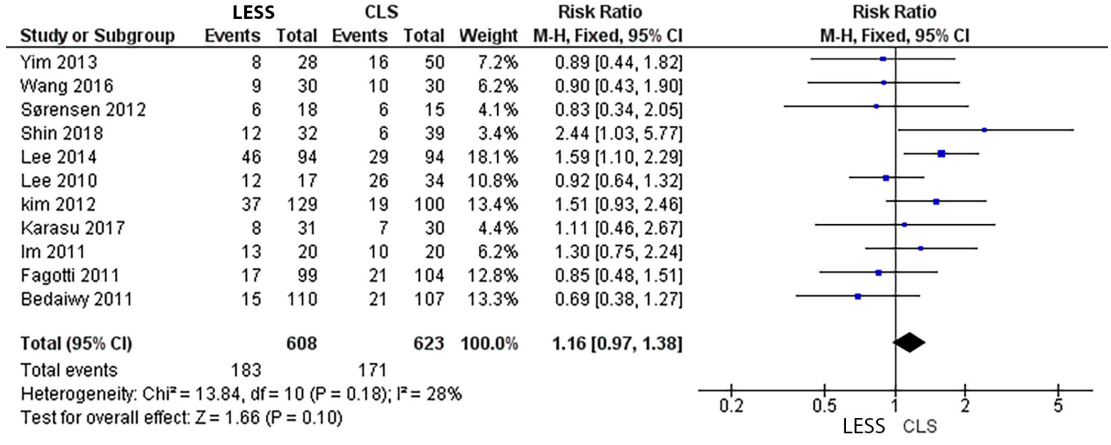

Figure 12. Meta-analysis of patient history of previous abdominal surgery

LESS: Laparoendoscopic single-site surgery, CLS: Conventional laparoscopy, CI: Confidence interval, SD: Standard deviation, IV: Inverse variance

comprehensive comparison highlights that while LESS may offer benefits such as reduced blood loss and analgesic use, it also presents challenges including longer operative times and higher complication rates. This may challenge the feasibility of LESS in some situations.

Salpingectomy using LESS was first performed by Ghezzi et al.[25] in 2005, but has not been completely implemented, likely due to the technical difficulties encountered. Innovations in techniques and devices have expanded single port applications to various gynecological procedures[26]. Some of the limitations of LESS include reduced triangulation, instrument interference, and reduced visualization. Considering these limitations, this consideration may explain why the incidence of complications after LESS adnexectomy was higher than with CLS as reported by our analysis. These issues can make it more difficult for surgeons compared to standard laparoscopy, and present a steeper learning curve for surgeons in training[27]. Therefore, patient selection may be key in certain circumstances[28]. Patients with smaller adnexal masses, normal BMI and without an extensive history of abdominal surgery may be preferred[29]. However, the current study did not identify significant differences in the above patient characteristics between the LESS and conventional laparoscopy groups, and therefore did not find evidence of patient selection bias affecting results. We performed an analysis comparing BMI and history of previous operations, ensuring there was no significant baseline difference between the LESS and CLS groups. This indicates that disease and patient features do not necessarily limit the applicability of LESS[26].

The most recent meta-analysis on this topic, Lin et al.[30], also found an increase in perioperative complications in the LESS group. This study differed from our study in that it was compelled to include ovarian cystectomy surgeries because of the limited number of studies available at that time dealing with adnexectomy. The fact that our study also shows an increase in perioperative complications seems to convincingly suggest

that LESS is more dangerous than CLS for adnexectomy. Furthermore, it is possible that more of the risk that was found in Lin et al.[30] came from the adnexectomy studies than from the cystectomy studies.

In addition to increased complications, LESS was found to have a longer operative time compared to CLS. Long operative time results in increased time spent under pneumoperitoneum and anesthesia and raises the risk of postoperative complications including paralytic ileus[31]. Jeung et al.[32] concluded that it was significantly more common for a postoperative ileus to occur in patients who underwent laparoendoscopic single port hysterectomy with operative times >150 minutes, whereas no ileus occurred during surgeries lasting ≤150 minutes. While this topic remains controversial and requires further investigation, it suggests a potential relationship between ileus and LESS.

Another point of view was reported in a recent case series by Fagotti et al.[6] and Escobar et al.[33], who sought to establish the feasibility of LESS for performing salpingo-oophorectomy in patients with *BRCA* gene mutations for the purpose of cancer risk reduction. Regarding LESS, they found the surgical competency can be attained in 10-15 cases, with a mean operative time of 38.1 minutes. This indicates that LESS may be as safe or safer than CLS in certain patient subgroups.

Study Limitations

This meta-analysis has several limitations. We could only find three RCTs, and they had a relatively small sample size. This creates a ROB. To overcome this, we included RCTs and non-RCTs in our study to achieve a larger sample size and greater statistical power. The resulting evidence was then highly heterogeneous, likely secondary to the differences concerning the tumor types, their size, the age of the patient, indications for surgery, and criteria used for matching. Unfortunately, we could not subgroup by the histologic type of ovarian mass, as very few studies gave data on this parameter.

