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TURKISH JOURNAL OF OBSTETRICS AND GYNECOLOGY

LETTER FROM THE PRESIDENT

Dear Turkish Gynecology and Obstetrics Family,

We are very happy to bring our esteemed colleagues together our June issue, which has a high scientific content. In our June issue, which is the second issue of 2024, we have tried to include scientific studies, mostly from abroad. I would like to remind you that there is a very serious effort behind the articles that have been examined very carefully among the many studies sent to our journal and I would like to thank our journal team.

As it is known, we held the 21st National Turkish Society of Gynecology and Obstetrics Congress in Limak Hotel, Cyprus between 15-19 May. In the congress, which welcomed 2180 participants, 392 scientific guests and 65 scientific sessions and brought together Turkey's largest obstetricians, 7 satellite symposiums and 9 oral presentation sessions were held. Our congress supported by 128 sponsoring companies also included 93 company stands. I would like to express my gratitude to our colleagues who participated in our congress with a rich social program and high scientific value.

As the TJOD family, I would like to express that we will pay utmost attention to both our scientific activities and the professional problems of our physicians.

Ismail Mete Itil, Prof. MD
President of TJOD

TURKISH JOURNAL OF OBSTETRICS AND GYNECOLOGY

EDITORIAL

Dear Colleagues,

We are glad and honored to be in front of our esteemed colleagues with the June issue which is the 2nd issue of 2024. Our June issue has been prepared as a result of a busy three-month effort and presented to our esteemed colleagues. I would like to express that the recognition of our journal has increased day by day, the reading rate has increased, and it has been cited from scientific journals that have a respected place in our field. I would like to thank our team, who have made significant contribution to this success and used their scientific knowledge for our journal.

We would like to state that June issue has a special importance for the TJOG family. We are planning to apply to the DOAJ index as of the publication of this issue. In fact, we have carried out and completed the preliminary processes of this application since the last year.

We believe that our journal, which is the scientific publication of the Turkish Society of Gynecology and Obstetrics, will constantly work and reach the highest levels it deserves.

Ercan Yilmaz, Prof. MD
Fatih Sendag, Prof. MD

Clinical Investigation / Araştırma

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Investigation of *PD-1* gene variants in patients with endometrial cancer: A case-control study

Endometriyum kanserli hastalarda PD-1 gen varyantlarının araştırılması: Olgu kontrol çalışması

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Abstract

Objective: To assess the possible association of two single-nucleotide polymorphisms (SNPs), PD-1.3 (+7146G/A) and PD-1.5 (+7785C/T), with endometrial cancer (EC) susceptibility. In addition, the correlations between these SNPs and available clinicopathologic characteristics of patients with EC were investigated.

Materials and Methods: In this case-control study, 147 women with pathologically confirmed EC and 258 age- and ethnically matched healthy women were enrolled between June 2019 and May 2022. Genomic DNA was extracted, and genotyping of PD-1.3 (+7146G/A) and PD-1.5 (+7785C/T) SNPs was performed. Haplotype analysis was also performed. Pearson's chi-square test with Yates correction was used to evaluate differences in allele and genotype distributions. The 95% confidence interval and odds ratio were determined using an unconditional logistic regression model.

Results: There were no remarkable differences in the allele and genotype distributions of PD-1.3 (rs11568821) and PD-1.5 (rs2227981) between healthy controls and EC patients. However, there was a remarkable difference in the AC haplotype between the control and EC groups. No association was found between the investigated SNPs and the clinicopathologic features of EC.

Conclusion: Our results indicated that the aforementioned SNPs were not related to the risk of EC in the southern Iranian population.

Keywords: Endometrial cancer, programed cell death-1, polymorphism, single-nucleotide polymorphisms

Öz

Amaç: Tek nükleotid polimorfizmlerinden (SNP) PD-1,3 (+7146G/A-rs11568821) ve PD-1,5 (+7785C/T-rs2227981 ile endometriyal kanser (EK) duyarlılığı arasındaki olası ilişkiyi değerlendirmektir. Ayrıca bu SNP'ler ile EK'li hastaların mevcut klinikopatolojik özellikleri arasındaki korelasyonlar araştırıldı.

Gereç ve Yöntemler: Bu olgu-kontrol çalışmasına Haziran 2019 ile Mayıs 2022 arasında patolojik olarak doğrulanmış EK'li 147 kadın ve yaş ve etnik açıdan uyumlu 258 sağlıklı kadın dahil edildi. Genomik DNA çıkarıldı ve PD-1,3 (rs11568821) ve PD-1,5 (rs2227981) SNP'lerinin genotiplemesi yapıldı. Haplotip analizi de yapıldı. Alel ve genotip dağılımlarındaki farklılıklar, Yates düzeltmeli Pearson ki-kare testi kullanılarak değerlendirildi. %95 güven aralığını ve olasılık oranını hesaplamak için koşulsuz bir lojistik regresyon modeli kullanıldı.

Bulgular: Tek nükleotid polimorfizmlerinden PD-1,3 (rs11568821) ve PD-1,5 (rs2227981) alel ve genotip dağılımları açısından EK'li hastalar ve sağlıklı kontroller arasında dikkate değer bir fark yoktu. Ancak EK'li hastalar ile kontrol grubu arasında AC haplotipinde dikkate değer bir fark vardı. Araştırılan SNP'ler ile EK'nin klinikopatolojik özellikleri arasında da bir ilişki bulunamadı.

Sonuç: Sonuçlarımız yukarıda bahsedilen SNP'lerin İran toplumunda EK riski ile ilişkili olmadığını gösterdi.

Anahtar Kelimeler: Endometriyum kanseri, programlanmış hücre ölümü-1, polimorfizm, tek nükleotid polimorfizmleri

PRECIS: Our results indicated that *PD-1* gene variants (PD-1.3 and PD-1.5) were not associated with the risk of endometrial cancer in the Iranian population.

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Introduction

Endometrial cancer (EC), which originates from the epithelium of the uterus, is the fourth most frequent malignancy in women worldwide and the most common cancer of the female reproductive system[1]. During the last two decades, mortality rates and the incidence of EC have increased. Thus, EC is a major concern for women's health, especially in developed countries. About 65,000 new cases of EC and 12,000 deaths are expected in the United States in 2022[2]. Although EC mainly affects postmenopausal women 60-70 years of age, approximately 5-10% of cases experience it under 40 years of age[3]. EC is divided into several molecular subtypes that show distinct clinical and pathological behavior[4]. Genetic alterations and dysregulated immune responses determine the risk level and prognosis of EC patients[5]. Despite current improvements in therapeutic protocols for other gynecologic malignancies, few improvements are available for the management of advanced-stage EC. Therefore, a deep understanding of the molecular changes associated with EC is needed to identify new biomarkers for the early diagnosis of EC and to identify new targets for prevention and more effective therapeutic approaches[5].

Programed cell death-1 (PD-1, CD279), a type I transmembrane glycoprotein, is one of the most important immune checkpoints belonging to the CTLA-4/CD28 subfamily of the immunoglobulin (Ig) superfamily. It is encoded by the *PDCD1* gene, which is located on chromosome 2q37.3[6] and is a co-inhibitory receptor that downregulates the activation of T-cells and leads to the maintenance of peripheral tolerance. It is expressed on activated immune cells, including B cells, $CD8^+$ and $CD4^+$ T-cells, Natural killer T-cells, regulatory T-cells (Treg), monocytes, and some DC subsets. PD-1 is also a marker of exhausted T lymphocytes[6,7]. PD-1 ligands (PD-L1/2) are expressed on a broad range of human hematopoietic and non-hematopoietic cells, as well as tumor cells. When PD-1 binds to its ligands, it induces inhibitory signals that suppress cytokine production and T-cell proliferation and attenuate tumor immunity[6]. Recent studies have revealed that antibodies that block immune checkpoints, such as anti-PD-1/PD-L1, are one of the most promising immunotherapy approaches for the treatment of some refractory tumors[8]. Despite the clinical success of immune checkpoint inhibitor therapies, some patients with EC do not respond well to these treatments; therefore, markers predicting the efficacy of anti-PD-1/PD-L1 immunotherapy may aid in patient selection and decision making by differentiating responders from non-responders[9].

One of the most frequent sources of genetic diversity in the human genome is single-nucleotide polymorphisms (SNPs). Based on where SNPs are located, within gene sequences or in regulatory regions near a gene, they might have different outcomes at the phenotypic level[10]. They can also be considered as molecular markers in association studies related to complicated human diseases such as autoimmune diseases and cancer. Genetic polymorphisms may affect *PDCD1* and *PD-L1* gene expression[7,11]. Previous studies have found that some PD-1 functional SNPs are related to different types of cancer, including brain tumors, thyroid cancer, colon cancer, and gastric cancer[12-15]. However, there are also some conflicting results[16,17].

Therefore, in this study, we evaluated the possible association of two known SNPs in the *PDCD1* gene, PD-1.3 (+7146G/A) and PD-1.5 (+7785C/T), with EC susceptibility in a southern Iranian population. In addition, correlations between these SNPs and existing clinicopathologic features of the patients were evaluated.

Materials and Methods

Study Population

In this case-control study, we selected 147 women with pathologically confirmed EC as a case group who were enrolled at Shahid Faghihi Hospital affiliated with Shiraz University of Medical Sciences (Shiraz, Iran) between June 2019 and May 2022. All patients with EC were staged using the International Federation of Gynecology and Obstetrics (FIGO) staging criteria. The control group included 258 age, ethnically matched healthy women who were selected from blood donors referred to the Fars Blood Transfusion Organization (Shiraz, Iran) without any history or evidence of clinical problems, especially gynecological disorders, autoimmune diseases, and cancer, and without any history of medication as inclusion criteria. The Shiraz University of Medical Sciences Research Ethics Committee approved this study (approval number: IR.SUMS.REC.1398.1160, date: 28.12.2019).

DNA Extraction and Molecular Analysis

After written informed consent, 4 mL of peripheral blood was obtained from healthy women and EC patients in tubes containing EDTA. QIAamp DNA Mini Kit (Qiagene, Germany) was used to extract genomic DNA. Genotyping of PD-1.3 (+7146G/A-rs11568821) and PD-1.5 (+7785C/T-rs2227981) SNPs was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) using specific primers and *Pst1* (Fermentas, Lithuania) and *PvuII* (Fermentas, Lithuania) restriction enzymes, respectively (Table 1). The digested products were separated by electrophoresis on an agarose gel (3%) stained with a safe stain for visualization under UV light.

Statistical Analysis

Haplotype analysis and deviation from Hardy-Weinberg equilibrium were assessed using the Arlequin software package algorithms. The SPSS software package (version 20, Chicago, IL, USA) was used to analyze the data. Differences in allele and genotype frequencies were calculated using Pearson's chi-square test with Yates correction. An unconditional logistic regression model was used to calculate the 95% confidence interval (CI) and odds ratio (OR). $P<0.05$ was statistically significant.

Results

Study Population

The demographic and clinicopathological data of the 147 patients with EC and 258 healthy controls are presented in Table 2. There were no remarkable differences between the mean age (p=0.26), age at menarche (p=0.07), age at menopause (p=0.38), and body mass index (p=0.09) of healthy controls and EC patients. The tumor type in 104 (70.4%) out of 147 EC patients was endometrioid adenocarcinoma, 124 (84.3%) of the EC patients were in FIGO stage I, and 82 (55.7%) EC patients were diagnosed with grade I carcinoma. The prevalence of diabetes and hypertension in patients with EC was 20 (13.6%) and 34 (23.1%), respectively.

PDCD1 Gene Variants and the Risk of EC

In this study, genotype distribution at positions PD-1.5 (rs2227981) and PD-1.3 (rs11568821) in both controls and EC patients was in Hardy-Weinberg equilibrium. As presented in Table 3, the frequencies of PD-1.3 (rs11568821) genotypes were 112 (76.2%) for GG, 33 (22.5%) for GA, and 2 (1.3%) for AA out of 147 patients, and in controls, there were 204 (79.1%) GG, 50 (19.4%) GA, and 4 (1.5%) AA out of 258 participants. Our results showed no remarkable differences in the frequencies of PD-1.3 alleles and genotypes between healthy controls and patients, and PD-1.3 (rs11568821) did not change the overall risk of EC overall (Table 3).

The frequencies of PD-1.5 (rs2227981) genotypes were 65 (44.2%) for CC, 61 (41.6%) for CT, and 21 (14.3%) for TT. In controls, the frequencies were 109 (42.2%) for CC, 107 (41.5%) for CT, and 42 (16.3%) for TT, with no remarkable differences between the two groups (Table 3). Statistical analysis also showed no remarkable differences in the allele frequency of PD-1.5 (rs2227981) between cases and healthy controls (Table 3).

Table 1. Primer sequences and PCR-RFLP conditions for amplification of *PDCD1* gene

Locus	Primer sequence	Annealing temperature	RE	Length of digested fragments
PD-1.3 (+7146G/A- rs11568821)	F: 5′-CCAGGCAGCAACCTCAATC-3′ R: 5′-GGTGTCCCCAGATCACACAG-3	58 °C	Pst1	G: 381 bp A:277 bp, 104 bp
PD-1.5 (+7785C/T-rs2227981)	F: 5′-GACGGAGTATGCCACCATTGTC-3′ R: 5′-AAATGCGCTGACCCGGGCTCAT- 3′	58 °C	PvuII	C: 196 bp T: 125 bp, 71 bp

RE: Restriction enzyme, RFLP: Restriction fragment length polymorphism, PCR: Polymerase chain reaction

Table 2. Demographic and clinicopathologic information of the study population

Variables		EC patients (n=147)	Healthy controls (n=258)	p-value
Age, mean ± SD		57.42±10.86	55.43±8.36	0.26
Age at menopause, mean ± SD		50.92±4.59	51.51±2.39	0.38
Age at menarche, mean ± SD		12.16±1.33	12.59±1.61	0.07
BMI, kg/m^2		30.25±5.79	28.8±3.67	0.09
Diabetes, n (%)		20 (13.6%)	-	-
Hypertension, n (%)		34 (23.1%)	-	-
Histological grade	I	82 (55.7%)	-	
	II	35 (23.8%)	-	
	III	30 (20.4%)	-	
FIGO stage	I	124 (84.3%)	-	
	II	9 (6.1%)	-	
	III	14 (9.5%)	-	
	IV	-	-	
Lymph node involvement	Yes No	22 (14.9%) 125 (85.0%)	- -	
Tumor size	≥3 cm ≤3 cm	60 (40.8%) 87 (59.2%)	- -	

EC: Endometrial cancer, FIGO: International Federation of Gynecology and Obstetrics, SD: Standard deviation, BMI: Body mass index

The allele and genotype frequencies of PD-1.3 (+7146G/A) and PD-1.5 (+7785C/T) were analyzed according to the clinicopathological features of patients with EC. The results showed that neither of the two SNPs was associated with any of the clinicopathological features of the disease, including lymph node (LN) involvement status, stage, histological grade, and tumor size.

PD-1 Haplotype Distributions in Controls and EC Patients

GC, GT, AC, and AT haplotypes were obtained from PD-1 SNPs using algorithms from the Arlequin software package. The GC haplotype was the most common haplotype in both EC patients (58.50%) and healthy controls (55.81%). Statistical analysis revealed that AT, GC, and GT haplotype distributions were not associated with EC (Table 4). At the same time, it was found that the AC haplotype frequency was remarkably different in EC patients compared with controls (Table 4). This haplotype was found to play a protective role in the development of EC (OR=0.57, 95% CI=0.33-0.96, p=0.04) (Table 4).

Discussion

In this study, we did not detect remarkable differences in the allele and genotype distributions of PD-1.3 (+7146G/A) and PD-1.5 (+7785C/T) between the control group and EC patients. However, there was a remarkable difference in the AC haplotype between healthy controls and EC patients. The results also showed no association between the evaluated SNPs and the clinicopathological features of EC.

Several studies regarding the association of PD-1.3 (+7146G/A) and PD-1.5 (+7785C/T) with the risk and/or progression of various types of cancer have yielded inconsistent results. Haghshenas et al.[17] could not find an association between PD-1.3 (+7146G/A) and PD-1.5 (+7785C/T) and the risk of breast cancer in the Iranian population. Their results also showed no correlation between the evaluated genotypes and clinicopathologic features of breast cancer. Furthermore, another study by Piredelkhosh et al.[16] found that the SNPs mentioned above had no remarkable association with non-small-cell lung cancer (NSCLC) susceptibility. Li et al.[18] also demonstrated no remarkable association between PD-1.5 (+7785C/T) and the risk of ovarian cancer. In contrast, several recent studies have revealed an association between *PDCD1* gene variants, both in terms of genotypic and allelic frequencies, and different types of cancer[19].

The human genome contains nearly 10 million SNPs. Some SNPs play a role in susceptibility to environmental factors,

Table 3. Genotype and allele frequencies of PD-1 SNPs in EC patients and healthy controls

SNPs	Genotype/allele	Healthy controls n (%)	Patients n (%)	p-value	OR	95% CI
PD1.3	GG	204 (79.1%)	112 (76.2%)	1.0	1.0	Reference
	GA	50 (19.4)	33 (22.5%)	0.41	0.83	0.53-1.29
	AA	4 (1.5%)	2 (1.3%)	0.90	1.04	0.48-2.29
	G	458 (88.75%)	257 (87.4%)	1.0	1.0	Reference
	A	58 (11.25%)	37 (12.6%)	0.56	0.93	0.75-1.16
PD1.5	CC	109 (42.2%)	65 (44.2%)	1.0	1.0	Reference
	CT	107 (41.5%)	61 (41.6%)	0.82	1.05	0.70-1.55
	TT	42 (16.3%)	21 (14.3%)	0.53	1.09	0.82-1.44
	C	325 (63.0%)	191 (65.0%)	1.0	1.0	Reference
	T	191 (37.0%)	103 (35.0%)	0.57	1.04	0.89-1.21

OR: Odds ratio, CI: Confidence interval, SNPs: Single nucleotide polymorphisms, EC: Endometrial cancer

Table 4. PD-1 haplotype distributions in EC patients and healthy controls

Haplotypes		Patients n (%)	Healthy controls n (%)	p-value	OR	95% CI
PD1.3	PD1.5					
G	C	86 (58.50%)	144 (55.81%)	0.45	1.03	0.80-1.31
G	T	49 (33.45%)	85 (32.94%)	0.27	1.13	0.85-1.47
A	C	11 (7.50%)	26 (10.07%)	0.04	0.57	0.33-0.96
A	T	1 (0.69%)	3 (1.16%)	0.80	0.76	0.24-2.56

OR: Odds ratio, CI: Confidence interval, EC: Endometrial cancer, *p<0.05

including toxins. Others increase the risk of developing certain diseases, affect a patient's response to certain medications, and are associated with some complex diseases such as cancer[7]. Recent genome-wide association studies have implicated that SNPs in genes that encode immunoregulatory molecules are involved in the inability of immune responses to control tumor growth and thus contribute to the risk of developing various types of tumors. They contribute to the molecular pathogenesis of complex diseases through various functional mechanisms[20]. EC is the most prevalent gynecological cancer in developed countries. SNPs within different genes are involved in endometrial carcinogenesis. In a review study by Bafligi et al.[21], SNPs in KLF, SOX4, HNF1B, CYP19A1, EIF2AK, and MYC were found to be closely associated with EC. In the current study, the association between two SNPs in the *PDCD1* gene (PD-1.5 and PD-1.3) and EC was evaluated.

rs11568821 (PD-1.3 G/A) is localized within intron 4 of the *PDCD1* gene[12]. The rs11568821 (PD-1.3 G/A) polymorphism can alter the expression of the *PDCD1* gene through a substitution of A for G, which may result in a loss of PD-1 inhibitory functions in individuals carrying the PD-1.3 A allele[20]. Parakh et al.[22] reported that melanoma patients with the GG genotype of PD-1.3 had more complete responses than those with the AG genotype, and the G allele remarkably correlated with a longer median progression-free survival than the A allele. However, our results did not show any remarkable differences in the frequency of PD-1.3 alleles and genotypes between the control group and EC patients. In other words, the PD-1.3 polymorphism did not modify the overall risk of EC. In line with our results, a recent study also reported no remarkable association between PD-1.3 and NSCLC[16]. Another study demonstrated a trend toward an association of PD-1.3 genotypes with skin basal cell carcinoma, although this association was not remarkably significant[23]. Furthermore, no significant association was found between hepatocellular carcinoma and breast cancer in a Turkish population[24] and an Iranian population[17], respectively. However, the PD-1.3 polymorphism was correlated with colorectal cancer in the Iranian population[25].

Another *PDCD1* gene polymorphism investigated in this study was rs2227981 (PD-1.5 C/T). It is located in exon 5 of PDCD1 and is a synonymous SNP. Because of the linkage disequilibrium between the rs2227981 (PD-1.5 C/T) polymorphism and other *PDCD1* gene polymorphisms, PD-1.5 may affect *PDCD1* expression at the mRNA and protein levels[7]. The possible association between the rs2227981 (PD-1.5 C/T) polymorphism and the risk of developing cancer was evaluated in three meta-analyses. The results showed that the T allele of the rs2227981 (PD-1.5 C/T) polymorphism remarkably reduced susceptibility to cancer[26-28]. The results from the Chinese Han population also suggested that PD-1.5 was potentially related to NSCLC susceptibility[29], and the results from the Iranian population demonstrated its association with gastric cancer risk. However, our findings revealed no remarkable differences between controls and EC patients regarding the allele and genotype distribution of PD-1.5. Consistent with our study, Ma et al.[30] and Fathi et al.[20] failed to show a PD-1.5 association with the risk of NSCLC and head and neck squamous cell carcinoma (HNSCC), respectively, and Li et al. could not show a PD-1.5 association with ovarian cancer in the Chinese population[18]. The inconsistency in results may be due to differences in the molecular pathology of the diseases studied and/or differences in minor allele frequency (MAF) in different populations.