Conclusion

Compared with CLS, LESS needed more operative time, but offered significantly less estimated blood loss. Hospital stays and VAS pain scores at 6 and 24 hours postoperatively were similar between the two techniques. LESS resulted in significantly lower postoperative analgesic use and comparable changes in HGB levels. However, the incidence of perioperative complications was higher in the LESS group. These findings challenge the feasibility and safety of LESS for adnexectomy when compared to CLS.

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Footnotes

Authorship Contributions

Surgical and Medical Practices: G.J.M., A.Az., Concept: G.J.M., Design: G.J.M., B.H., Data Collection or Processing: H.U., A.A., D.G., B.H., K.R., M.D., Analysis or Interpretation: K.R., A.Az., Literature Search: H.U., A.A., D.G., B.H., K.R., M.D., Writing: G.J.M.

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

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Guideline / Kılavuz

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PERİDER-TJOD joint review on threatened abortion and guideline for its treatment

PERİDER-TJOD düşük tehdidi ve tedavisi kılavuzu

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Abstract

Objective: Although there are several guidelines in the literature on "recurrent abortion", there is no comprehensive guideline on "threatened abortion". The overall purpose of this guideline is to provide healthcare providers with the best available evidence for examination and treatment of pregnant women with threatened abortion.

Materials and Methods: The scope of the guideline and the first version of the questions were prepared by the Perinatology and High Risk Pregnancies Association (PERİDER) guideline development group in January 2024. Meetings were held to discuss key questions and redefine them. A final list of 8 key questions was created. Keywords were defined for each question and ranked in order of importance and used in searches for all English-language publications in PubMed/Medline and Cochrane libraries. These databases were thoroughly scanned for publications that were published until February 1, 2024. Literature reviews were conducted as an iterative process. In the first step, systematic reviews and meta-analyses were collected. If no results were found, the research was expanded to randomized controlled trials and then to cohort studies and case reports, following the hierarchy of evidence levels.

Results: This guideline was presented to the board of directors of the Turkish Gynecology and Obstetrics Society (TJOD). With their suggestions, guideline was finalized, and it was decided to be published as a joint guideline of PERİDER-TJOD.

Conclusion: This guideline provides an overview of threatened abortion and the recommended treatments. In addition, by recognizing the deficiencies in the literature, suggestions were made regarding research that could help clinicians' decisions in the future.

Keywords: Threatened abortion, progesterone, threatened miscarriage

Öz

Amaç: Literatürde "tekrarlayan düşük" ile ilgili çok sayıda kılavuz bulunmakla birlikte, "düşük tehdidi" ile ilgili kapsamlı bir kılavuz bulunmamaktadır. Bu kılavuzun genel amacı, sağlık hizmeti sağlayıcılarına düşük tehdidinde muayene ve tedavi için mevcut en iyi kanıtları sağlamaktır.

Gereç ve Yöntemler: Kılavuzun kapsamı ve soruların ilk versiyonu Ocak 2024'te Perinatoloji ve Riskli Gebelikler Derneği (PERİDER) kılavuz geliştirme grubu tarafından hazırlandı. Temel soruların tartışılması ve yeniden tanımlanması için toplantılar yapıldı ve 8 sorudan oluşan son liste oluşturuldu. Her soru için anahtar kelimeler belirlenerek önem sırasına göre sıralandı ve PubMed/Medline ve Cochrane kütüphanelerindeki tüm İngilizce yayınlar için yapılan aramalarda kullanıldı. Bu veri tabanları, 1 Şubat 2024 tarihine kadar yayımlanan yayınlar için kapsamlı bir şekilde tarandı. Literatür taramaları

PRECIS: The overall purpose of this guideline is to provide healthcare providers with the best available evidence for examination and treatment of pregnant women with threatened abortion.

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yinelemeli bir süreç olarak yürütüldü. İlk adımda, sistematik derlemeler ve meta-analizler incelendi. Sonuç bulunamazsa, araştırma randomize kontrollü çalışmalara ve daha sonra kanıt düzeyi hiyerarşisini takip ederek kohort çalışmalarına ve olgu raporlarına kadar genişletildi.

Bulgular: Bu kılavuz hazırlanarak Türk Jinekoloji ve Obstetrik Derneği (TJOD) yönetim kuruluna sunuldu. Onların da önerileri ile kılavuza son şekli verildi ve PERİDER-TJOD ortak kılavuzu olarak yayınlandı.

Sonuç: Bu kılavuz, düşük tehdidine ve önerilen tedavilere genel bir bakış sunmaktadır. Ayrıca literatürdeki eksiklikler fark edilerek, gelecekte klinisyenlerin kararlarına yardımcı olabilecek araştırmalara ilişkin önerilerde de bulunulmuştur.