Aside from the above findings, our investigation showed that none of the two SNPs (PD-1.3 and PD-1.5) correlated with any of the clinicopathological features of EC patients, including LN involvement status, tumor size, stage, and histological grade. In line with our results, another study showed no association between the investigated SNPs and tumor size, tumor grade, tumor stage, LN involvement, or other clinicopathologic characteristics of breast cancer[17]. Moreover, Li et al.[18] found that *PDCD1* gene polymorphisms may be associated with the development of epithelial ovarian cancer but not with its clinical outcome in these patients. In addition, although our statistical analysis revealed that the AT, GC, and GT haplotype distributions were not associated with EC, the AC haplotype frequency was remarkably different in patients with EC compared with controls. This haplotype was found to play a protective role in the development of EC. Another study by Fathi et al.[20] suggested that although *PDCD1* gene polymorphisms at positions PD-1.5 and PD-1.3 did not correlate with HNSCC susceptibility, haplotype combinations resulting from these polymorphisms may confer susceptibility. In contrast, in a previous study evaluating four haplotypes derived from PD-1.5 and PD-1.3 polymorphisms in an Iranian population, no differences in haplotype distributions were observed between breast cancer patients and the control group[17].

Study Limitations

There are several limitations to our study that need to be considered when interpreting the results. First, only two functional SNPs in the *PDCD1* gene were selected to evaluate their associations with EC susceptibility, which may not reflect the effect of all genetic variants in *PDCD1*. Second, the sample size was relatively small. To elucidate the exact role of *PDCD1* gene polymorphisms in the pathogenesis of EC, it is necessary to study the full range of PD-1 genetic variants, perform a complete haplotype analysis in a larger sample size and in different ethnic groups, and perform a functional study of the haplotypes that emerge.

Conclusion

This study offered insight into the roles of PD-1.5 and PD-1.3 polymorphisms in the etiology of EC and their association with the clinicopathological features of patients with EC. The

results showed that these SNPs are not related to the risk of EC in the southern Iranian population. Current studies have shown that managing EC can be challenging; therefore, a profound knowledge of genetic variation and the mechanisms of its pathogenesis will lead to the achievement of therapeutic and diagnostic precision in this complicated cancer, which continues to increase in incidence and mortality.

Ethics

Ethics Committee Approval: The Shiraz University of Medical Sciences Research Ethics Committee approved this study (approval number: IR. SUMS. REC.1398. 1160, date: 28.12.2019).

Informed Consent: All participants provided informed consent before entering the study.

Authorship Contributions

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

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Hysteroscopic tubal electrocoagulation versus laparoscopic tubal disconnection for the management of hydrosalpinx and subsequent pregnancy outcomes: A randomized clinical trial

Hidrosalpinksin yönetiminde histeroskopik tubal elektrokoagülasyon ile laparoskopik tubal bağlantı kesilmesinin karşılaştırılması ve sonraki gebelik sonuçları: Randomize bir klinik çalışma

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Abstract

Objective: Hydrosalpinx impairs the success of in vitro fertilization (IVF) embryo transfer. Various surgical approaches, such as fluid aspiration or isolation of the affected fallopian tubes, have been used to enhance the outcome. This study was conducted to evaluate and compare the efficacy of laparoscopic tubal disconnection (LTD) and hydroscopic tubal electrocoagulation (HTE) for hydrosalpinx before IVF.

Materials and Methods: After obtaining ethical committee approval, we assessed 112 women who were subfertile due to hydrosalpinx to check their adherence to our selection criteria. Eligible patients were allocated into two groups (LTD vs. HTE). Both groups underwent extensive assessment before the operative procedure. IVF and subsequent embryo transfers were performed in both groups. Live birth and pregnancy rates were evaluated.

Results: Patients who underwent LTD prior to IVF embryo transfer had significantly higher live birth (41%), clinical pregnancy (57%), and chemical pregnancy (61%) rates in the LTD group than in the HTE group (12%, 35%, 41%, respectively). However, we could not find a significant difference between the two groups regarding the miscarriage (17% vs. 28%, p=0.33) and multiple pregnancy (14% vs. 12%, p=0.79) rates. No major complications with HTE were observed, except for a case of uterine perforation, whereas two cases of surgical complications occurred in the LTD group. Additionally, we found a significantly shorter operative time and hospital stay (0.5±0.7 days, p=0.012) in the HTE group.

Conclusion: LTD may be a more effective approach compared with hysteroscopic tubal electrocoagulation for improving birth and pregnancy rates in patients with IVF and hydrosalpinx.

Keywords: Hydrosalpinx, tubal infertility, IVF, embryo transfer, laparoscopy

Öz

Amaç: Hidrosalpinks, in vitro fertilizasyon (IVF) embriyo transferinin başarısını bozar. Sonucu iyileştirmek için sıvı aspirasyonu veya etkilenen fallop tüplerinin izolasyonu gibi çeşitli cerrahi yaklaşımlar kullanılmıştır. Bu çalışma, IVF öncesinde hidrosalpinks için laparoskopik tubal bağlantının kesilmesinin (LTBK) hidroskopik tubal elektrokoagülasyona (HTE) karşı etkinliğini değerlendirmek ve iki yöntemi karşılaştırmak için yapıldı.

PRECIS: Hysteroscopic tubal electrocoagulation versus laparoscopic tubal disconnection for the management of hydrosalpinx and subsequent pregnancy outcomes: A randomized clinical trial.

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Gereç ve Yöntemler: Etik kurul onayını aldıktan sonra, hidrosalpinks nedeniyle kısır olan 112 kadını, seçim kriterlerimize uygunluklarını kontrol etmek için değerlendirdik. Uygun hastalar 2 gruba ayrıldı (LTBK ve HTE). Her iki gruba da operasyon öncesi kapsamlı bir değerlendirme yapıldı. Her iki grupta da IVF ve ardından embriyo transferleri yapıldı. Canlı doğum ve gebelik oranları değerlendirildi.

Bulgular: Tüp bebek embriyo transferi öncesinde LTBK uygulanan hastalarda, HTE grubuna (sırasıyla %12, %35 ve %41) kıyasla canlı doğum (%41), klinik gebelik (%57) ve kimyasal gebelik (%61) oranları anlamlı derecede daha yüksekti. Ancak düşük (%17 vs. %28, p=0,33) ve çoğul gebelik (%14 vs. %12, p=0,79) oranları açısından 2 grup arasında anlamlı bir fark yoktu. HTE grubunda uterus perforasyonu dışında majör bir komplikasyon görülmezken, LTBK grubunda 2 olguda cerrahi komplikasyon meydana geldi. Ayrıca HTE grubunda anlamlı olarak daha kısa ameliyat süresi ve hastanede kalış süresi (0,5±0,7 gün, p=0,012) saptandı.

Sonuç: Hidrosalpinksli IVF hastalarında doğum ve gebelik oranlarının iyileştirilmesinde LTBK HTE'ye göre daha etkili bir yaklaşım olabilir.

Anahtar Kelimeler: Hidrosalpinks, tubal infertilite, IVF, embriyo transferi, laparoskopi

Introduction

Hydrosalpinx is the most serious type of tubal disease, accounting for more than 35% of female infertility cases[1,2]. It occurs when the ampullary part of the fallopian tube is abnormally enlarged and filled with fluid due to blockage. This blockage causes tubal fluid to build up and reverse its flow into the uterus. Symptoms of hydrosalpinx include dyspareunia, persistent pelvic discomfort, and pelvic pressure. It can be diagnosed by hysterosalpingography (HSG), sonohysterosalpingography, or vaginal ultrasonography (TVUS)[3].

With regard to infertility treatments, the development of in vitro fertilization (IVF) was initially motivated by tubal factor infertility. However, individuals with hydrosalpinx, compared with those without hydrosalpinx, have worse outcomes with IVF[4,5]. The reason for this is that the transferred embryos can be washed away by the tubal-uterine reflux of the fluid present in the hydrosalpinx. Additionally, the hydrosalpinx can affect endometrial receptivity, leading to lower implantation rates[6].

The standard treatment for women with hydrosalpinx is laparoscopic salpingectomy, which involves removing the affected fallopian tube[1]. This procedure increases the chances of a successful pregnancy after IVF by approximately 50%[7]. Alternatively, laparoscopic proximal tubal ligation can also improve IVF success rates. However, both procedures have invasive risks, such as anesthesia-related complications and technical difficulties due to pelvic adhesions[8].

To mitigate the risks associated with laparoscopic surgery, a less invasive method for treating hydrosalpinx before IVF may be beneficial[3]. One such method is the hysteroscopic implantation of Essure® intratumoral devices. These devices block the tubes, preventing fluid leakage into the uterine cavity. Essure® is a safe and effective method of permanent tubal ligation with minimal invasiveness[9].

In cases where laparoscopy is not feasible, hysteroscopy with an Essure implant or electrocoagulation can be considered as an alternative to the traditional treatment. This provides a viable option for individuals who cannot undergo laparoscopic procedures because of technical difficulties or contraindications[10].

Overall, the treatment options for hydrosalpinx vary from surgical interventions to less invasive methods. The choice of treatment depends on the individual's circumstances and the expertise of the medical professionals. In the current study, we investigated the efficacy of hydroscopic tubal electrocoagulation (HTE) compared with laparoscopic tubal disconnection (LTD) in patients with tubal infertility due to hydrosalpinx.

Materials and Methods

Ethical approval was obtained from the Ethical Committee and Institutional Review Board (IRB) of Al-Azhar University Hospitals under the reference code (430) to conduct a prospective clinical trial on women with infertility due to hydrosalpinx diagnosis. The study was conducted from September 2022 to January 2023 at Al-Hussein University Hospital, Cairo, Egypt.

Eligibility Criteria

Patients were eligible to be enrolled if they fulfilled the following inclusion criteria; (1) women aged 18-34 years, (2) diagnosed with tubal factor infertility due to hydrosalpinx, (3) an indication for assisted reproductive technology (e.g., IVF). HSG and TVUS confirmed the diagnosis. Patients were excluded if they had infertility due to other factors, such as uterine or male factors. Women with tubal obstruction were also excluded.

Patient Allocation

Following history taking, clinical examination, and confirmatory investigations, including routine and fertility lab profiles and imaging studies, patients were allocated to one of two groups (A or B) by a nurse who was not involved in the research team. We used sealed opaque envelopes to randomly allocate the enrolled patients to one study group. Group A (LTD) included 51 patients and group B (HTE) included 49 patients. The enrolled women underwent further evaluation and laboratory investigations to ensure their adherence to our preselected eligibility criteria.

HTE Surgical Technique

In cases where confirmatory laparoscopy was not possible because of contraindications, HTE was directly planned for patients during the second week of their menstrual cycle. The procedure involved the use of a 4-mm diameter hysteroscope and a lipotrope 5 Fr reusable bipolar electrode. During the procedure, the ocular end of the fallopian tube and the surrounding area of the uterine horn were subjected to unilateral or bilateral electrocoagulation.

To optimize embryo development and implantation, hysteroscopic fulguration was performed to treat the internal orifice of the fallopian tubes. This technique used hysteroscopic bipolar coagulation with a power of 40 W for 20 s. The goal was to degrade the tissue of the diseased tube's internal orifice using electric heat energy, resulting in scar formation. Instead of dilating the cervix, it was softened with misoprostol tablets taken 3 h before the procedure. The surgical duration and anesthesia time were minimized by inserting a bipolar electrode into the operative channel before admission. During the procedure, a saline solution was used as an irrigating medium in the uterine cavity to enhance the conductivity of the bipolar electrode. The electrode was inserted at the tubal ostea, and closure was ensured through coagulation using a 40-W electric current. Patients were closely monitored for 24 h after the operation for any signs of bleeding, severe discomfort, or fever, and they were prescribed antibiotics and anti-inflammatory pain relievers before being discharged[11].

LTD Surgical Technique

Laparoscopy was scheduled for patients to confirm the presence of hydrosalpinx, but it was not always possible because of certain factors. Tubal occlusion, either on one or both sides, was performed during laparoscopy using bipolar coagulation and a proximal tubal incision. However, laparoscopy is not recommended for patients with severe obesity or pelvic and abdominal adhesions resulting from prior surgery, pelvic inflammatory disease, or endometriosis. These conditions made the procedure technically challenging or unsuitable[11,12].

The afflicted fallopian tube(s) were located under general anesthesia by employing 5 mm ports for entrance at the right and left lower quadrants. Gripping the tube, about 2-3 cm away from the cornua, we administered monopolar diathermy. We then used scissors to cut off the diathermize tip[3].

Statistical Analysis

The data obtained from the history, clinical examination, and outcome measures were coded, entered, and analyzed using Microsoft Excel software. Subsequently, the data were imported into SPSS software for further analysis. Qualitative data are presented as numbers and percentages, while quantitative data are presented as mean ± standard deviation. To determine significant differences, various statistical tests were employed. The chi-square test was used to assess the difference and relationship of qualitative variables, whereas the t-test was used to compare differences between quantitative independent groups. A level of significance of 0.05 was set for significant results, and a significance level of 0.001 was set for highly significant results.

Results

Patients' Characteristics

Of the 112 assessed women, 101 were found to be eligible for inclusion. Fifty-one patients were assigned to group A (LTD), whereas group B (HTE) included 49 patients. There was no statistically significant difference between the study groups regarding all baseline assessed data, including age, body mass index, cycle duration, hormonal profile related to infertility (e.g., follicle-stimulating hormon, luteinizing hormone, progesterone, prolactin), antral follicle count, and hydrosalpinx laterality. The duration and type (secondary) of subfertility were significantly higher in the HTE group. Table 1 summarizes the baseline characteristics of the study participants.

Birth and Pregnancy Outcomes

Thirty-one women tested positive for β-hCG (chemical pregnancy) in the LTD group. The live birth rate was found to be statistically significantly higher in the LTD group (41%) than in the HTE group (p<0.001). Both chemical (61%) and clinical (57%) pregnancy rates were also significantly higher in women treated with LTD compared with patients in the HTE group (p-values were 0.004 and 0.046, respectively). A higher but not significant difference was found among pregnant women in the HTE group regarding the miscarriage rate (p=0.33).

Table 1. Baseline characteristics of study participants

		Group A (LTD) (n=51)	Group B (HTE) (n=49)	p-value
Age (years)†		26.3±4.7	29.6±3.4	0.06
Subfertility duration (months)†		28.6±2.8	39.2±3.9	0.03*
Subfertility type	**Primary**	29 (57%)	16 (34%)	0.01*
	Secondary	22 (43%)	33 (67%)	
Hydrosalpinx type	**Unilateral**	31 (61%)	37 (75%)	0.13
	Bilateral	20 (39%)	12 (25%)	
BMI (kg/m²)†		28.7±1.9	27.9±2.6	0.09
AFC†		20.1±2.4	15.8±6.3	0.11
Cycle duration (days)		28.6±3.1	29.1±4.4	0.32

LTD: Laparoscopic tubal disconnection, HTE: Hydroscopic tubal electrocoagulation, BMI: Body mass index, AFC: Antral follicle count, †: Continuous variables reported in (mean ± standard deviation), *: Statistically significant p-values

No significant difference was observed between the two study groups regarding the multiple pregnancy rate (14% vs. 12%, p=0.79). Table 2 shows the difference between the two study groups regarding live birth and pregnancy outcomes.

Complications and Secondary Outcomes

No surgical or procedure-related complications were reported in patients treated with HTE except for one woman who developed uterine perforation, whereas two patients in the LTD group developed serosal bowel injury and one patient reported port site infection. Regarding the operative time, a statistically significantly decrease was observed in HTE patients compared with the LTD group (6.6±2.1 vs. 16.9±4.3 mins, p<0.001). The average hospital stay duration in LTD was 1.32±0.57 days which was significantly higher than that duration among HTE patients 0.81±0.4 days, p=0.012).

Discussion

Findings Summary

We can summarize our study findings in 3 points; (1) Significantly higher live birth, clinical pregnancy, and chemical pregnancy rates in the LTD group than in the HTE group, (2) no significant difference between the two groups in miscarriage and multiple pregnancy rates, (3) no major complications with HTE except for a case of uterine perforation, while two cases of surgical complications occurred in the LTD group. However, we found a significantly shorter operative time and hospital stay in the HTE group.

Our Findings in the Context of Previous Literature

Hydrosalpinx, a common symptom of infertility, can result from various diseases like vaginal infections, endometriosis, or pelvic surgeries[1]. Enlargement of the fallopian tube's distal portion due to fimbrial blockage leads to hydrosalpinx, which potentially impacts pregnancy rates after IVF[13]. Surgical procedures that block communication between the fallopian tubes and the uterus may enhance the chances of successful pregnancy in individuals with hydrosalpinx. Diagnostic techniques, such as HSG and laparoscopy, aid in assessing the prognosis of pregnancy success. Laparoscopic surgeries like salpingectomy or tubal ligation, have shown better pregnancy outcomes post-IVF for patients with hydrosalpinx[14,15].

Rosenfield et al.[16] examined the impact of intracytoplasmic sperm injection on pregnancy outcomes following hysteroscopic tubal electrocoagulation versus LTD in individuals with hydrosalpinges, aligning with the current study. The hysteroscopic tubal electrocoagulation and LTD groups did not display significant differences in age or type of infertility (p>0.05), as reported in the study.

Our results are consistent with those of Bao et al.[2], who conducted a comparative analysis of hysteroscopic and laparoscopic tubal occlusion in hydrosalpinx before IVF. The study did not observe a significant discrepancy in the age distribution of infertility between the laparoscopic and hysteroscopic groups (p>0.05). When assessing the duration of infertility and the presence of hydrosalpinx on either side, no statistically significant differences were found between the two groups (p>0.05).

Dreyer et al.[8] also reported similar outcomes; 85 women were analyzed and divided into two groups. Forty-two women underwent laparoscopic salpingectomy, whereas 43 women underwent hysteroscopic proximal occlusion with the insertion of an intratubal device (Essure). The group that underwent hysteroscopic proximal occlusion had a significantly shorter median operation time than the laparoscopic salpingectomy group (7 vs. 41 minutes, p<0.001). While there was agreement on the duration of the procedures, there was no statistically significant difference in the length of postoperative hospital stays between the two groups. Only one woman who underwent laparoscopic salpingectomy experienced postoperative infection at the umbilical incision site, which resolved spontaneously.

In terms of complication rates, no statistically significant distinction was observed between the two groups, consistent with the findings of Dreyer et al.[8]. Among those who underwent hysteroscopic proximal occlusion using Essure, three women experienced complications (two failures and one case of pelvic inflammatory disease). However, only one woman in the laparoscopic salpingectomy group experienced a postoperative infection at the umbilical incision site, which resolved spontaneously.

In addition, Wu et al.[17] explored alternative hysteroscopic tubal occlusion methods to Essure in a separate study involving 56 women with hydrosalpinx. They assessed the efficacy of platinum fiber coil placement in 106 fallopian tubes of 55

Table 2. Post-interventional birth and pregnancy outcomes in each group

	Group A (LTD) (n=51)	Group B (HTE) (n=49)	p-value
Live birth rate†	21 (41%)	6 (12%)	0.001*
Clinical pregnancy rate	29 (57%)	17 (35%)	0.004*
Chemical pregnancy rate	31 (61%)	20 (41%)	0.046*
Miscarriage rate	9 (17%)	14 (28%)	0.33
Multiple pregnancy rate	7 (14%)	6 (12%)	0.79

LTD: Laparoscopic tubal disconnection, HTE: Hydroscopic tubal electrocoagulation, †: Live birth rate was calculated per clinical pregnancy, *: Statistically significant p-values

patients undergoing IVF, with successful complete proximal occlusion detected in 52 patients via HSG examination after 3 months. Among them, 44 proceeded with IVF-ET, achieving a clinical pregnancy rate of 60.5% and a live birth rate of 60.87%. This study concluded that platinum fiber coil insertion is a safe and beneficial option for hysteroscopic proximal tubal occlusion in hydrosalpinx patients preparing for IVF.

Furthermore, a systematic review by Xu et al.[18] involving over 3000 patients indicated that individuals with hydrosalpinx managed through hysteroscopic Essure device placement before IVF exhibited lower clinical pregnancy and live birth rates than those managed through laparoscopic salpingectomy and proximal tubal occlusion. Although our study did not directly compare pregnancy outcomes between the two groups, our results aligned with the aforementioned studies in highlighting the superiority of laparoscopic over hysteroscopic tubal occlusion for hydrosalpinx in patients undergoing IVF, particularly concerning the success of tubal occlusion.

Hysteroscopic tubal occlusion, particularly for the proximal portion of the hydrosalpinx, is a successful method to prevent backflow of fluid and improve implantation during assisted reproduction with minimal complications. Studies have supported the comparable effectiveness of hysteroscopic and laparoscopic procedures in addressing hydrosalpinx-related infertility and enhancing pregnancy outcomes after IVF[19-21]. Despite differences in some outcomes, such as tubal occlusion rates and procedure durations, both hysteroscopic and laparoscopic methods offer viable options to manage hydrosalpinx before IVF, tailored to individual patient conditions and preferences.

Conclusion

LTD may be a more effective approach than hysteroscopic tubal electrocoagulation for improving pregnancy rates in IVF patients with hydrosalpinx. However, individual patient factors, surgical expertise, and potential risks should be carefully considered when choosing the optimal treatment modality.

Ethics

Ethics Committee Approval: Ethical approval was obtained from the Ethical Committee and Institutional Review Board (IRB) of Al-Azhar University Hospitals under the reference code (430) to conduct a prospective clinical trial on women with infertility due to hydrosalpinx diagnosis.

Informed Consent: All participants provided informed consent before entering the study.

Authorship Contributions

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

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Comparison of laparoscopic and hysteroscopic surgical treatments for isthmocele: A prospective cohort

İstmoselde laparoskopik ve histeroskopik cerrahi tedavilerin karşılaştırılması: Prospektif bir kohort

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Abstract

Objective: To evaluate the clinical outcomes of laparoscopic and hysteroscopic surgical approaches for treating symptomatic isthmocele and identify their associated factors.

Materials and Methods: Forty-six patients with symptomatic isthmocele diagnosed using transvaginal saline infusion sonohysterography were enrolled in this prospective cohort study. Patients underwent either laparoscopic or hysteroscopic isthmoplasty based on their residual myometrial thicknesses and fertility desires and were subsequently followed by clinical and ultrasonographic examinations.

Results: Twenty-two patients underwent laparoscopy and 24 underwent hysteroscopic surgery. At baseline, there was no significant difference in the mean age and years since the last cesarean section between the two groups. However, the hysteroscopy group had a higher mean parity and previous cesarean sections (p=0.00, 0.03). The most common symptoms were abnormal uterine bleeding, infertility, and dysmenorrhea. The mean baseline residual myometrial thickness was significantly higher in the laparoscopy group (p=0.00), and only laparoscopic surgery led to a significant increase in residual myometrial thickness in patients (p=0.00). Both procedures significantly reduced abnormal uterine bleeding (p=0.00), but only laparoscopy reduced infertility (p=0.00) and hysteroscopy reduced dysmenorrhea (p=0.03). Hysteroscopy showed better symptom resolution in younger patients (p=0.01), whereas age did not affect laparoscopy outcomes.

Conclusion: Both approaches showed similar effectiveness in resolving abnormal uterine bleeding, with laparoscopy excelling in infertility resolution and hysteroscopy excelling in dysmenorrhea resolution.

Keywords: Cesarean section, hysteroscopy, laparoscopy, postcesarean section, scar

Öz

Amaç: Bu çalışmanın amacı semptomatik istmosel tedavisinde laparoskopik ve histeroskopik cerrahi yaklaşımların klinik sonuçlarını değerlendirmek ve ilişkili faktörleri belirlemektir.

Gereç ve Yöntemler: Bu prospektif kohort çalışmasına transvajinal salin infüzyon sonohisterografi kullanılarak semptomatik istmosel tanısı konan 46 hasta dahil edildi. Hastalara rezidüel miyometrial kalınlık ve doğurganlık isteklerine göre laparoskopik veya histeroskopik istmoplasti uygulandı ve ardından hastalar klinik ve ultrasonografik muayenelerle takip edildi.

PRECIS: Our study found laparoscopic and hysteroscopic isthmoplasty equally effective for abnormal uterine bleeding, with laparoscopy superior in infertility resolution and hysteroscopy in dysmenorrhea relief.

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Bulgular: Yirmi iki hastaya laparoskopi, yirmi dört hastaya ise histeroskopik cerrahi uygulandı. Başlangıçta, iki grup arasında ortalama yaş ve son sezaryenden bu yana geçen yıllar açısından anlamlı bir fark yoktu. Ancak histeroskopi grubunda ortalama parite ve önceki sezaryenlerin sayısı daha yüksekti (p=0,00, 0,03). En sık görülen semptomlar anormal uterin kanama, kısırlık ve dismenore idi. Başlangıçtaki ortalama rezidüel miyometrial kalınlık laparoskopi grubunda anlamlı derecede yüksekti (p=0,00) ve yalnızca laparoskopik cerrahi hastalarda anlamlı rezidüel miyometrial kalınlık artışına yol açtı (p=0,00). Her iki prosedür de anormal uterin kanamayı önemli ölçüde azalttı (p=0,00), ancak yalnızca laparoskopi kısırlığı azalttı (p=0,00) ve histeroskopi dismenoreyi azalttı (p=0,03). Histeroskopi genç hastalarda semptomlarda daha fazla düzelme sağladı (p=0,01), yaş ise laparoskopi sonuçlarını etkilemedi.

Sonuç: Her iki yaklaşım da anormal uterin kanamanın çözümünde benzer etkinlik göstermiştir; laparoskopi kısırlığın çözümünde ve histeroskopi dismenorenin çözümünde üstündür.

Anahtar Kelimeler: Sezaryen, histeroskopi, laparoskopi, sezaryen sonrası, skar

Introduction

The cesarean section (C/S) is one of the most commonly performed surgeries worldwide, with a growing prevalence[1]. Elective C/S, in contrast to natural vaginal delivery, offers mothers the benefits of pain avoidance, control over delivery timing, and reduced maternal anxiety[2]. In addition, C/S can benefit neonates by reducing the risks of chorioamnionitis, fetal heart rate issues, and cord prolapse, contributing to the increasing preference for C/S over vaginal delivery[2].

Performing C/S when medically indicated (e.g., prolonged labor, or fetal distress) significantly reduces maternal and neonatal complications and mortality[3]. However, performing C/S without medical indications increases the risk of complications[3]. One emerging complication is isthmocele (or cesarean scar defect), where a pouch-like structure forms at the prior C/S scar site on the uterine wall[4,5]. Isthmocele affects up to 70% of women undergoing C/S, with approximately 30% of them experiencing symptoms like abnormal uterine bleeding (AUB), dysmenorrhea, secondary infertility, and chronic pelvic pain[6].

The primary approach to managing symptomatic isthmoceles involves surgical interventions[6]. Laparoscopy is typically the preferred surgical approach for patients with large isthmoceles, whereas hysteroscopy is commonly performed on patients with smaller isthmoceles[7]. Previous research indicates that both laparoscopic and hysteroscopic procedures effectively alleviate symptoms[7]. However, a knowledge gap exists regarding the comparative effectiveness and advantages of these two approaches in symptom resolution, necessitating further investigation.

Consequently, our objective was to assess and compare the clinical outcomes and symptom relief achieved through laparoscopic and hysteroscopic surgery for isthmocele treatment.

Materials and Methods

Design and Setting

This prospective study was conducted at Arash Hospital, Tehran, Iran. We included patients who underwent either laparoscopic or hysteroscopic excision of the isthmocele between December 2021 and September 2022. Before commencement, this research received approval from the Research Deputy and the Ethics Committee of Tehran University of Medical Sciences under the reference number IR.TUMS.MEDICINE.REC.1402.106 and strictly adhered to the ethical standards described in the 1964 version of the Declaration of Helsinki, as revised in 2013[8]. In addition, explicit informed verbal and written consent was obtained from all participants. This study was conducted in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology guideline for cohort studies[9].

Participants

This study examined consecutive patients who had undergone either laparoscopic or hysteroscopic isthmocele resection and met specific inclusion criteria. These criteria comprised women with a history of at least one C/S and confirmed isthmocele diagnosis by an experienced radiologist based on saline infusion sonohysterography (SIS) characteristics between December 2021 and September 2022 at Arash Hospital in Tehran, Iran. Indications for laparoscopic or hysteroscopic isthmocele excision were significant symptoms, such as AUB, dysmenorrhea, and secondary infertility, which could not be attributed to alternative causes. Patients with residual myometrial thicknesses (RMT) ≥3 mm at the C/S scar site underwent hysteroscopic surgery, whereas those with RMTs less than 2 mm underwent laparoscopic intervention[7]. For individuals falling within the RMT range of 2-3 mm, the choice between laparoscopic and hysteroscopic approaches was based on reproductive intentions; laparoscopic procedures were preferred for patients desiring fertility, whereas hysteroscopic procedures were administered to those without the fertility desire[10].

The exclusion criteria encompassed patients whose symptoms could be explained by other gynecological conditions, including uterine polyps, cervical dysplasia, cervical infection, atypical endometrial cells, or endometriosis, as well as those with uncontrolled comorbidities.

Data Collection

The patients' baseline characteristics were assessed, including age, parity, number of previous C/S surgeries, time elapsed since their last C/S, and the presence or absence of AUB, dysmenorrhea, and secondary infertility. This information

was gathered through direct interviews with the patients and a thorough review of their medical records. Secondary infertility was defined as the inability to conceive after 6 months of unprotected sexual intercourse. Subsequently, patients were reevaluated 3 following the ischiocele excision procedure to determine the presence of AUB and dysmenorrhea. Furthermore, patients were visited 12 months after the surgery to evaluate the presence or absence of secondary infertility.

In addition, patients underwent transvaginal SIS conducted by an experienced radiologist from the center. Data regarding the RMT and dimensions (length, width, and depth) of the C/S scar site were collected at the initial assessment. In addition, according to previous studies, we calculated the volume of the C/S scar site using the formula "length*width*depth*0.52"[11]. Furthermore, the RMT of the C/S scar site was measured during the 3-month follow-up visits.

To determine the RMT during sonographic examination, the myometrial thickness at the C/S with the lowest measurement was assessed. The RMT at the C/S scar location was calculated by drawing a vertical line from the serosa to the isthmocele. In addition, we selected the sonographic view with the greatest length and depth to measure the residual myometrium dimensions. To measure the isthmocele length, we drew a line parallel to the cervical line. The isthmocele depth was calculated by measuring the vertical distance between the base of the lesion and the myometrial layer on the uterine fundus. Finally, the width of the isthmocele was measured. All measurements excluded endometrial thickness from isthmocele dimensions.

Procedures

a. Hysteroscopic resection of the isthmocele: Following procedural sedation analgesia, a hysteroscope was inserted into the uterine cavity to locate the isthmocele. Using a cutting current, we meticulously excised the upper and lower edges of the isthmocele until it was flattened and the muscular layer was exposed. Subsequently, the floor of the isthmocele was electrocoagulate using a roller ball. These procedures were performed under the direct vision of experienced obstetrics and gynecology specialists.

b. Laparoscopic isthmocele repair: Following the administration of general anesthesia, a hysteroscope was carefully inserted into the uterine cavity to precisely locate the ischiocele. Subsequently, trocars were placed in the peri-umbilical area to establish pneumoperitoneum, allowing assessment of the abdominal cavity. The isthmocele was then visually identified using the hysteroscope's light source, with the bladder gently pushed beneath the uterus. Once the isthmocele was located within the abdominal cavity, the uterine tissue was incised using a monopolar hook, and the upper and lower edges of the isthmocele were excised. After the excision, the incision site was meticulously repaired using a continuous suture technique employing 1/0 VICRYL® stitches. These surgical procedures were conducted by laparoscopic fellows with a minimum of 1 year of experience in laparoscopic surgeries, under the supervision of attending gynecologists with expertise in laparoscopic procedures.

Statistical Analysis

We used IBM® SPSS® version 25 for statistical analyses. Patients were categorized into two main groups: Laparoscopic and hysteroscopic surgeries. Baseline characteristics, isthmocele sonographic features, and clinical outcomes were compared between the groups. Continuous variables were summarized using mean and standard deviation. We compared the means of continuous variables between the groups using an independent t-test. Categorical data were compared using the chi-square test or Fisher's exact test, based on their characteristics. In addition, a non-parametric McNemar's test and paired sample t-test were used to compare paired categorial and continuous data between the two groups, respectively. The statistical significance level for all analyses was set at p-value <0.05.

In addition, we used binary logistic regression to assess the applicability values of different variables in predicting the response to surgical treatment, which was defined as the resolution of the symptoms of patients. The area under the receiver operating characteristic curve (AUROC) and 95% confidence interval (CI) were calculated to assess the strength of the predictive power of different variables. In addition, the statistical significance level in these analyses was set at p-value <0.05.

Results

Baseline Data

A total of 46 patients were included. Of them, 22 (47.8%) underwent laparoscopic surgery and 24 (52.2%) underwent hysteroscopic isthmocele excision surgery. The mean age of the patients was 38.6±4.2 years, with a range of 32-50 years, with no significant difference between the groups (p-value >0.05). The mean amount of parity and previous C/S surgeries in the studied population were 2.1±0.9 and 1.9±0.8, respectively. Notably, the hysteroscopy group exhibited notably higher values in both categories (p-values of 0.00 and 0.03, respectively). Additionally, the mean duration elapsed since the last C/S procedure was 8.7±5.9 years, with no statistically significant difference observed between the groups (p-value >0.05) Table 1. provides more detailed information regarding the baseline characteristics of the study participants.

In terms of isthmocele symptoms and surgical indications, among all patients, 35 (76.1%) presented with AUB, 22 (47.8%) experienced secondary infertility, and 14 (30.4%) reported dysmenorrhea at baseline assessment. Notably, there was a statistically significant difference between the groups in terms of the prevalence of infertility before surgery, with the laparoscopy group demonstrating a higher prevalence (p-value=0.00). However, no statistically significant differences were observed between the groups with regard to the prevalence of AUB and dysmenorrhea (p-value >0.05) (Table 1).

Table 1 overview of the baseline sonographic characteristics of the patient cohort. The mean isthmocele dimensions encompassed 8.2±3.8 mm in length, 7.1±3.8 mm in width, and 6.0±2.5 mm in depth for the entire cohort. Moreover, the mean calculated isthmocele volume amounted to 227.6±203.3 mm^3, with the laparoscopy group exhibiting markedly greater values (p-value <0.00). Furthermore, the mean RMT across the entire cohort was 2.8±1.4 mm, within a range of 1.5-8 mm. Notably, the laparoscopy group displayed a mean RMT of 2.0±0.2 mm (range: 1.5-2.7), whereas the hysteroscopy group exhibited a mean RMT of 3.6±1.6 mm (range: 2-8 mm). Significantly, the laparoscopy group demonstrated a notably higher mean RMT value (p-value <0.00).

Follow-up Data

All 46 patients participated in the follow-up visits (Figure 1). At the follow-up visits, 9 patients (19.5%) experienced AUB, whereas 8 (17.4%) and 5 (10.8%) reported infertility and dysmenorrhea after surgery, respectively. Specifically, 3 (13.6%), 5 (22.7%), and 2 (9.1%) of the laparoscopy group, and 6 (25%), 3 (12.5%), and 3 (12.5%) of the hysteroscopy group reported AUB, infertility, and dysmenorrhea after surgery, respectively (Table 2). The results of Fisher's exact test indicated no significant differences between the two groups in terms of AUB, infertility, and dysmenorrhea frequencies during follow-up visits (p-values >0.05) (Table 2).

The mean postoperative RMT of the patients was 5.3±2.0 mm (increased by 89.2%), with the laparoscopy group showing a mean of 6.7±1.3 mm (increased by 235%) and the hysteroscopy group 4.0±1.7 mm (increased by 13.9%). An independent

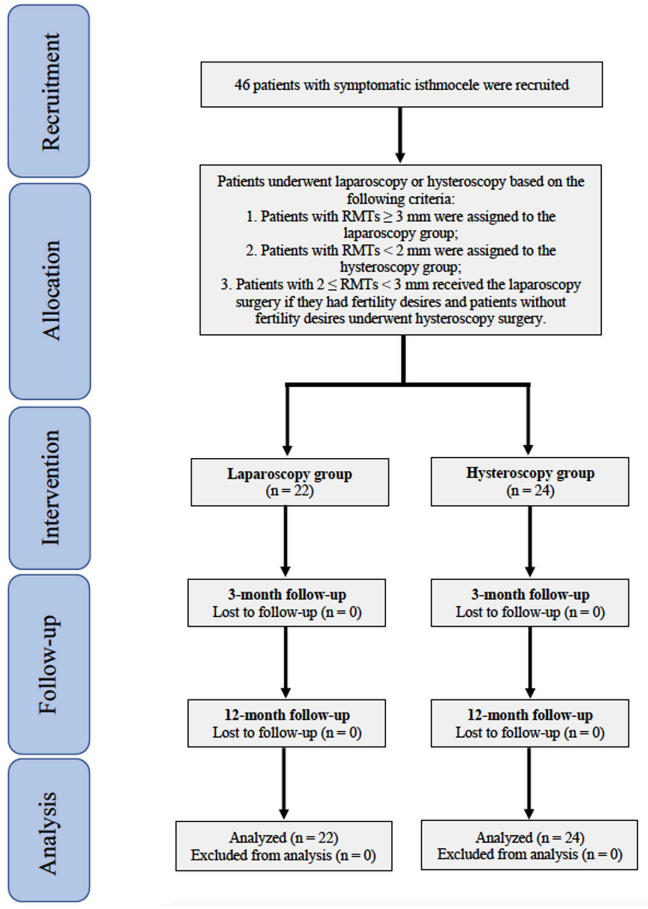

Figure 1. Flowchart of the study
RMT: Residual myometrial thickness

Table 1. Baseline characteristics of the included patients[a]

Variable	Total (n=46)	Laparoscopy (n=22)	Hysteroscopy (n=24)	p-value
Age, years	38.6±4.2	37.8±3.7	39.2±4.5	0.25[b]
Parity	2.1±0.9	1.7±0.6	2.5±1.0	0.00[b]
Number of C/S	1.9±0.8	1.6±0.6	2.1±0.8	0.03[b]
Years since last C/S, years	8.7±5.9	8.2±5.4	9.1±6.4	0.63[b]
AUB	35 (76.1)	15 (68.1)	20 (83.3)	0.30[c]
Infertility	22 (47.8)	16 (72.7)	6 (25)	0.00[d]
Dysmenorrhea	14 (30.4)	5 (22.7)	9 (37.5)	0.34[c]
Isthmocele length, mm	8.2±3.8	9.9±3.9	6.6±2.9	0.00[b]
Ishtmocele width, mm	7.1±3.8	8.4±2.6	5.8±4.3	0.01[b]
Isthmocele depth, mm	6.0±2.5	7.4±1.5	4.7±2.5	0.00[b]
Isthmocele volume, mm^3	227.6±203.3	347.9±203.7	117.4±128.3	0.00[b]
RMT, mm	2.8±1.4	2.0±0.2	3.6±1.6	0.00[b]

AUB: Abnormal uterine bleeding, C/S: Cesarean section, mm: Millimeter, RMT: Residual myometrial thickness
[a]: Data are presented as mean ± standard deviation or as number (percentage)
[b]: Independent t-test
[c]: Fisher's exact test
[d]: Chi-square test

Table 2. Follow-up characteristics of the included patients[a]

Variable	Total (n=46)	Laparoscopy (n=22)	Hysteroscopy (n=24)	p-value
AUB	9 (19.5)	3 (13.6)	6 (25)	0.46[b]
Infertility	8 (17.4)	5 (22.7)	3 (12.5)	0.45[b]
Dysmenorrhea	5 (10.8)	2 (9.1)	3 (12.5)	0.00[b]
RMT, mm	5.3±2.0	6.7±1.3	4.1±1.7	0.00[c]
ΔRMT, mm	2.5±2.5	4.7±1.2	0.4±1.6	0.00[c]

AUB: Abnormal uterine bleeding, RMT: Residual myometrial thickness, ΔRMT: Follow-up residual myometrial thickness - baseline residual myometrial thickness
[a]: Data are presented as mean ± standard deviation or as number (percentage)
[b]: Fisher's exact test
[c]: Independent t-test

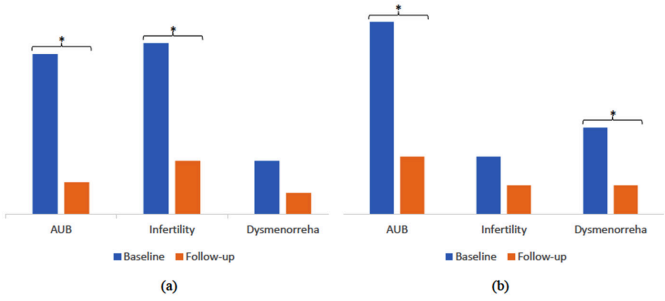

Figure 2. Prevalence of abnormal uterine bleeding, infertility, and dysmenorrhea at the baseline and follow-up points in the (a) laparoscopy and (b) hysteroscopy groups

t-test analysis revealed that the mean postoperative RMT in the laparoscopy group was significantly higher than that in the hysteroscopy group (p-value <0.00) (Table 2). Additionally, the mean RMT changes from baseline (ΔRMT: follow-up RMT-baseline RMT) were 2.5±2.5 mm for the entire cohort, 4.7±1.2 mm for the laparoscopy group, and 0.4±1.6 mm for the hysteroscopy group. Our analysis showed that laparoscopic surgery resulted in a significantly greater increase in RMT than hysteroscopic surgery (p-value <0.00) (Table 2).