Anahtar Kelimeler: Düşük tehdidi, progesteron, gebelik kaybı

Disclaimer

As Perinatology and High Risk Pregnancies Association (*Perinatoloji ve Riskli Gebelikler Derneği* - PERİDER), we developed the current clinical practice guideline to provide clinical recommendations in Türkiye, and the world to improve the quality of healthcare delivery to patients with threatened abortion. This guide represents the views of PERİDER obtained after careful consideration of the scientific evidence available at the time of its preparation. Due to the lack of sufficient scientific evidence on some issues, a consensus has been reached among the relevant PERİDER members. The purpose of clinical practice guidelines is to assist healthcare professionals in day-to-day clinical decisions regarding the appropriate and effective care of their patients. However, adherence to these clinical practice guidelines does not guarantee a successful or specific outcome or establish a standard of care. Clinical practice guidelines do not override the clinical judgment of the healthcare professional in the diagnosis and treatment of patients. Healthcare professionals should make their decisions on a case-by-case basis, using their own knowledge and skills and clinical reasoning. They should take into account the situation, circumstances, and wishes of each patient, and consult with the patient or, as appropriate, her guardian.

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About the Guideline

Although there are several guidelines in the literature on the recurrent abortion, there is no comprehensive guideline on "threatened abortion", so as PERİDER, we decided to prepare this guideline in 2024. The guideline development group selected from PERİDER determined the questions that needed to be answered in the guideline. They did a thorough literature review and created a guideline by reaching a consensus through regular meetings.

This guideline, prepared by PERİDER, was presented to the board of directors of the Turkish Gynecology and Obstetrics Society (*Türk Jinekoloji ve Obstetrik Derneği* - TJOD). With their suggestions, the guideline was finalized, and it was decided to be published as a joint guideline between PERİDER and TJOD.

The overall purpose of this guideline is to provide healthcare providers with the best available evidence for examination and treatment of pregnant women with threatened abortion.

This guideline provides an overview of threatened abortion and the recommended treatments. In addition, by recognizing the deficiencies in the literature, suggestions were made regarding research, that could help clinicians' decisions in the future.

This guideline is presented in two parts. In the first section, basic concepts and definitions regarding threatened abortion are summarized. Several up-to-date references have been used in the compilation of this part. In the second part, the main clinical questions, comprehensive literature data, and recommendations, especially for the treatment of threatened abortion, are included.

The scope of the guideline and the first version of the questions were prepared by the PERİDER guideline development group in January 2024. Guideline development group meetings were held to discuss key questions and redefine them. As a result of the meetings, a final list of 8 key questions was created. Keywords were defined for each question, ranked in order of importance, and used in searches for all English-language publications in PubMed/Medline and Cochrane libraries. These databases were thoroughly scanned for publications up to February 1, 2023. Literature reviews were conducted as an iterative process. In the first step, systematic reviews and meta-analyses were collected. If no results were found, the research was expanded to randomized controlled trials and then to cohort studies and case reports, following the hierarchy of evidence levels.

Part 1: Basic Concepts

What is miscarriage?

Miscarriage (Spontaneous Abortion) is the loss of pregnancy up to the 20th week of pregnancy. The World Health Organization also considers the loss of a fetus weighing less than 500 grams as an abortion. The most important symptoms in abortion are bleeding and pain; therefore, abortion can be mistaken for ectopic pregnancy or molar pregnancy[1].

What is threatened abortion?

Threatened abortion is bleeding in early pregnancy with no evidence of pregnancy loss[1].

What are the types of abortion?

Anembryonic pregnancy: It is a pregnancy that is not viable with the presence of a gestational sac without an embryo or yolk sac. In the past, the term "***blighted ovum***" was also used.

Recurrent pregnancy loss: It is the loss of two or more spontaneous pregnancies.

Septic abortion: A spontaneous or provoked abortion associated with a uterine infection.

Incomplete abortion: It is the presence of products of conception in the uterus after the diagnosis of pregnancy loss.

Inevitable abortion (abortus incipiens): Occurs when the abortion cannot be prevented as the cervix is open, accompanied by bleeding and pain. It is an old term that is not used much today.

Missed abortion: It is the loss of a fetus without symptoms. It can also be called asymptomatic pregnancy loss[2].

What is the frequency of threatened abortion in the world and in Türkiye?

Threatened abortion is an important cause of early pregnancy bleeding. Implantation bleeding, cervical lesions, and ectopic pregnancy should be kept in mind in the differential diagnosis of bleeding in the first trimester.

Although the studies are heterogeneous, in one study bleeding in the first trimester was observed in approximately 25% of pregnant women. In the same study, the rate of pregnancy loss was observed as 12% in pregnant women with bleeding in the first trimester[3]. In another observational study, bleeding was observed in 21% of pregnant women before 20 weeks of pregnancy and pregnancy loss was observed in 50% of this group[4].

There is no comprehensive epidemiological study on the incidence of threatened abortion in Türkiye.

What are the risk factors for threatened abortion?

Maternal Age: The risk of miscarriage varies with maternal age. There is a strong correlation between advanced maternal age (>35 years) and fetal chromosomal abnormalities[5]. Risk of miscarriage increases with advanced maternal age, and adolescent pregnancies are also a risk factor.

Paternal Age: The risk of pregnancy loss increases in advanced paternal age. However, this risk rate is lower than that of maternal age[6].

Previous pregnancy loss: Previous pregnancy loss increases the risk of loss in subsequent pregnancies. In one study the number of pregnant women with a history of at least one pregnancy loss was reported to be higher[7].

Maternal diseases: These are factors whose effects decrease when they are well controlled.

1. Some systemic infections (malaria, brucellosis, CMV, and HIV, travel sickness, influenza virus) and bacterial vaginosis increase the risk of miscarriage[8].
2. Pregnancy loss increases in case of diabetes, hypo/hyperthyroidism, obesity and chronic stress[9].
3. In women who become pregnant in the presence of an intrauterine device (IUD), the rate of miscarriage increases if the IUD is not removed[9].

Substance use: Smoking, alcohol, and drug use increase the risk of pregnancy loss[2].

Environmental factors: Exposure to excessive lead, arsenic, air pollution, and radiation are also associated with an increase in pregnancy loss[2].

Subchorionic hemorrhage: In the presence of subchorionic hemorrhage, the risk of pregnancy loss is two times higher[10].

Which etiologies should be considered in pregnant women with bleeding in the first trimester?

- Implantation Bleeding: A small amount of bleeding on the 10^{th}-14^{th} day of fertilization, spotting
- Threatened abortion
- Miscarriages (missed abortus, abortus incipiens)
- Ectopic pregnancy
- Pregnancy + IUD
- Pregnancy + Arteriovenous malformation
- Gestational trophoblastic disease (molar pregnancy)
- Genital infections
- Cervical and vaginal pathologies: Cervical polyp or myoma, cervical ectropion, cervical cancer, lacerations, disseminated vaginal condyloma acuminata[11,12].

How is threatened abortion diagnosed?

Vaginal spotting or bleeding can occur in approximately 25% of pregnancies in the first trimester. First trimester bleeding is often due to damage to the blood vessels in the decidua during implantation, or it can be caused by cervical or vaginal lesions. The diagnosis can be made by excluding other conditions in the differential diagnosis, by looking at the gestational week, bleeding severity and characteristics (spotting, mild or severe, intermittent or continuous, painful or painless). Initial diagnosis may be supported or revised after physical examination, laboratory tests, and imaging methods[3].

What are the symptoms suggestive of threatened abortion?

Symptoms such as bleeding and cramping are the most common in threatened abortion. A decrease in nausea and vomiting may also be seen[4,12,13].

Should pelvic and speculum examinations be performed in the diagnosis of threatened abortion?

A speculum examination is necessary during the physical examination. Bimanual pelvic and speculum examination allows one to distinguish between threatened abortion, inevitable miscarriage (abortus incipiens), and incomplete abortion.

In ectopic pregnancies, cervical and adnexal tenderness or a mass may be detected. Viewing the cervix with a speculum allows for estimating the severity of bleeding and removing any endocervical conception products. Rarely, cervical lesions and masses that may cause bleeding can also be identified[14].

What is the role of ultrasonography (USG) in the diagnosis of threatened abortion?

How often should USG be done?

Symptoms such as vaginal bleeding and uterine cramps can be observed in normal, ectopic and molar pregnancies, as well as early pregnancy loss. Therefore, it is important to distinguish threatened abortion from other early pregnancy complications. A thorough medical history and physical examination, along with a USG and, as the case may be, serum beta-hCG testing, can be helpful in making a diagnosis. If possible, USG should be performed to confirm the presence of a live intrauterine pregnancy. After it is determined that the pregnancy is intrauterine, USG can be considered again in case of findings such as severe bleeding, hemodynamic instability, and abdominal tenderness on physical examination. Otherwise, routine pregnancy follow-up protocols should be applied[15,16].

When can a gestational sac first be seen in USG?

With a transvaginal USG, the gestational sac can be seen between weeks 4.5-5 of gestation at the earliest according to the last menstrual date. These structures may be noticed slightly later in the transabdominal USG examination[17].

What is the minimum beta-hCG level necessary for the gestational sac to be seen with transvaginal and transabdominal USG?

In a retrospective study, the lowest serum beta-hCG level was measured using transvaginal USG as 390 mIU/mL in a live intrauterine pregnancy. The minimum threshold values for imaging the yolk sac and fetus using transvaginal USG were 1,094 mIU/mL and 1,394 mIU/mL, respectively[18]. However, in clinical practice, the widely accepted serum beta-hCG level for which the gestational sac can be visualized with transvaginal USG is 1,500-2,000 mIU/mL[19]. When beta-hCG level reaches 6,500 mIU/mL, the gestational sac can be visualized using transabdominal USG[20]. However, current studies show that as a result of developments in USG technology, values can be reduced to much lower limits[18].