Longitudinal Effects of Isthmocele Excision Surgery

Table 3 presents a comprehensive summary of our analyses regarding the impact of isthmocele excision surgery on symptom frequency among patients. The results of McNemar's test indicate a significant reduction in the frequencies of AUB (resolution rate: 74.2%, p-value <0.00), infertility (resolution rate: 63.6%, p-value <0.00), and dysmenorrhea (resolution rate: 78.5%, p-value: 0.00) in the entire cohort at follow-up compared with baseline. Both the laparoscopy and hysteroscopy groups experienced a substantial reduction in AUB frequency after surgery (resolution rates: 80% and 70%, p-value <0.00 for both). However, the frequency of infertility significantly decreased only in the laparoscopy group (resolution rate: 68.7%, p-value: 0.00), whereas the hysteroscopy group did not exhibit a significant reduction (resolution rate: 50%, p-values >0.05). Conversely, hysteroscopic surgery significantly reduced the frequency of dysmenorrhea (resolution rate: 60%, p-value=0.03), whereas laparoscopic surgery did not achieve a significant reduction (resolution rate: 67%, p-values >0.05) (Figure 2).

Furthermore, our paired sample t-test analysis demonstrated a significant increase in the mean RMT for the entire cohort after surgery (p-value <0.00) (Table 3). Subgroup analysis revealed that only the laparoscopy group experienced a significant post-surgical increase in RMT (p-value <0.00), whereas the mean RMT in the hysteroscopy group at the follow-up point did not significantly differ from their baseline mean RMT (p-values >0.05) (Table 3).

Effects of Different Variables on Treatment Response

To evaluate treatment responses and their associated factors, we categorized the study cohort into two groups: those with Complete Response to Treatment (CRT) and those with Incomplete Response to Treatment (IRT), based on their symptom status at follow-up compared with baseline. Patients without any of the three assessed symptoms (AUB, infertility, and dysmenorrhea) at follow-up were classified into the CRT group, whereas patients reporting any of these symptoms at follow-up were placed in the IRT group. This resulted in 29 patients in the CRT group and 17 patients in the IRT group (Table 4).

Based on the results of the chi-square test, neither the laparoscopic nor the hysteroscopic approaches demonstrated superiority in symptom resolution (p-values >0.05) (Table 4). Additionally, the CRT group had a significantly lower age compared with the IRT group in the entire cohort (p-value=0.00) and in the hysteroscopy group (p-value: 0.01). However, there was no significant difference in the mean age between the CRT and IRT groups among patients who underwent laparoscopic surgery (p-values >0.05) (Table 4). Furthermore, there were no significant differences between the CRT and IRT groups regarding parity, number of previous C/Ss, years since the last C/S, isthmocele dimensions (length, width, depth, and volume), and RMT in the entire cohort (p-values >0.05 for all) (Table 4).

We conducted an additional analysis to explore potential predictors of symptom resolution of each symptom individually in patients. The effects of the variables mentioned earlier were

Table 3. Comparison of the clinical and sonographic features before and after the isthmocele resection[a]

Variable		Total (n=46)	Laparoscopy (n=22)	Hysteroscopy (n=24)
AUB	Baseline	35 (76.1)	15 (68.1)	20 (83.3)
	1-year follow-up	9 (19.5)	3 (13.6)	6 (25)
	p-value	0.00[b]	0.00[b]	0.00[b]
Infertility	Baseline	22 (47.8)	16 (72.7)	6 (25)
	1-year follow-up	8 (17.4)	5 (22.7)	3 (12.5)
	p-value	0.00[b]	0.00[b]	0.25[b]
Dysmenorrhea	Baseline	14 (30.4)	5 (22.7)	9 (37.5)
	1-year follow-up	5 (10.8)	2 (9.1)	3 (12.5)
	p-value	0.00[b]	0.25[b]	0.03[b]
RMT, mm	Baseline	2.8±1.4	2.0±0.2	3.6±1.6
	1-year follow-up	5.3±2.0	6.7±1.3	4.0±1.7
	p-value	0.00[c]	0.00[c]	0.16[c]

AUB: Abnormal uterine bleeding, RMT: Residual myometrial thickness
[a]: Data are presented as mean ± standard deviation or as number (percentage)
[b]: McNemar's test.
[c]: Paired sample t-test

Table 4. Evaluating the effects of different variables on the treatment response[a]

Variable		Total (n=46)	Laparoscopy (n=22)	Hysteroscopy (n=24)
Surgical approach	CRT	29 (63.0)	15 (68.1)	14 (58.3)
	NCRT	17 (37.0)	7 (31.9)	10 (41.7)
	p-value	0.48[b]	-	-
Age	CRT	37.3±3.7	37.2±4.1	37.5±3.4
	NCRT	40.7±4.2	39.3±2.5	41.8±4.9
	p-value	0.00[c]	0.23[c]	0.01[c]
Parity	CRT	2.2±0.9	1.7±0.6	2.6±1.1
	NCRT	2.1±0.9	1.7±0.7	2.3±0.9
	p-value	0.69[c]	0.94[c]	0.43[c]
Number of C/S	CRT	1.8±0.7	1.6±0.6	2.1±0.8
	NCRT	1.0±0.8	1.7±0.7	2.2±0.9
	p-value	0.48[c]	0.71[c]	0.72[c]
Years since last C/S	CRT	7.8±5.1	8.4±5.4	7.2±4.9
	NCRT	10.2±7.0	8.0±5.9	11.7±7.6
	p-value	0.20[c]	0.87[c]	0.09[c]
Isthmocele length	CRT	7.8±3.6	9.5±3.4	6.0±2.9
	NCRT	8.8±4.1	10.8±5.0	7.5±3.0
	p-value	0.38[c]	0.47[c]	0.24[c]
Isthmocele width	CRT	6.7±3.4	8.4±2.9	4.8±3.0
	NCRT	7.7±4.4	8.5±2.0	7.1±5.6
	p-value	0.40[c]	0.95[c]	0.21[c]
Isthmocele depth	CRT	6.0±2.4	7.5±1.5	4.4±2.2

Table 4. Continued

Variable		Total (n=46)	Laparoscopy (n=22)	Hysteroscopy (n=24)
	NCRT	6.1±2.7	7.4±1.5	5.1±3.1
	p-value	0.92[c]	0.94[c]	0.51[c]
Isthmocele volume	CRT	210.1±195.6	330.6±187.2	82.6±101.9
	NCRT	256.2±218.9	384.9±247.1	166.0±150.2
	p-value	0.47[c]	0.57[c]	0.11[c]
RMT-pre	CRT	2.9±1.5	2.0±0.3	3.7±1.8
	NCRT	2.9±1.3	2.0±1.0	3.5±1.4
	p-value	0.99[c]	0.55[c]	0.71[c]

CRT: Complete response to the treatment, C/S: Cesarean section, NCRT: Incomplete response to the treatment, RMT-pre: Residual myometrial thickness at the baseline
[a]: Data are presented as mean ± standard deviation or as number (percentage)
[b]: Chi-square test
[c]: Independent t-test

separately assessed for the resolution of AUB (n=35), infertility (n=22), and dysmenorrhea (n=14). Our findings revealed that none of the variables significantly affected the resolution of AUB, infertility, and dysmenorrhea, except for the number of previous C/Ss (p-value=0.02) and isthmocele volume (p-value=0.00) in the dysmenorrhea group. This analysis indicated that a lower number of previous C/Ss and a smaller ischiocele volume are associated with the resolution of dysmenorrhea in patients.

Prediction of the Treatment Response

We conducted binomial logistic regression analysis to evaluate the predictive capabilities of variables that exhibited significant effects on treatment response in the independent t-test analysis mentioned earlier. Univariate logistic regression revealed that the age of patients was a significant predictor of symptom resolution (CRT vs. IRT) using a cutoff of 31 years, both in the entire cohort and the hysteroscopy group, with p-values of 0.01 (95% CI: 1.046-1.481) and 0.03 (95% CI: 1.014-1.678), and AUROCs of 0.74 and 0.75, respectively.

However, in contrast, our bivariate logistic regression analysis did not identify significant predictive values for either the number of previous C/Ss or ischiocele volume in predicting the resolution of dysmenorrhea (p-value >0.05).

Discussion

Our findings demonstrated that both laparoscopic and hysteroscopic surgeries can significantly reduce the prevalence of AUB in patients with isthmocele. However, only the laparoscopy group showed a significant decrease in infertility rates post-surgery, and only the hysteroscopy group exhibited significantly lower dysmenorrhea rates at follow-up compared with baseline. In addition, the mean RMT of the patients significantly increased after surgery in the laparoscopy group, whereas the hysteroscopy group did not experience such a change. In addition, both laparoscopy (68.1%) and hysteroscopy (58.3%) groups demonstrated significant symptom resolution after the surgery. Among the assessed variables, only the age of the patients in the hysteroscopy group could predict the treatment response, with a cut-off age of 31 years, whereas no other clinical or sonographic features could predict the response in either the hysteroscopy or laparoscopy groups.

Isthmocele, a common condition affecting up to 70% of women with prior C/S, commonly remains asymptomatic[12]. Research using transvaginal sonography and SIS has reported prevalence rates ranging from 24% to 70% and 56% to 84%, respectively, in women with previous C/S[12]. In patients with symptomatic isthmocele, AUB, infertility, dysmenorrhea, dyspareunia, and chronic pelvic pain are the most commonly reported symptoms[13].

Surgical excision is the primary treatment for symptomatic isthmocele[7], but there is an ongoing debate about the optimal surgical approach. Recent systematic reviews have indicated that both procedures can significantly alleviate AUB and infertility, with success rates between 71-100% and 25-100% for the laparoscopy and 86-100% and 30-100% for hysteroscopy, respectively[13]. While some studies suggest no substantial difference in effectiveness between laparoscopy and hysteroscopy[7,14], others lean toward laparoscopic surgery for better symptom resolution rates[15]. Considering this discrepancy regarding the effectiveness of laparoscopy and hysteroscopy in resolving different isthmocele-related symptoms, future research with larger sample sizes is essential.

Based on our findings, laparoscopic intervention led to a significant increase in RMT, whereas the hysteroscopy group did not experience a significant increase in RMT. This corroborates earlier research showing a substantial RMT increase after laparoscopy[16], whereas hysteroscopy was associated with a notably smaller RMT increase[14]. This divergence chiefly arises from the differing surgical techniques. Laparoscopic procedures involve the resection of the entire uterine tissue housing the isthmocele lesion and suturing the remaining uterine tissues, whereas hysteroscopy excises the isthmocele

lesion from the uterine cavity, resulting in a significantly thinner residual myometrium at the C/S scar site[13]. Considering this, laparoscopy seems preferable for patients with larger isthmocles (lower RMT) aiming for future pregnancies because it reinforces the uterine wall and reduces the risk of rupture or dehiscence[14]. Nevertheless, smaller isthmocele lesions with sufficient RMT may be effectively managed with hysteroscopy, resulting in lower complications and favorable outcomes[10]. Given these divergent findings, there remains a crucial need to establish precise treatment guidelines based on isthmocele characteristics, symptoms, and fertility desires. Further comprehensive studies with larger populations are imperative for this purpose.

Furthermore, we assessed the predictive factors for treatment response in laparoscopic and hysteroscopic surgeries for isthmocele. Our findings reveal that, among hysteroscopy patients, age is a significant predictor of complete symptom resolution, with those 31 years experiencing better outcomes. This corresponds with the only published study in this field, which found a lower age (cut-off: 38 years) to predict infertility resolution after hysteroscopy surgery[13]. This underscores age as a vital factor for predicting surgical treatment response, particularly infertility resolution. However, more research is needed to explore the potential relevance of additional clinical and sonographic features in predicting treatment response for both laparoscopic and hysteroscopic isthmocele surgeries.

Study Limitations

Our study has some limitations. Small sample sizes, observational design, and baseline differences between groups can hinder the generalizability of our findings. Hence, further studies with larger samples and randomized controlled trials are needed to assess and compare the effectiveness of laparoscopic and hysteroscopic isthmocele treatments.

Conclusion

In conclusion, laparoscopic and hysteroscopic isthmoplasty showed similar effectiveness in addressing AUB, with laparoscopy excelling in infertility resolution and hysteroscopy in dysmenorrhea resolution. In addition, only age (younger) among all assessed demographic, clinical, and sonographic factors predicted better treatment outcomes in hysteroscopy patients.

Ethics

Ethics Committee Approval: Before commencement, this research received approval from the Research Deputy and the Ethics Committee of Tehran University of Medical Sciences under the reference number IR.TUMS.MEDICINE.REC.1402.106 and strictly adhered to the ethical standards described in the 1964 version of the Declaration of Helsinki, as revised in 2013.

Informed Consent: Explicit informed verbal and written consent was obtained from all participating individuals.

Authorship Contributions

Surgical and Medical Practices: R.H., N.R.A., Z.A., Z.V., N.H., S.M., M.B., Concept: R.H., Design: R.H., N.R.A., Data Collection or Processing: N.R.A., Z.A., Z.V., N.H., M.B., Analysis or Interpretation: M.P., Z.A., Literature Search: M.P., S.D., Writing: M.P., S.D.

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Comparison of metformin, myoinositol and metformin-myoinositol combined treatments for polycystic ovary syndrome

Polikistik over sendromunda metformin, miyoinozitol ve metformin-miyoinozitol kombine tedavilerinin karşılaştırılması

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**This study was Dr. Ceyda Karadag's specialization thesis.*

Abstract

Objective: The objective of this study was to assess the effectiveness of myoinositol (4 g myoinositol + 400 mcg folic acid/day) compared with metformin (average 1700 mg/day), as well as the combined efficacy of both treatments in managing insulin-resistant polycystic ovary syndrome (PCOS) among women.

Materials and Methods: We retrospectively analyzed the records of 68 reproductive-age PCOS patients with insulin resistance over a 3-month period. Oral glucose tolerance tests (OGTT) (75 gr) were conducted to measure glucose levels at 0 and 120 min. Moreover, changes in prolactin, thyroid stimulating hormone, high-density lipoprotein, low-density lipoprotein, triglyceride levels, total cholesterol, follicle-stimulating hormone, luteinizing hormone, total testosterone, free testosterone, and dehydroepiandrosterone sulfate (DHEA-S) levels were evaluated pre- and post-treatment over a 3-month period.

Results: Statistically significant improvements were observed in menstrual regularity, body mass index (BMI), modified Ferriman Gallwey scores, OGTT glucose levels at 0 and 120 min, total testosterone, free testosterone, and DHEA-S levels across all groups ($p<0.005$).

Conclusion: No significant variances were observed in terms of BMI, modified Ferriman Gallwey scores, or androgen levels across the three treatment cohorts. The combination of myoinositol and metformin did not confer additional benefits compared with either treatment alone.

Keywords: Hirsutism, insulin resistance, metformin, myoinositol, polycystic ovary syndrome

Öz

Amaç: Bu çalışmada, polikistik over sendromlu (PCOS) kadınlarda insülin direnci olanlarda myoinositol tedavisinin (günde 4 g myoinositol + 400 mcg folik asit) metformin (ortalama 1700 mg/gün) ile karşılaştırılması ve bu iki tedavinin kombinasyonunun etkinliğinin incelenmesi amaçlanmıştır.

Gereç ve Yöntemler: Üreme çağındaki 68 insülin direnci olan PCOS hastasının 3 aylık hasta kayıtları incelenmiştir. Oral glikoz tolerans testleri (OGTT) (75 gr) 0 ve 120 dakikada glikoz düzeylerini, prolaktin, tiroid uyarıcı hormon, yüksek yoğunluklu lipoprotein, düşük yoğunluklu lipoprotein, trigliserid, total kolesterol, folikül stimüle edici hormon, luteinizan hormon, total testosteron, serbest testosteron, dehidroepiandrosteron sülfat (DHEA-S) düzeylerini ölçülmüş ve tedavinin ardından 3 ay sonra değerlerle karşılaştırılmıştır.

PRECIS: This study examines the efficacy of myoinositol, metformin, and their combination in treating polycystic ovary syndrome among patients with insulin resistance. It reveals that all three treatments demonstrate similar short-term effects on parameters such as oral glucose tolerance, lipid profile, body mass index, and hirsutism. Notably, improvements in clinical and metabolic outcomes were observed across all treatment groups, suggesting myoinositol's comparable effectiveness to metformin without additional benefits from combination therapy.

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Bulgular: Tüm gruplarda adet düzeni, vücut kitle indeksi (VKİ), modifiye Ferriman Gallwey skorları, OGTT'nin 0. ve 120. dakikadaki glikoz düzeyleri, total testosteron, serbest testosteron, DHEA-S düzeylerinde istatistiksel olarak anlamlı bir iyileşme gözlenmiştir (p<0,005).

Sonuç: VKİ, modifiye Ferriman Gallwey skorları ve azalan androjen düzeyleri açısından üç grup arasında fark bulunmamıştır. Myoinositolün metformin ile kombinasyonu, metformin veya yalnızca myoinositol kadar fayda sağlamamıştır.

Anahtar Kelimeler: Hirsutizm, insülin direnci, metformin, myoinositol, polikistik over sendromu

Introduction

Polycystic ovary syndrome (PCOS) is an endocrine disorder impacting around 6-14% of women in their reproductive years, with its exact cause remaining unidentified[1,2]. It is characterized by oligomenorrhea, symptoms of hyperandrogenism, and the presence of polycystic ovaries on ultrasonography[3]. Although its pathophysiology remains unclear, it is believed to be multifactorial, involving genetic factors, insulin resistance, hyperinsulinemia, and various endocrinological abnormalities[4]. Insulin resistance and the consequent hyperinsulinemia are pivotal factors in the pathophysiology of PCOS[5]. Insulin stimulates androgen production from the ovaries, with its effects manifesting directly in the ovary and indirectly in the pituitary gland[5].

The goal of treatment in patients with PCOS who do not desire gestation is to correct the symptoms of androgen excess and regulate menstrual cycles. Oral contraceptives (OCs) are the preferred choice of medication because of their efficacy in treating hirsutism and acne, as well as their protective effect against estrogen-induced effects on the endometrium. Nevertheless, OCs may exert adverse effects on insulin resistance, glucose tolerance, vascular reactivity, and coagulability[6].

Insulin-sensitizing agents are commonly used in PCOS treatment, especially in cases of insulin resistance and hyperinsulinemia. Metformin, a well-studied biguanide derivative antidiabetic, reduces gastrointestinal glucose absorption, inhibits gluconeogenesis, and enhances peripheral insulin sensitivity[7]. Myoinositol, a stereoisomer of carbon-6 sugar alcohol and member of the vitamin B group, serves as an intracellular secondary messenger in lipid synthesis, cell membrane structure, and cell growth, thereby influencing cell morphogenesis and cytogenesis[8,9]. Studies have shown that myoinositol activates enzymes that regulate glucose metabolism, and its deficiency has been linked to insulin resistance in patients with PCOS[10]. Myoinositol therapy has been shown to increase ovulation and decrease testosterone and insulin levels in PCOS patients with insulin resistance[11].

This study aimed to compare the effects of myoinositol, metformin, and their combination on clinical and laboratory parameters in patients with PCOS and insulin resistance.

Materials and Methods

The study included 68 patients aged 16-40 years who were admitted to the Akdeniz University Faculty of Medicine, Gynecology and Obstetrics Outpatient Clinic between January 2016 and August 2017 with hirsutism and/or menstrual irregularities, diagnosed with PCOS according to the European Society of Human Reproduction and Embryology (ESHRE)/ Rotterdam 2003 diagnostic criteria, and had concurrent impaired fasting glucose or impaired glucose tolerance. Before the start of the study, written approval was obtained from the Local Ethics Committee of the Faculty of Medicine of Akdeniz University (approval number: 618 - 2012-KAEK-20; date: 01.11.2017).

PCOS diagnosis was established in individuals who met two of the three criteria outlined in the ESHRE diagnostic criteria.

1. Oligomenorrhea and/or anovulation
2. Clinical and/or biochemical hyperandrogenemia findings
3. Detection of polycystic ovaries using pelvic and/or vaginal ultrasonography.

Exclusion criteria included a history of medication use for PCOS in the previous 6 months, pregnancy, diabetes, hypertension, liver or kidney diseases, systemic diseases such as heart disease for those receiving corticosteroids, use of medications affecting insulin resistance, ongoing infections, and severe insulin resistance or heart valve disease.

Participants underwent a 75-g oral glucose tolerance test (OGTT) at baseline and at the third month of treatment, following a three-day 150-200 g/day carbohydrate diet and a 12-h fasting period post-normal daily activity. Diagnostic criteria for diabetes mellitus (DM), impaired glucose tolerance, and impaired fasting glucose were based on the American Diabetes Association 2011 guidelines[12], defining the following:

- Impaired fasting glucose: fasting plasma glucose levels of 100-125 mg/dL
- Impaired glucose tolerance: 2nd hour plasma glucose levels in OGTT of 140-199 mg/dL
- DM: fasting plasma glucose levels ≥126 mg/dL or 120th minute blood sugar levels ≥200 mg/dL during OGTT.

Patients were divided into three groups according to the treatment protocols they received.

Patients were allocated into three treatment groups: Group 1 (n=22): Metformin monotherapy (oral administration of 850 mg metformin twice daily for 3 months). Group 2 (n=28): Myoinositol monotherapy (oral administration of 2 g myoinositol twice daily for 3 months). Group 3 (n=18): Combination therapy with myoinositol and metformin (oral administration of 850 mg metformin twice daily and 2 mg myoinositol twice daily for 3 months).

Metformin administration started at a dosage of 850 mg tablets, with dose titration from the first day of treatment to minimize side effects, gradually increasing to 1700 mg daily after 2 weeks.

Patients were provided detailed information regarding potential side effects associated with metformin.

Data collection included measurements of height and body weight at baseline and 3 months after treatment. Body mass index (BMI) was calculated by dividing body weight (kg) by the square of height (m). Hirsutism scores were determined and recorded using the modified Ferriman-Gallwey scoring method at initial patient assessment and at the 3-month follow-up. Hair density was assessed on a scale from 0 to 4 in nine different regions using this method: The upper lip, chin, chest area, back, waist, lower and upper abdomen, and upper arms and legs. Those with a modified Ferriman-Gallwey score of 8 were diagnosed with hirsutism. Pelvics or transvaginal ultrasonography was performed using a Toshiba-Applio 500 ultrasound device at the Akdeniz University Faculty of Medicine Obstetrics and Gynecology outpatient clinic. Polycystic ovary changes were defined as the presence of 12 or more follicles with a diameter of 2-9 mm and/or increased ovarian volume (>10 mL) on ultrasonographic examination. Clinical parameters [ovulation presence and hirsutism (Ferriman-Gallwey) scores] and biochemical parameters (75 g OGTT, high-density lipoprotein, low-density lipoprotein, triglyceride, free and total testosterone, DHEA-S) were retrospectively compared with pretreatment values after 3 months of treatment.

Statistical Analysis

Data were analyzed using the SPSS Statistics 20 statistical package. The Shapiro-Wilk test was used to determine whether the data were normally distributed. Nonparametric data are presented as median minimum-maximum, while parametric data are presented as mean ± standard deviation. In independent samples, one-way analysis of variance was used to compare normal distributed groups, and the Kruskal-Wallis test was used to compare non-normally distributed groups. Intra-group comparisons were conducted using paired samples t-tests for normally distributed data and Wilcoxon tests for non-normally distributed data. Post-hoc analysis was performed using Bonferroni correction. A significance level of $p<0.05$ was considered statistically significant.

Results

Pre-treatment demographic, clinical, and laboratory parameters were similar across all groups (Table 1). Table 2 presents the clinical and laboratory parameters of the groups after 3 months of treatment. No significant differences were observed between the groups in terms of BMI, menstrual regularity, or laboratory parameters after 3 months of treatment ($p>0.05$). The modified Ferriman-Gallwey scores of the patients after treatment were 8.64±3.82 in Group 1, 8.11±3.85 in Group 2 and 8.67±5.68 in Group 3. Hirsutism scores of 8 or higher were observed in 14 (63.6%) patients in Group 1, 14 (50%) patients in Group 2, and 8 (44.4%) patients in Group 3 after treatment.

In Group 1, significant differences were noted between pre-treatment and post-treatment values for BMI, menstrual regularity, hirsutism scores, 0-min and 120-min OGTT values, triglycerides, total cholesterol, free testosterone, total testosterone, and DHEA-S ($p<0.016$). Similar significant differences were observed in Group 2 and Group 3 between pre-treatment and post-treatment values for the aforementioned parameters ($p<0.016$). Intra-group changes and statistical comparisons of groups before and after treatment are summarized in Table 3.

There was no significant difference between the groups in terms of the incidence of impaired glucose tolerance or impaired fasting glucose after treatment ($p=0.378$). Improvements in OGTT values were observed in all groups regardless of the type of medication used. Additionally, no significant difference was found in the improvement of hirsutism scores among the three treatment groups ($p=0.265$). Similarly, there was no significant difference in menstrual cycle improvement or total weight loss between the three treatment groups ($p=0.057$, $p=0.356$, respectively).

Discussion

This study revealed no significant differences in the short-term effects of metformin, myoinositol, and their combination on OGTT, lipid profile, BMI, and hirsutism in patients with PCOS and insulin resistance. Positive changes in clinical and laboratory outcomes were observed in all three treatment groups before and after the intervention. Few studies have directly compared the effects of myoinositol, metformin, and combination therapy on clinical and metabolic parameters in women with PCOS and insulin resistance.

Previous research by Legro et al.[13] demonstrated that myoinositol treatment for 6 months led to improvements in menstrual cycles in 88% of patients with PCOS. Similarly, Gerli et al.[14] found that 70% of patients with PCOS achieved a normal menstrual pattern after 14 weeks of myoinositol therapy. Consistent with these findings, we observed improvements in menstrual parameters across all three treatment groups.

Studies investigating hirsutism scores with myoinositol treatment in PCOS have reported a significant decrease in Ferriman-Gallwey scores after 6 months of treatment[15-17]. Interestingly, our study found a statistically significant reduction in Ferriman-Gallwey scores in all three groups after only 3 months of treatment. This early response suggests that lifestyle changes and weight loss associated with the 3-month treatment regimen contributed to the observed improvement.

Several agents used in PCOS treatment target elevated serum androgen levels, which play a significant role in the clinical manifestations of the condition. Zacchè et al.[18] observed a significant decrease in free and total testosterone levels after 3 months of myoinositol treatment.

Similarly, metformin therapy reduces insulin and androgen levels, improve insulin sensitivity, and induce ovulation[19,20]. Our study corroborates these findings, demonstrating reductions in fasting glucose levels, OGTT values, and insulin

Table 1. Demographic, clinical and laboratory parameters of groups before treatment

		Group 1 (n=22)	Group 2 (n=28)	Group 3 (n=18)	p
Age (years)		26.9±6.07	23.1±4.4	25.6±5.86	0.054
Gravida		0 (0-3)	0 (0-3)	0 (0-2)	0.434
Parity		0 (0-2)	0 (0-2)	0 (0-1)	0.097
Abortus		0 (0-1)	0 (0-1)	0 (0-1)	0.735
Menstrual regularity	Oligomenorrhoea	20 (90.9%)	26 (92.9%)	14 (77.8%)	0.269
	Amenore	2 (9.1%)	2 (7.1%)	4 (22.2%)	
USG	Normal	4 (18.2%)	2 (7.1%)	2 (11.1%)	0.483
	PCOS	18 (81.8%)	26 (92.9%)	16 (88.9%)	
BMI (kg/m^2)		27.03±6.17	24.55±2.91	27.3±5.16	0.218
Hirsutism score		9 (3-18)	9 (2-19)	9 (3-27)	0.999
OGTT 0 minute		94.2±11.4	92.8±12.6	99.3±10.6	0.281
OGTT 120 minute		168.6±17.4	162±17.9	154.7±24.3	0.111
Triglycerides (mg/dL)		112±51.3	98.1±31.9	127.2±55.8	0.207
LDL (mg/dL)		100.7±26.1	101.7±15.9	105±17.2	0.833
HDL (mg/dL)		46.5±8.4	49.2±6.5	45.6±7.9	0.128
Total cholesterol (mg/dL)		168.3±32.5	176.8±22.8	168.6±23.8	0.398
Free testosterone (pg/mL)		2.71±0.89	2.41±1.05	2.52±0.88	0.293
Total testosterone (ng/mL)		0.6±0.17	0.52±0.18	0.54±0.21	0.421
DHEA-S (ug/dL)		287.2±123.6	255.3±80	303±141.4	0.523
FSH (mIU/mL)		6.14±1.73	6.09±1.86	5.2±1.31	0.059
LH (mIU/mL)		7.79±7.04	8.58±5.75	7.37±4.62	0.480
Estradiol (pg/mL)		62.5±46.5	55.1±23.4	77.3±53.3	0.377
Prolactin (ng/mL)		10.7±4.2	11.6±4.9	13.2±4.6	0.185
TSH (uIU/mL)		1.79±1.03	1.49±0.65	1.96±1.09	0.162

PCOS: Polycystic ovary syndrome, OGTT: Oral glucose tolerance tests, DHEA-S: Dehydroepiandrosterone sulfate, LDL: Low density lipoprotein, HDL: High density lipoprotein, LH: Luteinizing hormone, FSH: Follicle stimulating hormone, USG: Ultrasonography, BMI: Body mass index

Table 2. Clinical and laboratory parameters of the groups after 3 months of treatment

		Group 1 (n=22)	Group 2 (n=28)	Group 3 (n=18)	p
BMI (kg/m^2)		26.03±5.71	23.77±2.58	26.4±4.14	0.100
Menstrual regularity	Oligomenorrhoea	10 (45.5%)	14 (50%)	4 (22.2%)	0.153
	Normal	12 (54.5%)	14 (50%)	14 (77.8%)	
Hirsutism score		8 (3-17)	7.5 (2-16)	8 (3-24)	0.767
OGTT 0 minute		85±12.6	83.1±12.7	82.7±9.9	0.667
OGTT 120 minute		108±24.4	106.7±28.7	107.1±17.9	0.729
Triglycerides (mg/dL)		103.8±43	94.6±28	115.7±44.8	0.289
LDL (mg/dL)		96.4±23.7	92.3±12.8	95.7±12.3	0.602
HDL (mg/dL)		47.4±7.9	53.1±9.9	46.7±6.7	0.062
Total cholesterol (mg/dL)		161±31.6	163.5±17.8	155.1±19.5	0.336

Table 2. Continued

	Group 1 (n=22)	Group 2 (n=28)	Group 3 (n=18)	p
Free testosterone (pg/mL)	2.38±0.68	2.03±0.92	2.11±0.63	0.196
Total testosterone (ng/mL)	0.47±0.07	0.41±0.11	0.45±0.13	0.101
DHEA-S (ug/dL)	248.6±71.2	228.5±63.6	236±81.4	0.679
FSH (mIU/mL)	4.71±0.94	4.08±1.03	4.49±0.86	0.097
LH (mIU/mL)	4.56±1.97	5.36±2.52	5.11±2.62	0.169
Estradiol (pg/mL)	41.5±16.2	35.7±9.15	47.1±18.8	0.118
Prolactin (ng/mL)	10.6±3.03	9.95±4.13	10.4±3.23	0.593
TSH (uIU/mL)	1.97±0.78	1.69±0.71	2±0.67	0.138

OGTT: Oral glucose tolerance tests, DHEA-S: Dehydroepiandrosterone sulfate, LDL: Low density lipoprotein, HDL: High density lipoprotein, LH: Luteinizing hormone, FSH: Follicle stimulating hormone, BMI: Body mass index

Table 3. Intra-group changes before and after treatment

Pre-treatment		Group 1 (n=22)		p Pre-treatment	Group 2 (n=28)		p Pre-treatment	Group 3 (n=18)		p
		Post-treatment			Post-treatment			Post-treatment		
BMI		27 ±6.1	26 ±5.7	**0.002**	24.5 ±2.9	23.7 ±2.5	**0.001**	27.3 ±5.1	26.4 ±4.1	**0.002**
Menstrual regularity	Amenore	2 (%9.1)	-	**0.001**	2 (7.1%)	-	**0.001**	4 (22.2%)	-	**0.001**
	Oligomenorrhoea	20 (90.9%)	10 (45.5%)		26 (92.9%)	14 (50%)		14 (77.8%)	4 (22.2%)	
	Normal	-	12 (54.5%)		-	14 (50%)		-	14 (77.8%)	
Hirsutism score		9 (3-18)	8 (3-17)	**0.004**	9 (2-19)	7.5 (2-16)	**0.001**	9 (3-27)	8 (3-24)	**0.001**
OGTT 0 minute		94.2 ±11.4	85 ±12.6	**0.003**	92.8 ±12.6	83.1 ±12.7	**0.001**	99.3 ±10.6	82.7 ±9.9	**0.001**
OGTT 120 minute		168.6 ±17.4	108 ±24.4	**0.001**	162 ±17.9	106.7 ±28.7	**0.001**	154.7 ±24.3	107.1 ±17.9	**0.001**
Triglycerides (mg/dL)		112 ±51.3	103.8 ±43	**0.006**	98.1 ± 31.9	94.6 ±28	**0.091**	127.2 ±55.8	115.7 ±44.8	**0.003**
LDL (mg/dL)		100.7 ±26.1	96.4 ±23.7	0.038	101.7 ±15.9	92.3 ±12.8	**0.004**	105 ±17.2	95.7 ±12.3	**0.009**
HDL (mg/dL)		46.5 ±8.4	47.4 ±7.9	0.034	49.2 ±6.5	53.1 ±9.9	**0.002**	45.6 ±7.9	46.7 ±6.7	**0.193**
Total cholesterol (mg/dL)		168.3 ±32.5	161 ±31.6	**0.004**	176.8 ±22.8	163.5 ±17.8	**0.001**	168.6 ±23.8	155.1 ±19.5	**0.001**
Free testosterone (pg/mL)		2.71 ±0.89	2.38 ±0.68	**0.003**	2.41 ±1.05	2.03 ±0.92	**0.001**	2.52 ±0.88	2.11 ±0.63	**0.012**
Total testosterone (ng/mL)		0.6 ±0.17	0.47 ±0.07	**0.001**	0.52 ±0.18	0.41 ±0.11	**0.001**	0.54 ±0.21	0.45 ±0.13	**0.007**
DHEA-S (ug/dL)		287.2 ±123.6	248.6 ±71.2	**0.007**	255.3 ±80	228.5 ±63.6	**0.001**	303 ±141.4	236 ±81.4	**0.001**

OGTT: Oral glucose tolerance tests, DHEA-S: Dehydroepiandrosterone sulfate, LDL: Low density lipoprotein, HDL: High density lipoprotein, LH: Luteinizing hormone, FSH: Follicle stimulating hormone, BMI: Body mass index

resistance parameters with both metformin and myoinositol therapy.

A study by Nas and Tűű[21] comparing combined treatments of metformin, myoinositol, and their combination found no significant differences in effectiveness between the myoinositol and metformin groups. Similarly, a recent meta-analysis comparing metformin and myoinositol reported similar metabolic effects in the short term, with myoinositol being better tolerated because of fewer gastrointestinal side effects[22].

Study Limitations

The limitations of our study include its retrospective nature and the lack of a control group receiving only lifestyle recommendations. Nonetheless, our findings support the efficacy of myoinositol as a treatment for PCOS and underscore its potential advantages over metformin, particularly in terms of tolerability and side effect profile.

Conclusion

Given the uncertain etiopathogenesis of PCOS, treatment primarily focuses on symptom management. However, addressing underlying factors such as hyperandrogenemia and insulin resistance can lead to normalization of the clinical picture. Our study suggests that myoinositol therapy is at least as effective as metformin in improving clinical and laboratory parameters in patients with PCOS and insulin resistance. Furthermore, combining myoinositol with metformin did not provide any additional benefits. Considering the reported side effects associated with metformin, myoinositol has emerged as a promising treatment option for patients with insulin-resistant PCOS.

Ethics

Ethics Committee Approval: Before the start of the study, written approval was obtained from the Local Ethics Committee of the Faculty of Medicine of Akdeniz University (approval number: 618 - 2012-KAEK-20; date: 01.11.2017).

Informed Consent: Retrospective study.

Authorship Contributions

Surgical and Medical Practices: C.K., Ö.B., Concept: C.K., Design: C.K., M.S., Data Collection or Processing: M.S., S.S., Analysis or Interpretation: Ö.B., M.S.B., Literature Search: B.K., S.S., Writing: C.K.

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

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Effect of human umbilical cord stem cells (HUMSC) administration on collagen expression in the anterior vaginal wall in menopausal rats

İnsan göbek kordonu kök hücresi (İGKKH) uygulamasının menopozdaki sıçanlarda vajinal ön duvarın kollajen ekspresyonu üzerine etkisi

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Abstract

Objective: To evaluate the outcome of human umbilical cord stem cells (HUMSC) administration on collagen expression within the frontal vaginal wall of menopausal rats.

Materials and Methods: We conducted an experimental, randomized post-test-only controlled group design. The study samples were 40 healthy female Winstar rat with the age of 8-12 weeks that had been ovariectomized, had never mated, and weighed 18-22 grams. The umbilical cord was obtained from voluntary donors who did not have a history of hepatitis B, hepatitis C, HIV, cytomegalovirus infection, treponema pallidum infection, or a history of other infections transmitted through the blood, placental tract, and genitals. Data collection (frontal vaginal wall of the rat) was carried out in a controlled environment with the consideration that all conditions were maintained equally and could be controlled.

Results: There were 36 samples. A total of 13 menopausal rats (72%) had strong collagen expression and 5 rats had weak-to-moderate collagen expression (28%). On the other hand, 18 menopausal rats (100%) that belonged to the control group had weak-moderate collagen expression, and no menopausal rats appeared to have strong expression (0%). The administration of collagen to the anterior vaginal wall of postmenopausal rats proved to be effective by increasing the strong collagen expression in the damaged anterior vagina of postmenopausal female rats ($p<0.05$).

Conclusion: Administration of HUMSC resulted in an increase in collagen levels in the anterior vaginal tissue of postmenopausal female rats. These results demonstrate significant therapeutic potential for the treatment of pelvic floor dysfunction.

Keywords: Human umbilical cord stem cells, collagen expression, anterior vaginal wall, menopause rats

Öz

Amaç: Bu çalışmanın amacı insan göbek kordonu kök hücresi (İGKKH) uygulamasının menopozdaki sıçanlarda vajinal ön duvarın kolajen ekspresyonu üzerine etkisini belirlemektir.

Gereç ve Yöntemler: Rastgele son test kontrollü grup tasarımıyla deneysel bir çalışma yürütülmüştür. Örnekler, dahil etme kriterlerine uyan; yumurtalıkları alınmış, hiç çiftleşmemiş, 8-12 haftalık ve 18-22 gram ağırlığında 40 sağlıklı dişi Winstar sıçanlarıydı. Göbek kordonu; hepatit B, hepatit C, HIV, sitomegalovirüs enfeksiyonu, treponema pallidum enfeksiyonu veya kan, plasenta yolu ve cinsel yolla bulaşan diğer enfeksiyon geçmişi olmayan gönüllü

PRECIS: This study developed a novelty administration of HUMSC was proven to result in an increase in collagen levels in the anterior vaginal tissue of postmenopausal female rat.

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bir donörden alınmıştır. Veri toplama (sıçanın ön vajinal duvarı) kontrollü bir ortamda, tüm koşulların eşit şekilde sağlanması ve kontrol edilebilmesi durumunda gerçekleştirildi.

Bulgular: Otuz altı örnek vardı. Toplam 13 menopozal sıçanda (%72) güçlü, 5 sıçanda zayıf-orta düzeyde kolajen ekspresyonu (%28) vardı. Buna karşılık, kontrol grubunda 18 menopozal sıçanda (%100) zayıf-orta düzeyde kolajen ekspresyonu vardı ve hiçbir menopozal sıçanda güçlü ekspresyon (%0) görülmedi. Postmenopozal sıçanların ön vajinal duvarına kollajen uygulanması, postmenopozal dişi sıçanların hasarlı ön vajinasındaki güçlü kolajen ekspresyonunu önemli ölçüde artırabilmektedir ($p<0,05$).

Sonuç: İnsan göbek kordonu kök hücresi uygulamasının postmenopozal dişi sıçanın ön vajinal dokusunda kolajen düzeylerinde artışa neden olduğu kanıtlanmıştır. Bu sonuçlar pelvik taban hastalıklarının tedavisinde önemli terapötik potansiyel olduğunu göstermektedir.

Anahtar Kelimeler: İnsan göbek kordonu kök hücreleri, kolajen ekspresyonu, ön vajinal duvar, menopoz sıçanları

Introduction

Pelvic floor dysfunction (PFD) refers to various clinical conditions, such as pelvic organ prolapse (POP), stress urinary incontinence (SUI), overactive bladder, fecal incontinence, and sexual dysfunction[1-4]. The rate of POP and SUI increases with age, especially during menopause, affecting women's quality of life. The prevalence report shows that POP affects 30% of middle-aged women in China, 19% in Australia, and over 50% of women aged over 60 years in the United States. By 2050, symptomatic POP is expected to affect at least 46% of the women population in the United States[5]. Although in Denpasar City of Indonesia, POP was reported in approximately 11.38% of cases within 2 years of evaluation[6,7].

The cause of PFD is complex, with many factors involved: Pregnancy, childbirth, and the structure of the pelvic floor's connective tissue[8]. However, despite the pregnancy and childbirth that most women experienced, not all developed PFD. Any disruptions or conditions affecting connective tissue or neuromuscular support can cause increased PFD[9]. Pelvic bones, pelvic floor muscles, and nerves that serve as supporting tissue components of the pelvic floor work together to suitably function. In addition, various cells and extracellular matrix composing pelvic floor muscle play a crucial role in supporting the pelvic organs. The extracellular matrix consists mainly of collagen, glycoproteins, and proteoglycans. Collagen makes up approximately 70% of this matrix and is essential for the development of PFD. In a study, women with uterine prolapse and cystocele had lower collagen levels than those without POP. The study also found increased collagen degradation in patients with POP, and a higher collagen I/III ratio strengthens connective tissue to reduce POP risk[10].

Currently, treatment for PFD relies on managing symptoms once they appear, but alternative options for preventive treatment are needed[1,2]. PFD typically occurs during menopause; therefore, PFD prevention is highly beneficial at that time. Unsuccessful treatment leads to surgery in women. Autologous fascia or tissue repair has a 11% recurrence rate[11]. Common complications after Transvaginal Mesh (TVM) may include constant pain and deterioration of the urethra/bladder. According to the Food and Drug Administration, over 1000 complications were reported from 2005 to 2007 for POP and SUI treatment, increasing from 2008 to 2010[4]. POP repair with TVM does not have significant clinical benefits, as 30% of patients finish off needing additional surgery[12].