At which gestational weeks are the yolk sac and embryo typically seen?

With transvaginal USG, the yolk sac is typically monitored between the 5th and 6th weeks of pregnancy, and with transvaginal USG, the measurable embryo is monitored between the 6th and 7th weeks according to the last menstrual period[21].

What is the minimum gestation time, embryo size and gestational sac size expected for fetal cardiac activity?

Based on the date of the last menstrual period, embryonic cardiac activity is expected to be monitored with transvaginal USG around the 6th week of pregnancy. Fetal cardiac activity should be observed when the embryo reaches ≥7 mm in size or when the gestational sac size is ≥25 mm[21].

What are the USG criteria for the diagnosis of a nonviable pregnancy?

There are strict criteria for the diagnosis of a nonviable pregnancy:

- No fetal cardiac activity when the embryos head-rump distance is ≥7 mm
- Failure to monitor the embryo when the gestational sac is ≥25 mm in size
- In an early pregnancy in which a gestational sac was observed but no yolk sac was observed, an embryo with a heartbeat could not be monitored in the control performed 2 weeks later.
- Failure to monitor an embryo with a heartbeat after ≥11 days in early pregnancy in which the yolk sac was also observed in the gestational sac[21].

What is the role of laboratory tests in the diagnosis of threatened abortion?

How often is beta-hCG testing recommended for a woman with vaginal bleeding and pain to make a differential diagnosis of threatened abortion and ectopic pregnancy?

In cases where intrauterine and ectopic pregnancies cannot be differentiated with transvaginal USG, beta-hCG follow-up at 48-hour intervals may be beneficial. An increase of more than 50% in beta-hCG levels at this 48-hour interval can be interpreted in favor of an intrauterine pregnancy. The expected rate of increase also differs according to the initial beta-hCG level. The baseline beta-hCG value shows different percentages depending on the concentration: 49% if it is <1,500 mIU/mL, 40% if it is in the range of 1,500-3,000 mIU/mL, and 33% if it is in the range of >3,000-<10.000 mIU/mL[22].

Is serum progesterone level useful in the diagnosis of threatened abortion?

Although it has been used in the past, the use of serum progesterone levels in cases with no observed intrauterine gestational sac is controversial and has not found a routine place in clinical practice. The difference in serum progesterone levels between spontaneous pregnancies and assisted reproductive technology pregnancies also limits their use in the diagnosis of threatened abortion.

In a recent meta-analysis, a one-time serum progesterone measurement of <12 ng/mL in the first trimester was found to be effective in predicting the probability of a miscarriage

in pregnant women with threatened abortion[23]. Therefore, measurement of serum progesterone levels can be considered to predict the prognosis in pregnant women with threatened abortion rather than diagnosing it.

What are the possible obstetric consequences of a pregnancy with threatened abortion?

Bleeding and cramps are common in early pregnancy. Studies have shown that about 25% of pregnant women experience vaginal bleeding before the 20th week of pregnancy, and between 12% and 57% of these pregnant women will eventually experience early pregnancy loss. Subchorionic hematomas are associated with threatened abortion; risk of early pregnancy loss is higher in pregnancies with large subchorionic hematomas, but the presence of a subchorionic hematoma does not increase birth complications in ongoing pregnancies[24]. Heavy bleeding, especially when accompanied by pain or cramping, has a significantly worse prognosis than light bleeding or spotting. In addition, pregnant women with heavy vaginal bleeding should be evaluated for hemorrhagic anemia; this is usually a precursor of early pregnancy loss and is associated with a poor prognosis[25,26].

In pregnancies with threatened abortion, the risk of adverse outcomes such as pregnancy loss, premature rupture of membranes, premature birth, fetal growth restriction, placental abruption, cesarean delivery, postpartum uterine atony, and the need for neonatal intensive care increases in the later stages of pregnancy[27]. In addition, women who experienced an early pregnancy loss had an increased risk of depression, sleep disturbance, feelings of anger and guilt[28,29].

Part 2: Treatment of Threatened Abortion

Is bed rest and/or hospitalization beneficial in the treatment of threatened abortion?

High-quality evidence supporting bed rest to prevent miscarriages in women with confirmed fetal viability and vaginal bleeding in the first half of pregnancy is insufficient[30]. Bed rest does not improve outcomes and may cause psychological harm to pregnant women who later experience early pregnancy loss[31]. Since most cases of early pregnancy losses are due to chromosomal or fetal anomalies, activity restriction is unlikely to affect the final outcome of these pregnancies[32].

The effectiveness of bed rest to prevent early pregnancy loss in pregnant women experiencing threatened abortion has not been proven[25,33-35]. While there are no randomized controlled trials or meta-analyses proving that bed rest improves patient outcomes, there is only one retrospective study suggesting its effectiveness. In this study, women who adhered to bed rest had fewer spontaneous abortions (9.9% *vs.* 23.3%, p=0.006) and a higher rate of term pregnancy (89% *vs.* 70%, p=0.004) than those who did not. However, large, prospective, randomized studies are needed to confirm whether bed rest has a true therapeutic effect (Table 1)[36].

Therapeutic bed rest continues to be widely used, even though it has no benefits and has known harms. The benefit of bed rest was only demonstrated in a single non-controlled non-randomized study[31].

Since differentiating the diagnosis of bleeding in the first trimester is not easy, hospitalization of pregnant women with unconfirmed diagnoses should be considered. However, there is not enough information in the literature to recommend hospitalization of pregnant women diagnosed with a threatened abortion.

Should restriction of sexual intercourse be recommended in threatened abortion?

Although sexual intercourse restriction is recommended in threatened abortion as a general practice in Türkiye, there is no study on this subject in the literature. Therefore, it is rational to recommend avoiding sexual intercourse during the acute bleeding period until randomized controlled trials are conducted, or until solid evidence is available.

Table 1. Bed rest at threatened abortion

Author, year	n	Study design	Treatment group	Control group	Efficacy of bed rest
Diddle et al.[33] 1953	9742	Prospective, observational	Bed rest ± sedation	Normal physical activity	No difference
Hamilton et al.[34] 1991	23	Randomized controlled	Bed rest	Normal physical activity	Three miscarriages were reported, but it was not specified which group they belonged to. The study has been terminated.
Harrison[35] 1993	61	Double-blind randomized controlled	Bed rest	Placebo and hCG injection	In the prevention of miscarriage, hCG treatment was found to be significantly better than bed rest
Ben-Haroush et al.[36] 2003	230	Retrospective analysis	Compliant with bed rest	Non-compliant with bed rest	Fewer spontaneous abortions (p=0.006) and more term pregnancies (p=0.004) were observed in those who were compliant with bed rest

Does natural progesterone therapy have a place in the treatment of threatened abortion?

The use of natural progesterone in threatened abortion has not been shown to be beneficial in increasing live birth rate[37-41]. In a randomized controlled trial, in those with a history of ≥1 miscarriage (from diagnosis to 16th gestation week), use of vaginal 400 mg progesterone, twice daily, has been shown to increase the rate of live birth[39]. In an interim analysis of another recent randomized controlled trial, it was concluded that the use of 400 mg vaginal progesterone did not change the live birth rate, even in those with a history of miscarriage, and the study was therefore terminated before the targeted number of patients was reached[38]. A study which shows that oral use of natural progesterone is more effective than vaginal use has a low level of evidence due to the small number of patients and it was not designed to be a double-blind study[42]. Similarly, there is a randomized controlled trial in the literature showing that the rate of miscarriage decreases with the use of vaginal progesterone, but the level of evidence is low due to the small sample size (30 controls, 30 patients)[43].

Therefore, use of natural progesterone is not recommended after the diagnosis of threatened abortion in pregnant women without a history of miscarriage. However, in pregnant women who have had a previous miscarriage, use of vaginal progesterone 400 mg twice daily, can be considered until the 16th week of gestation.

Does synthetic progesterone therapy have a place in the treatment of threatened abortion?

Two recent (2018 and 2021) meta-analyses on the use of progesterone in the treatment of threatened abortion with different designs have been published in the Cochrane Library[29,40]. In the 2018 analysis, data from 7 studies containing a total of 696 pregnant women were compiled. In threatened abortion, the use of progesterone was evaluated to be more effective in reducing the rate of miscarriage compared to placebo or control groups [relative risk (RR): 0.64, confidence interval (CI): 0.47-0.87]. When pregnant women who used oral progesterone were compared with those who did not receive treatment, the rate of miscarriage was reduced with oral progesterone (RR: 0.57; CI: 0.38-0.85); while there was no difference in those using vaginal progesterone compared to placebo (RR: 0.75; CI: 0.47-1.21). In this meta-analysis, it was reported that progesterone use did not increase the risk of congenital anomalies, although its evidence level was low (RR: 0.7, 95% CI: 0.1-4.82)[29]. In the more recent 2021 meta-analysis, data from 7 studies involving 5,682 pregnancies were examined[40]. Subgroup analysis was also conducted excluding cases with recurrent miscarriage. In these analyses comparing the effectiveness against placebo, data from two studies including 4,090 pregnant women for vaginal micronized progesterone and the data from one study including 406 pregnant women for dydrogesterone were evaluated. Vaginal natural progesterone did not provide a statistically significant reduction in the miscarriage rate compared to placebo (RR: 0.9, CI: 0.8-1.01). Similarly, no significant difference was observed in the data of the only study in which dydrogesterone was compared with placebo (RR: 0.9, CI: 0.55-1.47)[44]. In the only study comparing the effectiveness of oral micronized progesterone and dydrogesterone, no difference was found in the reduction of miscarriage rates (RR: 0.76, CI: 0.25-1.75)[45]. This review stated that there are no data available to evaluate the effectiveness of 17-α hydroxyprogesterone or oral micronized progesterone in threatened abortion[45].