In recent years, stem cell use as an alternative method for PFD has attracted much attention from researchers, and many ***in vivo*** tests have been performed to evaluate its use. Furthermore, human umbilical cord stem cell (HUMSC), the stem cell selection in this study, is proven to be easily accessible and can be obtained in large quantities. HUMSC transplantation leads to the formation of organized connective tissue and increased collagen, facilitating the healing process[13]. This suggests that HUMSC may be beneficial and effective in treating POP[14]. HUMSC is expected to alter the collagen content of the extracellular matrix, improving pelvic floor strength and PFD outcome. In other words, the researchers wanted to evaluate the alteration of collagen expression in the anterior vaginal wall of menopausal model rats in the effect of HUMSC administration. This research carried out the potential of being an initial step to be developed in collaboration with other researchers as well, so that the proposed intervention may further be studied in humans and could later be implemented in daily practice.

Materials and Methods

This research is an experimental study with a randomized post-test-only controlled group design in the Animal Lab. Units, Biomedicine, Udayana Faculty of Medicine, Denpasar.

Sampling

The samples were female Wistar rats that had undergone ovariectomy and were in the Animal Lab. Units, Biomedicine, Udayana Faculty of Medicine, Denpasar. The sample size in the study was calculated on the basis of a calculation formula. Added to the possibility of dropping out of 10% of the study during the research, the minimum number of samples per group is 11. Samples were taken using simple random sampling techniques (random number tables in the Microsoft Excel for Windows program). Rat numbers 1-20 were assigned to the treatment group with HUMSC administration, and rat numbers 21-40 were assigned to the control group without HUMSC administration. Sample inclusion criteria were healthy female Wistar rats with the age of 8-12 weeks, who had been ovariectomized, who had never mated, and weighed 18-22 grams. The exclusion criteria were rats that had previously been used in other experiments, and the dropout criteria were sick rats, including rats who died or were lost in the research.

Research Procedure

Animals were fed pellets (BR 1) and tap water during the acclimatization process for a week before treatment. Body weight was measured, grouping was carried out according to the code with random distribution, and rats that were ready to be ovariectomized for the research process were defined as those whose body weight ranged from 200 to 350 g.

Rat ovariectomy was performed based on the modified method of Ingle DJ and Griffith JQ, 1971. The rat's body weight was measured and then anesthetized with i.m. ketamine at a dose of 40 mg/kgBW. The fur in the abdomen was shaved, the rat was laid on the operating table, sterilized with betadine solution and 70% alcohol, and covered with a sterile drape. A transabdominal incision (1.5-2 cm) is made approximately above the uterus. The incision is made layer by layer until it penetrates the peritoneal wall. The incision wound was pulled to the right and left sides. The fat pads are removed to make it easier to find the oviducts and ovaries. The ovaries look like a bunch of translucent grapes. The ligation is carried out in two places (proximal and distal ovaries) and then continues with the removal of the right and left ovaries. While searching and removing the ovaries, other organs must be kept moist by dripping them with physiological fluids. Before suturing, nebacetin powder is sprinkled in the abdominal cavity. After the wound is stitched, the rat is placed in a cage. Each cage contains only 1 rat. On days 1, 2, and 3 after oophorectomy, a dose of gentamicin i.m. was injected on 5 mg/kg BW/day. During maintenance, adequate drinking and food are provided, with alternating light/dark light for 12 h and at room temperature. Signs of successful oophorectomy can be seen from the appearance of diestrus in the vaginal smear.

Test rats were partitioned into two groups, comprising 18 rats each. The step-by-step procedures included the following: 1) labeling P3-5 HUMSC with DiR dye (XenoLight); 2) subepithelially injecting $3x10^6$ HUMSC into the frontal vaginal wall of test rats, 2 weeks post-ovariectomy; 3) incubating $1 x 10^6$ HUMSC with 1 mL of 80 g/mL DiR at 37 °C for 5 minutes; 4) flushing the HUMSC twice with PBS; 5) euthanizing samples at 12 weeks post-infusion. Following euthanasia, the stomach of the sample rats was dissected to expose the pubic symphysis, while the perineal skin was removed. The frontal vaginal wall was then collected and divided into two fragments: Proximal and distal. The proximal fragment is cut into two parts; one half is for histology and immunohistochemistry use, so it has to be immersed in 10% neutral buffered formalin and embedded in paraffin, while the other half is cryopreserved at 80 °C in OCT compound. In contrast, the distal section is used for mRNA expression analysis; therefore, it must be frozen-stored at 80 °C in liquid nitrogen. Furthermore, before biomechanical testing (4 hours after collecting speciment), the frontal vaginal wall has to be wrapped in a wet gauze and soaked in 0.9% saline.

Preparation of HUMSC from the Infant Umbilical Cord

The research was conducted after obtaining ethical clearance approval from the health research ethics commission of the Faculty of Medicine, Udayana University (approval number: 12704'N14'2'2 vII'l4lLTDo23, date: 15.05.2023). The umbilical cord was obtained from a voluntary donor who signed an informed consent form. Voluntary donors are mothers without a history of hepatitis B, hepatitis C, HIV, cytomegalovirus infection, treponema pallidum infection, or a history of other infections transmitted through the blood, placental tract, and genitals[8].

After the baby is born, the umbilical cord is cut at around 3-5 cm using a sterile knife and stored in a container containing 0.9% normal saline solution, stored at 4 °C and handled aseptically. The surface of the umbilical cord is rinsed with a phosphate salt buffer solution to remove blood adhering to the surface.

Human umbilical cord mesenchymal stem cell extract was prepared using the Quick-DNA Universal Kit, produced by Zymo Research. The sample was human umbilical cord tissue that had been cut negligible and weighed 25 mg.

Statistical Analysis

All data analysis above uses SPSS version 25.0 computer software. Descriptive statistical analysis aimed to describe the results of measuring research variables based on treatment and control groups. The Normality test assesses the distribution of data in each group. The homogeneity of variance test aims to assess whether the data variance between groups is homogeneous or not. The mean comparison test compares the average collagen levels between the groups and assess the treatment of these three levels. The mean comparison is said to be significant if the p-value <0.05.

Results

Characteristics of the Research Subjects

36 samples were divided into 18 control and 18 treatment groups. In this study, the average age of rats when the study began in the treatment group was 76.95±1.31 days, whereas it was 76.45±1.60 days (Table 1). Based on their age, the research subjects from these two groups have passed the reproductive maturity period. The reproductive maturity period for rats begins at the age of 28-42 days, and those aged more than 60 days are classified into the adult stage where the growth and development of their reproductive organs is complete.

Table 1. Demographic characteristics of the treatment and control groups

Characteristics	Treatment group		Control group		p*
	Mean	SD	Mean	SD	
Age (day)	76.95	1.31	76.45	1.60	0.293
Body weight (gram)	211.24	1.73	211.85	1.61	0.275

*: Kolmogorov-Smirnov test, SD: Standard deviation

The results of the Kolmogorov-Smirnov test showed that both groups had normal data distribution (p>0.05), and Levene's test showed that the two groups had homogeneity of variance (p>0.05).

In this study, collagen expression in the frontal wall of menopausal rat vaginas was examined using immunohistochemical techniques. The mean H-score collagen level in the anterior vaginal wall of menopausal rats in the treatment group was 3.2±1.01, whereas it was 1.6±0 in the control group. The mean H-score in the treatment group was higher than that in the control group, and the Shapiro-Wilk test yielded a p-value of <0.05 (Table 2).

Effect of HUMSC Administration on Collagen Expression in a Female Rat Model of Menopause

In the treatment group, five (28%) menopausal rats showed weak-to-moderate collagen expression and 13 (72%) menopausal rats showed high collagen expression. In contrast, within the control group, 18 (100%) menopausal rat showed a weak-moderate collagen expression, and no menopausal rodent showed high collagen expression. With the chi-square test, it was found that the x^2 value=20.3 and the p<0.001, showing a substantial change of collagen expression between the treatment and control groups. Simultaneously, it proves a significant escalation of strong expression of collagen in the damaged anterior vagina of postmenopausal female rats (Figure 1).

Table 2. Distribution of the average H-score for collagen in the treatment and control groups

Variable	Treatment group		Control group		p*
	Mean	SD	Mean	SD	
H-score collagen	3.2	1.01	1.6	0.48	<0.05*

*: Shapiro-Wilk, SD: Standard deviation

Discussion

Due to the limitations of manufactured synthetic mashes for POP, stem cells combined with materials may be a viable therapeutic approach. Achieving therapeutic results *in vivo* is crucial by understanding the interaction between cells and materials and the microenvironment to regulate cell behaviors[15]. However, the limited number of studies evaluating these findings means that recommendations about the effectiveness of evidence-based modalities cannot be established yet. In this study, we evaluated the effect of HUMSC administration on collagen expression in the anterior vaginal wall of menopausal rats.

Table 3. Effect of HUMSC administration on collagen expression in a female rat model of menopause

	Treatment group (n=18)	Control group (n=18)	X^2	p*
Strong-very strong expression Weak-moderate expression	13 5	0 18	20.3	<0.001

HUMSC: Human umbilical cord stem cells

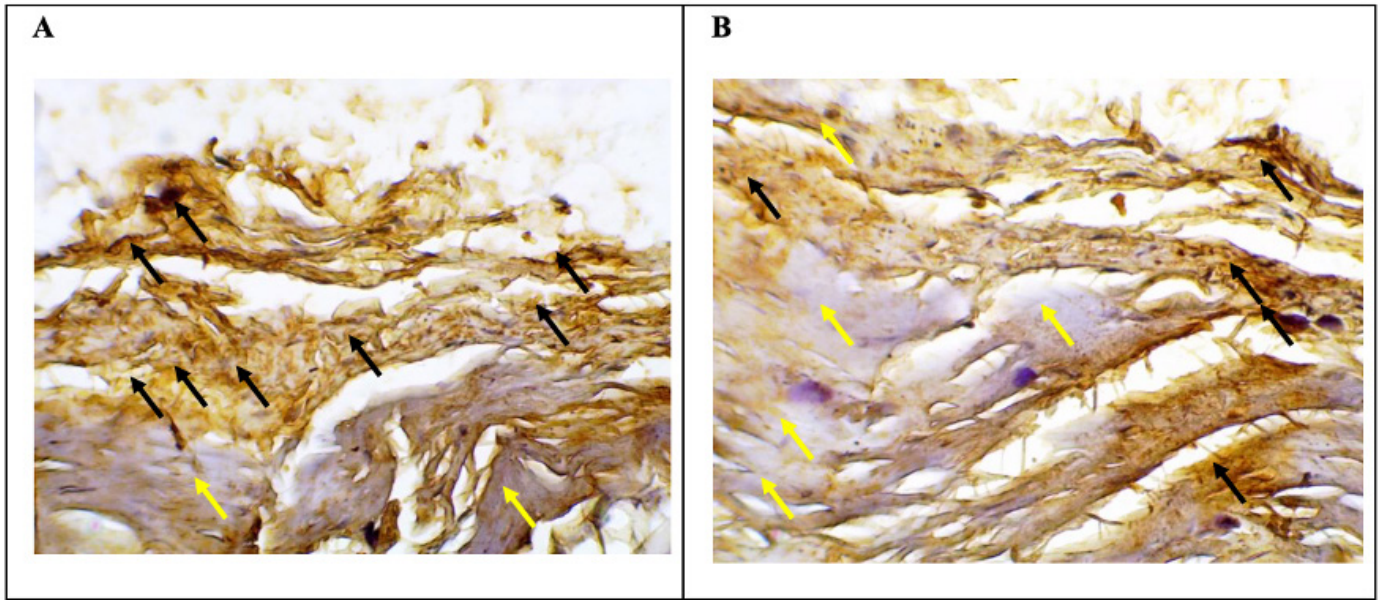

Figure 1. Collagen expression in the treatment group **(A)** and control group **(B)**. **A.** H-score = 4.3. Strong expression. Out of 346 endometrial epithelial cells, 118 epithelial cells were stained with strong intensity; 133 with moderate intensity; 64 with weak intensity; 31 cells were unstained. Magnification 400. Note: intensity 0 = weak, 1 = moderate, 2 = strong, 3 = strong. **(B)** H-score = 1.37. Weak moderate expression. Of the 345 epithelial cells, 7 were stained with strong intensity; 12 with moderate intensity; 82 with weak intensity; 244 unstained. Magnification 400. Note: intensity 0 = weak, 1 = moderate, 2 = strong, 3 = strong

Preclinical research on macaque monkey vaginas showed increased collagen and microvascular thickness in the vaginal lamina propria after transplantation of mesenchymal stem cells from the umbilical cord. In addition, smooth muscle in the vagina also increased. Mesenchymal stem cell transplantation enhances vaginal biomechanics by increasing the elastic modulus of the vagina and making it stiffer[10]. It is still difficult to develop animal models for POP-related studies. Strategies like vaginal extending, ovariotomy, or prolapse in rats/sheep are commonly performed. Insertion is typically found underneath the stomach or vaginal wall. Cell-based tissue plan strategies have improved vaginal repair in rats by obtaining epithelial cell phenotypes and reducing inflammation caused by TVM in sheep vagina[16]. We used female Wistar rats that were ovariectomized, never mated, 8-12 weeks, and weighed 18-22 grams. These rats can be used as a model in this test, and surgical procedures are available.

Research conducted on rat showed that application of HUCMSC to the vaginal walls of ovariectomized Sprague-Dawley rat increased collagen levels, and the collagen I:III ratio of HUMSC is thought to not only increase collagen but also the expression of genes that produce collagen based on research examining gene expression after administration of HUMSC[17].

Smooth muscle plays an important role in supporting the pelvic viscera and vaginal wall. In women with POP, there is a decrease in smooth muscle. Our study showed that increasing collagen in the anterior vaginal wall of postmenopausal female rats can significantly improve smooth muscle expression in the damaged anterior vaginal wall. Matrix metalloproteinases (MMPs) and issue inhibitors of metalloproteases (TIMPs) affect extracellular component homeostasis. Mesenchymal stem cells can create MMPs and TIMPs from the extracellular matrix. The study found a decrease in MMP2, MMP9, and MMP13, which break down collagen types I and II. Mesenchymal stem cells aid in reducing collagen degradation[16].

Smooth muscle morphology changes are crucial in pelvic organ prolapse. The increase in smooth muscle cells is due to MSCs differentiating into them. However, studies have shown that MSCs can also control smooth muscle cells through paracrine effects. The paracrine impact is due to the release of exosome and various active substances from stem cells through exosome or microvesicles, including proteins, RNA, hormones, and chemicals[16].

Changes in vaginal biomechanics may occur with an increase in the elastic modulus during MSC transplantation. This aligns with research on POP patients, which reduces type I collagen but simultaneously increases type III collagen. The changes occur due to changes in the extracellular matrix and smooth muscle regeneration of vaginal walls[16].

Advanced glycation end (AGE) are non-enzymatic products of the glycation and oxidation of proteins and lipids. AGE affects POP by increasing cross-linking in the prolapsed tissue. This is because AGE affects collagen metabolism through AGE receptors without affecting expression or structure. AGE activates the P-P38 pathway, along with MAPK and NF-kB-p-p65, to regulate collagen metabolism[18].

In vivo studies have shown that human stem cells can affect AGE. The ability of HUMSCs to release anti-inflammatory cytokines (such as IL-4, IL-6, and IL-10) might protect against the toxic effects of AGE-induced fibroblasts. The use of MSC treatment for POP has been studied in preclinical studies, with possible mechanisms being to activate the P13K/AKT/PTEN pathway to protect against AGEs' cytotoxic effects[18]. MSCs transplantation in preclinical studies with intravenous or urethral administration also reported the potential to treat urinary and fecal incontinence by increasing the smooth muscle cells, vascular thickness, and connective tissue in the periurethra[10]. Tissue engineering combines cells with materials or mesh to provide support to pelvic tissue, immune modulating, and anti-inflammatory capacities[19].

The spectrum of trophic components created by MSCs consists of exosomes, cytokines, and chemokine, or secretomes. Recent studies have focused on the secretory function rather than the differentiation ability of these cells. This is called acellular therapy, which does not require live cell transplants. This decreases transportation and simplifies standardizing the work. *In vivo* research revealed the effects of exosomes on increasing type I collagen and collagen degradation in vaginal fibroblasts[19].

Study Limitations

This study has some limitations: It did not investigate the fate of HUMSCs after transplantation *in vivo*; it had a small sample size of animal groups due to ethical concerns; and the ovariectomized model used in this study lacked symptoms of prolapse. In addition, further evaluation of the possible interaction of pro- and anti-inflammatory factors on the effectiveness of HUMSC in treating POP is needed, and a longer postoperative follow-up assessment is also necessary to monitor the efficacy of this procedure. Furthermore, because of the small size of the rat vagina, evaluating postoperative complications and biomechanical properties is challenging. We recommend that future research should focus on building and validating POP models with clinical phenotypes, and more studies in larger animal models or human patients and proteomic analysis are needed.

Conclusion

Administration of HUMSC to postmenopausal female rats resulted in an escalation toward collagen levels in the anterior vaginal wall. These results demonstrate significant therapeutic potential for treating PFD cases (possibly referring to a condition or disease). The therapeutic implications of the increase in collagen levels could be an important basis for developing more effective therapeutic strategies for PFD cases (if referring to a medical condition).

Ethics

Ethics Committee Approval: The research was conducted after obtaining ethical clearance approval from the Health Research Ethics Commission of the Faculty of Medicine, Udayana University (approval number: 12704'N14'2'2 vII'l4lLTDo23, date: 15.05.2023).

Informed Consent: The umbilical cord was obtained from a voluntary donor who signed an informed consent form.

Authorship Contributions

Surgical and Medical Practices: K.F.M., I.W.P.S.Y., A.J.K., I.N.M.A., Concept: K.F.M., I.W.P.S.Y., A.J.K., I.N.M.A., Design: K.F.M., I.W.P.S.Y., A.J.K., I.N.M.A., Data Collection or Processing: K.F.M., I.W.P.S.Y., A.J.K., I.N.M.A., Analysis or Interpretation: K.F.M., I.W.P.S.Y., A.J.K., I.N.M.A., Literature Search: K.F.M., I.W.P.S.Y., A.J.K., I.N.M.A., Writing: K.F.M., I.W.P.S.Y., A.J.K., I.N.M.A.

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Is there a relationship between the psychological state of infertile patient and ovarian reserve indicators?

İnfertilite hastalarının psikolojik durumu ile over rezervi göstergeleri arasında bir bağlantı var mı?

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Abstract

Objective: This study explored the relationship between reduced ovarian reserve and the psychological state of infertile women.

Materials and Methods: This cross-sectional, single-center study was conducted with 106 infertile women. The Beck Depression Inventory (BDI) was used to assess patients' propensity for depression. The data relating to infertility, such as causes of infertility, type of infertility (primary or secondary), duration of infertility, and treatment status [previous assisted reproductive technologies (ART) treatment and ART treatment failure] were recorded for each patient. The ovarian reserve was determined using laboratory tests [anti-Mullerian hormone (AMH); follicle-stimulating hormone (FSH)] and transvaginal ultrasonography to measure the antral follicle count (AFC) in each ovary.

Results: There was no significant relationship between the total score obtained from the Beck depression scale and AFC, AMH, thyroid-stimulating hormone, FSH, estradiol, and prolactin measurements (p>0.05). There was no significant difference between the groups regarding depression levels based on the cause of infertility (p=0.412). Additionally, the type of infertility (primary, secondary) did not differ between the groups (p=0.586). There were no differences on the BDI scale regarding the level of depression between patients who underwent in vitro fertilization (IVF) treatment (history of previous IVF treatment failure) and those who did not.

Conclusion: There was no significant association between AFC and AMH levels and the depression state of infertile patients.

Keywords: Infertility, ovarian reserve, mood disorders, depression

Öz

Amaç: Bu çalışmanın amacı, azalmış over rezervi ile infertil hastaların depresyon eğilimi arasındaki ilişkiyi araştırmaktır.

Gereç ve Yöntemler: Bu kesitsel tek merkezli çalışma 106 infertil kadın ile yürütülmüştür. Hastaların depresyon eğilimini değerlendirmek için Beck Depresyon Envanteri (BDE) kullanıldı. İnfertilite ile ilgili veriler: İnfertilite nedenleri, infertilite tipi (primer ve sekonder), infertilite süresi, tedavi durumu [önceki yardımcı üreme teknolojileri (YÜT) tedavisi ve YÜT tedavi başarısızlığı] her hasta için kaydedildi. Over rezervi laboratuvar testleri [anti-Mullerian hormonu (AMH); folikül uyarıcı hormon (FSH)] ve her bir overdeki antral folikül sayısını (AFC) ölçmek için transvajinal ultrasonografi kullanılarak belirlendi.

Bulgular: BDE'den elde edilen toplam puan ile AFC, AMH, tiroid uyarıcı hormon, FSH, estradiol ve prolaktin ölçümleri arasında anlamlı bir ilişki yoktu (p>0,05). İnfertilite nedenine göre depresyon düzeyleri açısından gruplar arasında anlamlı bir fark bulunmamıştır (p=0,412). Ek olarak infertilite tipine (primer, sekonder) göre gruplar arasında farklılık yoktu (p=0,586). YÜT tedavisi gören (daha önce YÜT tedavisi başarısızlığı öyküsü olan) ve görmeyen hastalar arasında depresyon düzeyi için BDE ölçeğinde fark yoktur.