In a recent study, an increased risk of malignancy was reported in individuals exposed to 17-α hydroxyprogesterone in the womb for the treatment of threatened abortion[46]. For this reason, the Türkiye Pharmaceuticals and Medical Devices Agency (*Türkiye İlaç ve Tıbbi Cihaz Kurumu*) suspended the licenses of drugs containing this molecule with a letter they published in 20.09.2024. As PERİDER, Due to this increased risk, we do not recommend the use of 17-α hydroxyprogesterone in the treatment of threatened abortion.

In addition, no increased risk of adverse effects and congenital anomalies has been detected with the use of progesterone (vaginal micronized, dydrogesterone), compared to placebo, but the level of evidence is low[40].

In another recent meta-analysis, data from 4907 pregnant women were examined[47]. In a subgroup analysis of this study (n=4833) in which threatened abortion treatments were evaluated, miscarriage rate was reduced with progesterone treatment, compared to placebo and control groups (RR: 0.7, CI: 0.52-0.95). However, in this meta-analysis, sub-analyses were not conducted in terms of progesterone form, dose, or application methods.

Another meta-analysis showed that the use of progesterone in threatened abortion significantly reduced the miscarriage rate compared to the control group (those receiving placebo and those receiving no treatment) (RR: 0.53, CI: 0.36-0.78). The least risk of miscarriage was detected in the oral dydrogesterone group (RR: 0.43, CI: 0.26-0.71). There was no significant difference in the risk of miscarriage between the use of vaginal micronized progesterone and the control group (RR: 0.72, CI: 0.39-1.34). In the subgroup analysis of two different studies comparing oral dydrogesterone and vaginal micronized progesterone treatments, no difference was found in terms of reducing the risk of miscarriage (RR: 1.06, CI: 0.42-2.66). As a result, progesterone therapy-especially oral dydrogesterone-has been reported to be effective in preventing pregnancy loss in women with threatened abortion[48].

In another meta-analysis that included 10 studies with a total of 5056 pregnancies, progesterone treatments were shown to reduce the risk of miscarriage, (RR: 0.73, CI: 0.59-0.92), but this benefit was detected only for oral progesterone (RR: 0.58, CI: 0.42-0.80) and was not observed for vaginal progesterone (RR: 0.90, CI: 0.80-1.01)[49].

A recent network meta-analysis including data of 10,424 pregnant women from 59 randomized controlled trials, in which threatened abortion was treated with progesterone, showed that oral dydrogesterone treatment was the most effective in preventing miscarriage (SUCRA 100%), followed by vaginal progesterone treatment (SUCRA 67.9%), while oral micronized progesterone treatment was the least effective (SUCRA 15.7%). As a result, it is reported that oral dydrogesterone treatment is the most effective treatment in threatened abortion and these results may help physicians in informing pregnant women and in making treatment choices[50].

In a prospective double-blind randomized controlled study including 50 pregnant women with threatened abortion, synthetic vaginal 8% 90mg, progesterone gel was compared with placebo, and it was reported that uterine contractility, pain, and miscarriage rates decreased significantly in the treatment arm[51].

In another randomized controlled study conducted in 2009, including 191 pregnancies, it was reported that dydrogesterone treatment (40 mg oral loading dose followed by 2x10 mg maintenance dose until the 16th week of gestation) in pregnant women with threatened abortion reduced the risk of miscarriage compared to placebo[52].

In a different randomized controlled study published in the same year, including 146 threatened miscarriages, dydrogesterone treatment (2x10 mg oral maintenance, until the end of one week after bleeding stops) reduced the miscarriage rate compared to placebo ($p \leq 0.05$), and no difference was found between the two groups in terms of congenital anomalies[53].

In another randomized controlled study published in 2005, where 154 pregnant women at risk of miscarriage were evaluated and it was found that the dydrogesterone group (40 mg oral loading dose followed by 2x10 mg maintenance dose until vaginal bleeding stops) resulted in fewer miscarriages compared to the control group without treatment ($p<0.05$)[54].

In a randomized controlled study in which 53 pregnant women with threatened abortion participated, the primary aim was to investigate the effects of progesterone use on utero-placental blood flow. The number of miscarriages was fewer with dydrogesterone (30 mg daily for 6 weeks) compared to micronized vaginal progesterone (300 mg daily for 6 weeks), but statistical analysis was not performed[55].

In a study published in 2021, in which dydrogesterone treatment (40 mg oral loading followed by 3x10 mg oral maintenance, continued until the 12th week of pregnancy or 1 week after bleeding stopped) was compared with placebo in threatened abortion (including 406 pregnant women), no difference was found between the groups in terms of the miscarriage rate ($p=0.772$)[44].