Sonuç: AFC and AMH düzeyleri ile infertil hastaların depresyon durumu arasında anlamlı bir ilişki saptanmadı.

Anahtar Kelimeler: İnfertilite, over rezervi, duygudurum bozuklukları, depresyon

PRECIS: We evaluated the relationship between reduced ovarian reserve and the psychological state of infertile patients.

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Introduction

Infertility is defined as the inability of a couple to conceive after 12 months of regular unprotected intercourse[1]. Worldwide, infertility is estimated to affect 8-12% of couples of reproductive age[2]. The importance of poor ovarian responders was reemphasized by Drakopoulos et al.[3] in 2020; in their study, the authors rated this condition as frustrating for both the patient and fertility expert. Low ovarian reserve is a cause of infertility, which is clinically associated with a poor response to gonadotropin stimulation and a low success rate of in vitro fertilization (IVF) cycles[4]. Several factors are associated with poor ovarian response. The Bologna Criteria for poor ovarian response has recently been defined as follows: patient age (⩾40 years), previous poor ovarian response experience with ⩽3 oocytes retrieved after conventional stimulation and/or an abnormal ovarian reserve test, antral follicle count (AFC) <7 or anti-Mullerian hormone (AMH) <1.1 ng/mL)[5].

Currently, AMH, follicle-stimulating hormone (FSH), and AFC are markers used to determine ovarian reserve. Unlike FSH, the most important feature of AMH is that it is a marker that can be used regardless of the menstrual cycle. This feature facilitates the use of AMH as a laboratory parameter for ovarian reserve determination on the day of the patient's admission. Therefore, the use of AMH for patients with infertility has increased in recent years in gynecological practice[6]. Another useful tool for investigating ovarian reserve is transvaginal ultrasonography (TVUS), which provides an instant (momentary) overview of AFC. Using TVUS, we can measure the number of primordial follicles in each ovary.

The association between hormonal dysfunction and mood-related disorders is a topic "under the magnifying glass" for many scientists worldwide[7]. Thyroid dysfunction is the most common autoimmune endocrine disease in women of reproductive age and is associated with menstrual irregularities, anovulation, and infertility. Hypothyroidism is considered a potential risk factor for female infertility. Atis et al.[8] showed that a significant percentage of women with clinical and subclinical hypothyroidism have sexual dysfunction. Impairment of sexual function is an important indicator of depression and related pathologies[9]. The study by Atis et al.[8] showed that hyperprolactinemia, hyperlipidemia, and depression were associated with female sexual dysfunction in clinical hypothyroidism. In another study, the authors explained an increased risk of depression in women compared with men because of fluctuations in estrogen levels that occur during reproductive cycle events, especially during the menopausal transition[10]. There are studies in the literature showing the relationship between depressive disorders and the premature ovarian aging process[11,12]. In the present study, we aimed to explore the relationship between poor ovarian reserve (POR) and the psychological state (tendency to depression) of infertile women.

Materials and Methods

This cross-sectional study, with 106 infertile women, was conducted in our public academic tertiary hospital between 08/15/2021 and 01/15/2022. All patients were divided into four groups according to the cause of infertility (female, male, both genders, and unexplained infertility). All participants were informed about the subjects of this study. The study inclusion criteria were volunteering to participate, being examined for infertility in our hospital, and patient data accessibility. Study exclusion criteria were patients who did not agree to participate in the investigation, those who applied to our hospital for infertility but did not undergo a detailed laboratory and ultrasonographic examination, and conditions or diseases that prevented filling questionnaires, such as loss of vision, hearing or sensory, motor, and cognitive (dementia, psychosis) skills.

Demographic and social characteristics (age, education level, employment status, financial status) and obstetric characteristics (number of gravidas, parities, history of abortion, and comorbid diseases) were evaluated in each group. The data relating to infertility, such as the causes of infertility (female, male, both, and unexplained infertility), type of infertility (primary or secondary), duration of infertility, duration of marriage (duration of the relationship with a partner), and treatment status [previous assisted reproductive technologies (ART) treatment, and ART treatment failure] were recorded for each patient. The ovarian reserve was determined using laboratory tests (AMH; FSH) and TVUS. We used TVUS to measure the number of antral follicles in each ovary[13]. Each patient underwent ultrasonography evaluation by two gynecologists, and AFC was determined for each ovary[14].

AFC was performed in the early follicular phase of the menstrual cycle; according to the assessment, we divided patients into three groups: normal ovarian reserve, POR, and patients with polycystic ovary morphology[15,16]. The study involved infertility patients whose levels of FSH, thyroid-stimulating hormone (TSH), estradiol (E2), and prolactin (PRL) were measured on the third day of the menstrual cycle. In addition, serum progesterone levels were measured in the midluteal phase of the menstrual cycle.

In our study, the Beck Depression Inventory (BDI) was used to identify the severity of depression[17]. We considered the BDI inventory "an indicator of the presence and degree of depressive symptoms" because of its worldwide use. BDI provides a psychiatric assessment of the depth of depression (severity of depressive symptoms). Inventory is a self-report questionnaire consisting of 21 questions (items). The answers were summed up to obtain a total score that ranged from 0 to 63, with the total score reflecting the severity of depression. There were four levels of depression according to the BDI scale:

- GROUP 1 (0-12): Minimal depression
- GROUP 2 (13-18): Mild depression
- GROUP 3 (19-29): Moderate depression
- GROUP 4 (30-63): Severe depression

After completing the questionnaire, the patient was referred to a psychiatrist for further evaluation, counseling, and treatment arrangements.

The study was approved by the Ethics Committee of University of Health Sciences Turkey, Bursa City Hospital (approval number: 2021-13/9, date: 14.07.2021).

Statistical Analysis

The Shapiro-Wilk test was used to decide whether or not a sample fit a normal distribution. Continuous variables were expressed as median (minimum: maximum) values, whereas categorical variables were expressed as n (%) values. The Kruskal-Wallis test was used if normality was not observed. Categorical variables were compared using the chi-square test and Fisher-Freeman Halton test. The relationships between AMH, TSH, FSH, E2, and prolactin parameters and the total score obtained from the depression scale were analyzed using correlation analysis, and the Spearman correlation coefficient was calculated. To calculate the sample size needed in our study, the article published by Nicoloro-SantaBarbara et al.[18] was used as a reference study, and a priori power analysis was performed. The authors examined the magnitude and predictors of emotional responses to the diagnosis of infertility in two groups of women: those with diminished ovarian reserve (DOR) and those clinically diagnosed with an anatomical cause of infertility. In their study, the authors reported the infertility distress level as 137.05±32.21 for the DOR group (n=51) and 118.05±31.90 for the ACI group (n=51), and the effect size value calculated using these values was determined as d=0.59. As a result of the a priori power analysis, using the relevant effect size value (d=0.59), considering the type I error as 5% and the targeted power level as 80%, a total of n=106 subjects were included in the study, taking into account possible losses. Analyses were conducted using G*Power[19] and SPSS (IBM Corp. Released 2019. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp.) program, and the significance level was set at α=5%.

Results

In total, 106 infertile women were included in the study. The median age of the participants in the study was 31 years. The median number for gravida and parity was 0. When the distribution was examined according to abortion status, the proportion of participants who had experienced abortion was 31.10%, whereas the proportion of participants who had not was 68.90%. The percentage of participants with chronic disease was 10.40%. The median marriage duration of the participants was 4 years, and the median infertility duration was 2 years. The proportion of participants with primary infertility was 65.10%, whereas that of those with secondary infertility-was 34.90%. When patients were examined according to the cause of infertility, it was found that 13.50% were due to male factors, 53.80% were due to female factors, 2.90% were due to both male and female factors, and 29.80% were due to unexplained infertility. It was determined that 56.60% of the participants had never received any treatment before, whereas 21.70% had previous treatment experience. The remaining 20.7% of the patients did not provide clear information about their previous treatment history. The median AMH (ng/mL) level of the patients was 2.87, whereas the median TSH (mIU/L) level was 2.28 (Table 1).

According to the BDI scale, no patients were found to be in group 4 with a score of more than 30, indicating severe depression. There was no significant difference between the groups regarding the depression state based on the cause of infertility (p=0.412). It was determined that there was no difference in marriage duration between the groups (p=0.264). Infertility duration also did not differ between the groups (p=0.169). The type of infertility did not differ between the groups (p=0.586). The treatment status did not differ between the groups (p=0.847), as was the case with the education level (p=0.645) and employment status (p=0.848). The financial status also

Table 1. General characteristics of participants in the study

Age, years (n=106)	31 (19:44)
Gravida (n=106)	0 (0:6)
Parity (n=106)	0 (0:3)
Abortion (n=106)	
Yes	33 (31.10%)
No	73 (68.90%)
Comorbid disease (n=106)	
Yes	11 (10.40%)
No	95 (89.60%)
Duration of marriage (n=106)	4 (0.33:21)
Duration of infertility (n=106)	2 (0.33:15)
Type of infertility (n=106)	
Primary	69 (65.10%)
Secondary	37 (34.90%)
Cause of infertility (n=104)	
Male	14 (13.50%)
Female	56 (53.80%)
Both	3 (2.90%)
Unexplained	31 (29.80%)
Treatment history (n=83)	
Absent	60 (56.60%)
Present	23 (21.70%)
AMH (ng/mL) (n=95)	2.87 (0.03:6.98)
TSH (mIU/L) (n=95)	2.28 (0.01:30.20)

Data were presented as median (minimum: maximum) and n%, AMH: Anti-Mullerian hormone, TSH: Thyroid-stimulating hormone

did not differ between the groups (p=0.487). There was no significant difference in AMH measurement (p=0.713), TSH measurement (p=0.520), or prolactin measurement (p=0.082) between the groups. Depression status did not differ between the groups based on the distribution of the right ovarian AFC (p=0.706) or left ovarian AFC (p=0.642) (Table 2).

There was no significant relationship between the total score from the depression scale and AMH, TSH, FSH, E2, and prolactin measurements (p>0.05) (Table 3).

Tablo 2. Comparisons of demographic and clinic characteristics according to depression status

	n	Group 1	n	Group 2	n	Group 3	p-value
Cause of infertility							
Male	55	7 (12.70%)	37	6 (16.20%)	12	1 (8.30%)	0.412[a]
Female		29 (52.70%)		17 (45.90%)		10 (83.30%)	
Both		1 (1.80%)		2 (5.40%)		0	
Unexplained		18 (32.70%)		12 (32.40%)		1 (8.30%)	
Duration of marriage	57	4 (0.33:21)	37	4 (0.50:17)	12	7 (1.17:20)	0.264[b]
Duration of infertility	57	2 (0.33:8)	37	1.50 (0.50:15)	12	2.50 (1:11)	0.169[b]
Type of infertility							
Primary	57	38 (66.70%)	37	22 (59.50%)	12	9 (75%)	0.586[c]
Secondary		19 (33.30%)		15 (40.50%)		3 (25%)	
Treatment history							
Absent	43	31 (72.10%)	34	24 (70.60%)	6	5 (83.30%)	0.847[a]
Present		12 (27.90%)		10 (29.40%)		1 (16.70%)	
Education level							
Below high school	57	14 (24.60%)	37	8 (21.60%)	12	5 (41.30%)	0.645[a]
High school		14 (24.60%)		8 (21.60%)		3 (25%)	
Above high school		29 (50.90%)		21 (56.80%)		54 (50.90%)	
Working status							
Employed	54	30 (55.60%)	36	18 (50%)	12	6 (50%)	0.848[c]
Unemployed		24 (44.40%)		18 (50%)		6 (50%)	
Financial status							
Below minimum wage	57	0	37	1 (2.70%)	12	0	0.487[a]
Minimum wage		20 (35.10%)		11 (29.70%)		4 (33.30%)	
Above minimum wage but less than 2 times		23 (40.40%)		14 (37.80%)		3 (25%)	
More than 2 times minimum wage		14 (24.60%)		11(29.70%)		5 (41.70%)	
AMH (ng/mL)	56	2.45 (0.03:9.11)	34	2.92 (0.02:6.40)	12	2.24 (0.03:5.90)	0.713[b]
TSH (mIU/L)	52	2.45 (0.01:30.20)	34	2.06 (0.34:6.41)	11	2.58 (0.01:7.90)	0.520[b]
Prolactin (µg/L)	51	21.60 (4.94:60.8)	28	18.90 (6.75:138)	12	12.15 (3.71:31.10)	0.082[b]
Right ovary							
POR	54	18 (33.30%)	34	14 (41.20%)	11	2 (18.20%)	0.706[a]
NOR		16 (29.60%)		10 (29.40%)		4 (36.40%)	
PCOM		20 (37%)		10 (29.40%)		5 (45.50%)	

Tablo 2. Continued

	n	Group 1	n	Group 2	n	Group 3	p-value
Left ovary							
POR	56	21 (37.50%)	36	14 (38.90%)	11	3 (27.30%)	0.642[a]
NOR		11 (19.60%)		11 (30.60%)		3 (27.30%)	
PCOM		24 (42.90%)		11 (30.60%)		5 (45.50%)	

POR: Poor ovarian reserve, NOR: Normal ovarian reserve, PCOM: Polycystic ovary morphology, The data are expressed as median (minimum: maximum) and n (%), [a]: Fisher Freeman Halton test, [b]: Kruskal Wallis test, [c]: Chi-square test, AMH: Anti-Mullerian hormone, TSH: Thyroid-stimulating hormone

Table 3. The relation between hormone levels and BDI score

n=106	BDI total score	
	r_s	p-value
AMH (ng/mL)	-0.44	0.662
TSH (mIU/L)	-0.04	0.650
FSH (mIU/L)	-0.02	0.807
E2 (ng/L)	-0.05	0.624
Prolactin (µg/L)	-0.14	0.170

r_s: Spearman correlation coefficient, AMH: Anti-Mullerian hormone, TSH: Thyroid-stimulating hormone, BDI: Beck Depression Inventory, FSH: Follicle-stimulating hormone, E2: Estradiol

Discussion

Infertility is a widespread concern that affects numerous couples globally. According to the World Health Organization, infertility is a "disease" of the reproductive system. It can be described as a "reproductive deficiency" that is not life-threatening and does not have physical impacts. However, reproductive deficiency may have detrimental effects on personality development, leading to frustration and weakening personality because most couples believe that having children is a life goal[20].

Infertility's consequences vary, including social impact, emotional breakdown, and severe distress in women's health. Birenbaum-Carmeli and Dirnfeld[21] highlight the importance of understanding the cultural and social context in which infertility occurs and is treated. However, the duration of the infertile period may play a key role in the psychological state of the infertile woman. Our study did not find a significant relationship between the duration of infertility and depression tendency (Table 2). We believe this is due to the short mean period of infertility in the patients in our study.

In some cultures, infertility is seen as a motherhood disability, which puts significant pressure on women to conceive and bear children[22]. This can lead to severe emotional distress, anxiety, and depression, especially if medical treatments are unsuccessful. This is why depression and other associated mood disorders can be seen in infertile women. Interestingly, the results of the present study did not show differences between primary and secondary infertility on the BDI scale in terms of the level of depression.

Hammarberg et al.[23] pointed out that while most women were satisfied with the treatment, many reported feeling anxious and stressed during the treatment process. Some women also reported experiencing depression and emotional difficulties, particularly if treatment was unsuccessful. Medicalization of infertility has inadvertently reduced the negative emotional reactions experienced by infertile couples[24]. Furthermore, advances in ART, such as IVF, offer hope to infertile couples who fear not becoming parents[25]. Perhaps this is how we can explain the lack of differences on the BDI scale for the level of depression between patients who underwent IVF treatment (history of previous IVF treatment failure) and those who did not in the present study. However, it is essential to acknowledge that infertility is a complex and emotionally charged issue that can significantly impact the mental health of individuals and couples. Atis et al.[8] found that hypothyroidism was associated with higher depression scores. In contrast, our study found no significant relationship between TSH levels and the BDI score. Atis et al.[8] also noted that mean PRL levels were significantly higher in patients with clinical and subclinical hypothyroidism. Our study found no difference in BDI scores based on hormone levels (Table 3).

One potential factor contributing to depression and other mood disorders in infertile women is ovarian dysfunction and POR. We know that a strong relationship exists between POR and treatment success. Infertile patients can have an idea about the success of the treatment because of doing individual research and being informed by the physician about the results of the tests. Considering all these conditions, a poor response to treatment with a POR may affect the psychological state of the patient compared with patients with normal ovarian reserve. Maki noted that women in the menopausal period commonly experience increased depressive symptoms and depressive disorders[26]. Frey et al.[27] also indicated that identifying individuals who might be at a higher risk for depression during the menopausal transition could guide preventive strategies for this population. The underlying mechanism for these symptoms is not fully understood, but it may involve the regulation of serotonin and norepinephrine by ovarian hormones. The authors also noted that this association requires

further research. Our study investigated the impact of impaired ovarian reserve on patients' psychological status. Following this goal, we examined the ovarian reserve of each patient using ultrasound and laboratory methods. Nicoloro-SantaBarbara et al.[18] reported that women with DOR had significantly higher infertility distress scores than women with anatomical causes of infertility. However, our study results did not show the impact of AFC and AMH values on the level (severity) of depression. This may be because this study's median duration of infertility was short (2 years), and the patients were well-informed about treatment regimens and treatment opportunities at our tertiary care center. Low ovarian reserve causes concern and may subsequently delay treatment and further evaluation of infertile patients[28]. However, the results of this study showed that there is no relationship between depression and low ovarian reserve. This is possible because of the development of ART worldwide and the easy availability of treatment methods in our country, which gives patients hope of conceiving.

Study Limitations

Our study has several limitations that should be considered when interpreting the results. First, the study's cross-sectional design does not allow us to conclude causality. Second, we only used self-report measures to assess depression and anxiety, which may not provide an accurate assessment of these conditions. Third, we assessed only the psychological state of women and did not consider the psychological state of their partners. Future research in this field should include a study with a control group.

Conclusion

Infertility is a complex issue with significant emotional and psychological consequences for both individuals and couples. Although medical procedures such as IVF can offer hope to infertile couples, they still experience stress and emotional instability during treatment. It is important to understand the cultural and social context in which infertility occurs and is treated and the potential factors that can cause depression and other mood disorders in infertile women. A factor facilitating the treatment process of women with infertility, and compliance with treatment, is the patient's psychological state. We examined the effect of ovarian reserve indicators on the psychological status of women with infertility. Our study results show that the impacted ovarian reserve is not a determinant of psychological status in infertile women.

Ethics

Ethics Committee Approval: The study was approved by the Ethics Committee of University of Health Sciences Turkey, Bursa City Hospital (approval number: 2021-13/9, date: 14.07.2021).

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

Authorship Contributions

Concept: S.R.O., E.Ü., Design: S.R.O., Data Collection or Processing: S.R.O., Z.A., E.O, M.A.T., Analysis or Interpretation: B.B.Y., Literature Search: S.R.O., Z.A., Writing: S.R.O., Z.A., B.A.

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Vitamin D evaluation in adenomyosis: A retrospective cross-sectional study

Adenomyoziste D vitamini değerlendirmesi: Retrospektif kesitsel çalışma

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Abstract

Objective: Adenomyosis is a chronic inflammatory illness that depends on estrogen. In addition to its immune regulatory effects in chronic diseases, vitamin D also plays roles in regulating normal cell growth. In the present study, the purpose was to evaluate the possible relationships between serum 25-OH vitamin D levels and clinical and laboratory parameters in patients who were histopathologically diagnosed with adenomyosis.

Materials and Methods: A total of 168 females with a history of hysterectomy between January 2019 and November 2022 who were histopathologically diagnosed with adenomyosis and 168 women who were not diagnosed with adenomyosis were retrospectively evaluated in the present study. Demographic, clinical, and laboratory data were recorded at the time of admission. Visual analogue scale (VAS) scores were calculated for each patient to evaluate the severity of dysmenorrhea.

Results: There was a significant difference between the groups in terms of VAS: the adenomyosis group scored an average of 6, whereas the control group scored an average of 3 ($p<0.001$). The average platelet volume value of the patients was 8.6 fL in the adenomyosis group, and that of the control group was 7.2 fL, and it was detected to be significantly elevated in the adenomyosis group ($p<0.001$). The CA-125 value of the patients was 63.5 U/mL in the adenomyosis group, and that of the control group was 15.6 U/mL and it was detected to be significantly rised in the adenomyosis group ($p<0.001$). The 25-OH vitamin D level of the patients was 12.6 ng/mL in the adenomyosis group and that of the control group was 19.1 ng/mL and it was detected to be significantly elevated in the control group.

Conclusion: The current investigation provides compelling evidence for the association between low vitamin D levels and adenomyosis, which agrees with other research in the field. The current study's findings agree with other research that suggests vitamin D regulates cellular and signaling networks, including those that control cytokines and gene expression during adenomyosis. However, further studies are needed because data assassing the therapeutic efficacy of vitamin D in adenomyosis are questionable.

Keywords: Adenomyosis, dysmenorrhea, hysterectomy, 25-OH vitamin D

Öz

Amaç: Adenomyozis, östrojen bağımlı kronik enflamatuvar bir durumdur. D vitamini, kronik hastalıklardaki immün düzenleyici etkilerinin yanı sıra normal hücre büyümesinin düzenlenmesinde de rol oynar. Çalışmamızda histopatolojik olarak adenomyozis tanısı koyulan hastaların serum 25-OH vitamin D düzeyleri ile klinik ve laboratuvar parametreler arasındaki olası ilişkileri değerlendirmeyi amaçladık.