In another study from 2018, in which 118 pregnant women with threatened abortion were randomized to dydrogesterone (2x10 mg oral, 2 weeks) and micronized progesterone (2x200 mg oral, 2 weeks) groups, the effectiveness of these treatments was assessed. No significant difference was observed between the treatment groups, but side effects, such as dizziness and lightheadedness, were more frequently observed in the micronized progesterone group ($p=0.003$)[45].

In a prospective cohort study, 1,285 pregnant women with threatened abortion were examined, and no significant difference was found in miscarriage rates when dydrogesterone (40 mg, oral loading followed by 3x10 mg, oral maintenance for 2 weeks) and progesterone (2x100 mg, oral for 2 weeks) treatments were compared ($p=0.566$)[56].

The effectiveness of oral micronized progesterone is less than that of dydrogesterone has not been shown except for cases with a previous history of miscarriage. Therefore, considering its possible side effects, it is not the first choice in threatened miscarriage.

Vaginal use of micronized progesterone should be considered due to its difficulty of use in bleeding patients and local side effects.

In line with all the evidence above, oral dydrogesterone treatment may be considered as the first choice in threatened abortion, since it has fewer side effects and is found to be more effective in most studies compared to micronized progesterone.

Do other treatments contribute to the treatment of threatened abortion?

No concrete data have been found in the literature regarding the benefits or harms of additional treatments such as magnesium, iodine, folic acid or vitamin D in pregnant women with threatened abortion. Thus, a recommendation on this subject cannot be given.

Can invasive diagnostic tests be performed on pregnant women with threatened abortion?

There is only one study in the literature that was published in 1979, which examines this question. In the study, 1600 pregnant women who underwent amniocentesis for prenatal diagnosis were included. Among these pregnant women, 73 had gone through threatened abortion in their current pregnancy, but none of them had a miscarriage after the following amniocentesis procedure[57].

There weren't any publications reporting negative results regarding an indicated amniocentesis in pregnant women who do not have active bleeding and have had a threatened miscarriage. There are no data regarding chorionic villus biopsy or cordocentesis.

Considering the limited data available; amniocentesis can be performed in pregnant women who have had a threatened abortion.

Should anti-D be administered to pregnant women diagnosed with threatened abortion in the presence of Rh incompatibility?

The information on this subject is contradictory. In addition to publications that do not recommend anti-D in early pregnancy, guidelines that recommend anti-D immunoglobulin for all bleedings also exist (Table 2)[58].

Table 2. A review of recommendations in the guidelines for the use of anti-D immunoglobulin in pregnant women with threatened abortion[58]

	ACOG	SOGC	RCOG	RANZCOG
Threatened abortion <12 weeks	Unspecified	Yes	Unspecified	No
Threatened abortion >12 weeks	Yes	Yes	Yes	Yes

ACOG: American College of Obstetricians and Gynecologists, SOGC: Society of Obstetricians and Gynaecologists of Canada, RCOG: Royal College of Obstetricians and Gynaecologists, RANZCOG: The Royal Australian and New Zealand College of Obstetricians and Gynaecologists

Although the cause of bleeding in pregnant women at risk of miscarriage is not always known, it is often due to separation of the placenta, which may cause fetomaternal bleeding. Due to the risk of alloimmunization, anti-D immunoglobulin is recommended, especially in pregnant women with significant bleeding[59]. For bleedings in the first three months, a dose of 50 micrograms is sufficient, since less bleeding may occur, but there is no harm in using the normal dose of 300 micrograms[60]. Most pregnant women presenting to the emergency department with threatened abortion at earlier than the 12th week of pregnancy have heavy or recurrent bleeding or accompanying abdominal pain. The risk of fetomaternal bleeding is high in these pregnant women, and as a result, Rh isoimmunization is possible. The Rh status of these pregnant women should be recorded in the emergency department. Anti-D immunoglobulin should be recommended to all Rh D negative pregnant women who present to the emergency department with threatened abortion before the 12th week of pregnancy[61].

Considering current data, the use of anti-D immunoglobulin should be recommended in pregnant women with threatened abortion and Rh incompatibility.

Recommendations

This guideline was compiled by PERİDER after a detailed screening and review process for all gynecologists and obstetricians. Clear recommendations could be made on certain topics thanks to the availability of robust evidence. All the literature data used in concluding these suggestions has been explained in detail so that clinicians can evaluate them as well. Therefore, when using this guideline, it would be ideal to make your Clinical decisions after examining the presented data in detail.

Studies and literature on some important topics of the guide are insufficient, and for some subjects, no evidence exists at all. The recommendations we give, especially on these issues, are based on general common sense and habits to make safer choices for our patients. Scientific studies are needed to make more accurate and evidence-based recommendations on these issues in the future. Researchers might consider taking on questions such as "What is the incidence and prevalence of threatened abortion in Türkiye?", "Is bed rest and/or hospitalization beneficial in the treatment of threatened abortion?", "Should sexual intercourse restriction be recommended in threatened abortion?". These future studies may shed light on medical practice, improve healthcare for our patients by changing several habits that are not evidence-based.

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Footnotes

Authorship Contributions

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