Gereç ve Yöntemler: Çalışmamızda Ocak 2019-Kasım 2022 tarihleri arasında histerektomi yapılan ve histopatolojik olarak adenomyozis tanısı konulan 168 kadın ile adenomyozis tanısı konulmayan 168 kadın retrospektif olarak değerlendirildi. Başvuru sırasında demografik, klinik ve laboratuvar verileri kaydedildi. Dismenorenin şiddetini değerlendirmek için her hastada vizüel analog skala (VAS) puanı hesaplandı.

PRECIS: Our study strongly supports the association between low 25-OH D vitamin levels and adenomyosis and deep infiltrative endometriosis.

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Bulgular: Adenomyozis grubunda VAS ortalaması 6, kontrol grubu vizüel ağrı skoru ortalaması ise 3 saptanmış olup gruplar arasında anlamlı fark saptanmıştır (p<0,001). Adenomyozis grubunda hastaların ortalama trombosit hacim değeri 8,6 fL, kontrol grubundakilerin ise 7,2 fL saptanmış olup, adenomyozis grubunda anlamlı yüksek saptanmıştır (p<0,001). Adenomyozis grubunda hastaların CA-125 değeri 63,5 U/mL, kontrol grubundakilerin ise 15,6 U/mL saptanmış olup, adenomyozis grubunda anlamlı yüksek saptanmıştır (p<0,001). Adenomyozis grubunda hastaların 25-OH D vitamini seviyesi 12,6 ng/mL, kontrol grubundakilerin ise 19,1 ng/mL saptanmış olup, kontrol grubunda anlamlı yüksek saptanmıştır.

Sonuç: Literatürdeki çalışmalar ile uyumlu olarak, çalışmamız düşük D vitamini düzeyleri ile adenomyozis arasındaki ilişkiyi güçlü bir şekilde desteklemektedir. Çalışmamız, adenomyozisde gen ekspresyonları ve sitokinleri içeren, hücresel ve sinyal yollarının düzenlenmesinde D vitamininin rolünü öne süren çalışmalarla tutarlı olmuştur. Bununla birlikte D vitamininin adenomyozisdeki terapötik etkinliğini değerlendiren veriler şüpheli olduğundan ileri çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Adenomyozis, dismenore, histerektomi, 25-OH D vitamini

Introduction

The development of endometrial glands and stroma in in the myometrium is a characteristic of the estrogen-dependent chronic inflammatory disease known as adenomyosis. Following reactive hypertrophic and/or hyperplastic changes to the surrounding myometrium, adenomyosis results in uterine enlargement[1]. The presence of endometrial tissue along the inner surface of the uterus outside the uterus is a characteristic of chronic endometriosis. The endometrial origin and the anatomical location of the lesions are thought to be the two main distinctions between endometriosis and adenomyosis[2]. Endometriosis and adenomyosis share several characteristics in terms of symptomatology, histology, and molecular changes[3,4]. There are distinctions in the pathogenesis and pathogenic mediators of these two separate diseases[5]. Invagination of the basalis endometrium into the myometrium and *de novo* development from metaplasia of embryonic mullerian remnants or endometrial stem/progenitor cells with the myometrium are the two most widely recognized ideas about the pathophysiology of adenomyosis. Previous investigations have identified a panel of pathways involving tissue damage or injury at the endometrial-myometrial interface, which can result in inflammation, localized production of estrogen, and the adenomyosis development[6]. Adenomyosis often develops in the 4th-5th week of life during the middle of a decade and after childbearing is finished. Nevertheless, newer imaging techniques, including magnetic resonance imaging and transvaginal ultrasonography, have revealed that adenomyosis can also affect younger females[7]. Adenomyosis patients have various issues, including severe painful symptoms and reduced quality of life due to irregular uterine bleeding. This progressive illness can lead to infertility or subfertility that requires appropriate treatment[8]. Although it is common and the symptoms are severe, little data are available about its pathogenesis. The reason for this may be because of the long-standing dependence on histopathological examination of uterine samples after hysterectomy and the lack of reliable preoperative diagnosis for disease diagnosis[9]. Because of variations in the diagnostic criteria and methods for obtaining myometrial samples, histological evaluation of hysterectomy tissues reveals a prevalence of adenomyosis ranging from 5% to 70%[10]. It has been reported that many risk factors such as alcohol use, smoking, body mass index (BMI), age at menarche, and parity may be responsible for the pathogenesis of adenomyosis[11]. In addition to these risk factors, other risk factors that are still controversial are included in the literature. One of these factors, vitamin D, plays a role in regulating both healthy cell development and immune system effects in chronic diseases[12]. vitamin D stimulates the synthesis of anti-inflammatory cytokines while decreasing the production of pro-inflammatory cytokines, in addition induce programed cell death, and suppresses neovascularization[12]. Considering these data, our purpose was to assess potential relationships between serum 25-OH vitamin D levels and laboratory and clinical characteristics in individuals with histopathologically confirmed adenomyosis diagnoses.

Materials and Methods

This study was designed in a retrospective cross-sectional fashion following the Helsinki Declaration. An informed consent form was received from the patients, and the rules regarding animal rights were followed. The study received approval from the Ethics Committee of Dokuz Eylül University School of Medicine (date: 13/09/2023, number: 2023/28-20). A total of 168 women who underwent hysterectomy and were histopathologically diagnosed with adenomyosis between January 2019 and November 2022 and 168 women who were not diagnosed with adenomyosis were retrospectively evaluated. The study group contained both premenopausal and postmenopausal females between the ages of 35 and 50 years with histopathologically detected adenomyosis. The control group contained 168 females who underwent hysterectomy because of abnormal uterine bleeding, postmenopausal bleeding, myoma uteri, and adnexal mass, and no adenomyosis was detected. Patients with vitamin D use during the last 6 months before operation, the presence of any systemic disease, the presence of known malignancy, menopause and hormone replacement therapy, oral contraceptive use, and a known diagnosis of bilateral or unilateral endometrioma were excluded from the study. Laboratory, clinical, and demographic data (e.g., age, BMI, smoking, age at first menarche, alcohol consumption, and obstetric history) were recorded at the time of admission. Visual analogue scale (VAS) scores were calculated for each patient to evaluate the severity of dysmenorrhea. A VAS value of "0" denoted the absence of pain, whereas a VAS value of "10" represented the maximum level of discomfort. The presence of

deep infiltrative endometriosis (DIE) detected during surgery was retrospectively obtained from patient files.

Statistical Analysis

The analyses were conducted using SPSSx26.0 (IBM Inc., Chicago, IL, USA). Normality analysis was conducted using the Kolmogorov-Smirnov test. The quantitative data of the patients were reported as mean ± standard deviation (minimum-maximum). Non-normally distributed variables were analyzed using the Mann-Whitney U test. These outcomes are presented as median (minimum-maximum) values for each group. Fisher's Exact test and the chi-square test were employed to assess the categorical data, and the results are presented as counts and percentages (%). A 95% confidence interval was used to analyze the results. A p-value less than 0.05 was regarded as statistically significant.

Results

In this research, the average age of the participants was 43±3.9, and no significant difference was detected between the adenomyosis groups and the control group (p=0.1). The average BMI of the participants was 22.6±1.5 kg/m^2, age at menarche was determined as 12±1, and no significant difference was detected between the groups in this respect (p=0.9, p=0.3, respectively). The average gravida of the participants in this research was 2.2±0.9, the parity mean 2±0.8, and no significant difference was observed between the groups (p=0.5, p=0.07, respectively). No significant difference was observed between the groups with regard to smoking and alcohol use of the patients (p=1, p=0.8, respectively). The average visual pain score in the adenomyosis group was 6, and the average visual pain score in the control group was 3. A significant difference was detected between the groups in this respect (p<0.001). No significant difference was detected about operation indications between the patients in the adenomyosis group and the control group (p=0.8). The presence of DIE was detected to be significantly elevated in the adenomyosis group than in the control group (p<0.001) (Table 1).

The average hemoglobin of the participants in the adenomyosis group was 11.1 g/dL, and that of the control group was 11.3 g/dL, and it was detected to be significantly elevated in the control group (p<0.001). The average hematocrit of the patients was 33.5% in the adenomyosis group and 34.1% in the control group, and it was detected to be significantly elevated in the control group (p<0.001). The average leukocyte level of the patients was detected to be 7.5±1.2 n/mL, and no significant difference was detected between the groups (p=0.09). The average platelet volume value of the patients in the adenomyosis group was 8.6 fL, and that of the control group was 7.2 fL, and it was detected to be significantly elevated in the adenomyosis group (p<0.001). The neutrophil/lymphocyte ratio (NLR) of the patients in the present study was 1.8±0.5, the platelet/lymphocyte ratio (PLR) was 98.8±34.5, and no significant difference was detected between the groups in this respect (p=0.7, p=0.6, respectively). The CA-125 value of the patients was 63.5 U/mL in the adenomyosis group, and that of the control group was 15.6 U/mL, and it was detected to be significantly increased in the adenomyosis group (p<0.001). The 25-OH vitamin D level of the patients in the adenomyosis

Table 1. Demographic and clinical characteristics of the groups

Variables	Group 1 (n=168, 50%)	Group 2 (n=168, 50%)	All patients (n=336, 100%)	p-value
Age (years)	43 (35-50)	44 (35-52)	43±3.9 (35-52)	0.1
BMI (kg/m^2)	22.6 (18.7-29.4)	22.6 (18.6-29.1)	22.6±1.5 (18.6-29.4)	0.9
Age at menarche (years)	12 (10-15)	12 (10-15)	12±1 (10-15)	0.3
Gravida	2 (0-6)	2 (0-4)	2.2±0.9 (0-6)	0.5
Parity	2 (0-5)	2 (0-4)	2±0.8 (0-5)	0.07
Smoking (10 packs/day)	28.6% (48/168)	28.6% (48/168)	28.6% (96/336)	1.0
Alcohol	29.8% (50/168)	28.6% (48/168)	29.2% (98/336)	0.8
VAS	6 (3-9)	3 (1-9)	4.8±1.8 (1-9)	<0.001
Operation indication				
*Abnormal uterine bleeding	32.1% (54/168)	27.4% (46/168)	29.8% (100/336)	0.8
*Postmenopausal bleeding	11.3% (19(168)	12.5% (21/168)	11.9% (40/336)	
*Myoma uteri	35.1% (59/168)	36.3% (61/168)	35.7% (120/336)	
*Adnexal mass	21.4% (36/168)	23.8% (40/168)	22.6% (76/336)	
Presence of deep infiltrative endometriosis	30.4% (51/168)	0% (0/168)	15.2% (51/336)	<0.001

BMI: Body mass index, VAS: Visual analog scale

group was 12.6 ng/mL, and that of the controligroup was 19.1 ng/mL, and it was detected to be significantly elevated in the control group (p<0.001) (Table 2).

The mean hemoglobin of the adenomyosis patients was 10.8±0.6 g/dL in the present study, and the average hematocrit was 32.8±2%, and no significant difference was detected between positive and negative patients for the presence of DIE (p=0.5, p=0.7, respectively). The mean leukocyte count of the adenomyosis patients was 7.6±1.1 n/mL, the mean platelet volume value was 8.6±0.5 fL, and no significant difference was observed between the groups (p=0.8, p=0.7, respectively). The NLR of the adenomyosis patients in this research was 1.7±0.5, the PLR was 96.2±29.4, and no significant difference was detected between the groups in this respect (p=0.8, p=0.5, respectively). The mean CA-125 of the adenomyosis patients was 66.2±20 U/mL, and no significant difference was detected between the groups (p=0.6). The 25-OH vitamin D level of patients with positive DIE presence was detected to be 12.3 ng/mL, and that of patients with negative DIE presence was 13.1 ng/mL, and it was detected to be significantly lower in the DIE positive group (p=0.03) (Table 3).

Discussion

The visual pain score was detected to be elevated in the adenomyosis group than in the control group in this study. The mean platelet volume level was elevated in the adenomyosis group compared with the control group. CA-125 level was elevated in the adenomyosis group compared with the control group. The average 25-OH vitamin D level in the DIE-positive group was lower than that in the DIE-negative group. The majority of studies conducted on vitamin D and adenomyosis in the literature suggest that increased dairy product consumption and omega-3 fatty acid consumption are linked to a lower incidence of endometriosis and adenomyosis by providing high levels of 1.25-OH vitamin D3 in the circulation[13-15]. Previous studies in the literature report that Vitamin D Binding Protein (VDBP) significantly increases ectopic endometrium and serum VDBP levels are elevated in females with endometriosis and adenomyosis[16,17].The pathophysiology of this association may include variation in VDBP (GC-2) levels, as proposed by Faserl et al.[17]. females with endometriosis are more likely to have the GC-2 polymorphism. The GC-2 polymorphism may be linked to insufficient macrophage phagocytism activation, perhaps leading to an inability to stop the implantation of endometriotic tissue in the peritoneum. Becker et al.[18] showed that 1α-hydroxylase expression in damaged endometrial tissue was significantly elevated compared with that in healthy tissues. However, there are conflicting reports in the literature regarding this relationship. Hartwell et al.[19] suggested that the serum 25-OH-D3 level increased in females with damaged endometrial tissue compared with the control group and that this difference was statistically significant. Delbandi et al.[20] reported that 25-OH-D3 levels were similar for females having and not having adenomyosis. However, these outcomes are not in line with the outcomes of other research suggesting that high dietary vitamin D intake decreases the risk of developing adenomyosis and endometriosis. A higher demand for vitamin D can be inferred from women with endometriosis who had elevated VDBP concentrations in both their blood and endometriotic tissue. Becker et al.[18] reported an increase in receptor synthesis and an acceleration of the conversion of vitamin D to its active form in endometriotic tissue. It was found in this study that serum 25-OH-D3 levels decreased in females with adenomyosis, in accordance with these findings. Skowrońska et al.[21] according to his suggestion, the severity of the sickness determines the level of vitamin D. This study demonstrated a correlation between DIE and 25-OH vitamin D levels. Therefore, our findings suggest that the 25-OH vitamin D level may be used as a marker to gauge the severity of the illness. According to Helde-Frankling and Björkhem-Bergman[22], vitamin D consumption is linked to a reduction in pain, which may be the consequence of prostaglandin (PG) inactivation and reduced production. 15

Table 2. Laboratory characteristics of the groups

Variables	Group 1 (n=168, 50%)	Group 2 (n=168, 50%)	All patients (n=336, 100%)	p-value
Hemoglobin (g/dL)	11.1 (8.1-12.2)	11.3 (8.6-12.3)	11±0.6 (8.1-12.3)	<0.001
Hematocrit (%)	33.5 (25.5-38.1)	34.1 (25.6-36.8)	33.3±1.8 (25.2-38.1)	<0.001
Leukocyte (n/mL)	7.6 (4.5-11.6)	7.5 (4.2-11.4)	7.5±1.2 (4.2-11.6)	0.09
MPV (fL)	8.6 (7.5-9.8)	8.2 (7.4-9.2)	8.4±0.5 (7.4-9.8)	<0.001
NLR	1.6 (0.9-3.9)	1.6 (0.9-3.8)	1.8±0.5 (0.9-3.9)	0.7
PLR	91.9 (39.3-194.5)	92.5 (41.4-233.3)	98.8±34.5 (39.2-233.3)	0.6
CA-125 (U/mL)	63.5 (19.8-152.6)	15.6 (8.6-54.1)	41.4±28.9 (8.6-152.6)	<0.001
25-OH vitamin D (ng/mL)	12.6 (8.9-31.2)	19.1 (10.8-34.5)	16.1±4.2 (8.9-34.5)	<0.001

NLR: Neutrophil/lymphocytei ratio, PLR: Platelet/lymphocytei ratio, MPV: Mean platelet volume

Table 3. Comparison of laboratory characteristics of adenomyosis cases according to the presence of deep infiltrative endometriosis (DIE)

Variables	Group 1 (DIE+) (n=51, 30.4%)	Group 2 (DIE-) (n=117, 69.6%)	All patients (n=168, 100%)	p-value
Hemoglobin (g/dL)	11.1 (8.1-12.1)	11.1 (8.8-12.2)	10.8±0.6 (8.1-12.2)	0.5
Hematocrit (%)	33.5 (25.5-36.2)	33.5 (25.6-38.1)	32.8±2 (25.5-38.1)	0.7
Leukocyte (mL)	7.8 (4.5-10.8)	7.6 (4.5-11.6)	7.6±1.1 (4.5-11.6)	0.8
MPV (fL)	8.6 (7.5-9.8)	8.6 (7.6-9.7)	8.6±0.5 (7.5-9.8)	0.7
NLR	1.6 (0.9-3.6)	1.6 (0.9-3.9)	1.7±0.5 (0.9-3.9)	0.8
PLR	94.9 (53.2-194.5)	90.7 (39.3-181.7)	96.2±29.4 (39.2-194.5)	0.5
CA-125 (U/mL)	68.4 (30.6-152.6)	61.5 (19.8-108.6)	66.2±20 (19.8-152.6)	0.06
25-OH-D vitamin (ng/mL)	12.3 (8.9-31.2)	13.1 (9.1-22.5)	13.1±2.5 (8.9-31.2)	0.03

NLR: Neutrophil/lymphocytei ratio, PLR: Platelet/lymphocytei ratio, MPV: Mean platelet volume

OH-PG dehydrogenase can be induced and cyclooxygenase 2 can be suppressed to accomplish this. Anastasi et al.[23] reported a significant relationship between insufficient 25-OH vitamin D levels and severe and moderate pelvic pain. Given that 25-OH vitamin D has anti-inflammatory properties and can reduce inflammation in endometriotic foci, it is possible that injured endometriotic tissues consume 25-OH vitamin D. A substantial linear association between blood 25-OH vitamin D levels and the width of ovarian endometrioma was observed by Ciavattini et al.[24]. This may be a possible explanation for the significant decreasing trend because there is more endometriotic tissue in bilateral endometrioma than in unilateral endometrioma. Considering the factors that may affect the development of adenomyosis, such as vitamin D form, receptor status of vitamin D, gene polymorphism, and immunological status, in addition to the inconsistent results in the literature, it can be concluded that several theories account for the effects of vitamin D on adenomyosis. Nevertheless, the precise vitamin D threshold level at which immune impairment occurs is unclear.

Study Limitations

The present study had some limitations, such as being retrospective, a limited number of patients, and the use of patient records in the healthcare system. This study sheds light on the previously unexplored link between 25-OH vitamin D and adenomyosis. It also had strengths, such as representing one of the few studies revealing the link between 25-OH vitamin D and dysmenorrhea.

Conclusion

The present study results are consistent with the data of other studies in the literature. This study strongly supports the link between low vitamin D levels and adenomyosis. This study is also consistent with studies reporting the role of vitamin D in regulating cellular and signaling pathways, including gene expression and cytokines, in adenomyosis. However, further studies are needed because there is doubtful evidence to assess the therapeutic effectiveness of vitamin D in adenomyosis. Further study is necessary because the evidence supporting the use of increasing dietary vitamin D consumption as a preventative strategy is limited, despite the encouraging statistics.

Ethics

Ethics Committee Approval: The study received approval from the Ethics Committee of Dokuz Eylül University School of Medicine (date: 13/09/2023, number: 2023/28-20).
Informed Consent: Retrospective study.

Authorship Contributions

Surgical and Medical Practices: U.A., S.E., Concept: O.Y., Design: O.Y., Data Collection or Processing: H.A.A., C.A., T.B.B., Analysis or Interpretation: T.B.B., Literature Search: H.A.A., C.A., Writing: U.A, S.E.
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Review / Derleme

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Correlation between the existence of serum autoantibodies and the risk of endometriosis: A systematic review and meta-analysis

Serum otoantikorlarının varlığı ile endometriozis riski arasındaki korelasyon: Sistematik bir derleme ve meta-analiz

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Abstract

Endometriosis is a common condition among women and can cause complications such as abdominal pain, dysmenorrhea, and infertility. One of the potential causes of this disease is autoimmunity. However, evidence regarding the role of autoimmunity is conflicting and inconclusive. The aim of this study was to investigate whether autoantibodies, a sign of autoimmunity, are present in people suffering from endometriosis. Relevant studies up to April 14, 2023 were identified by systematically searching Scopus, PubMed, Web of Science, Embase, and Google Scholar. This meta-analysis includes all qualified case-control studies of human populations that analyzed the association between serum autoantibodies and endometriosis. The odd ratios and 95% confidence intervals were calculated. In addition, heterogeneity and publication bias were examined, and subgroup analyses were performed based on region and target antigens. Forty-one studies were included, comparing 2,825 endometriosis patients with 4,158 healthy controls. The meta-analysis findings indicated a significant association between the presence of autoantibodies in the serum and an increased susceptibility to endometriosis (odds ratio: 4.242, confidence interval 95%: 3.824-4.706, p<0.001). In addition, there was a significant correlation between the presence of endometriosis and serum levels of anti-nuclear antibodies, B2 glycoprotein 1, CA125, carbonic anhydrase 1, cardiolipin, endometrial, laminin-1, smooth muscle, and syntaxin autoantibodies. Upon further analysis, it was found that the serum levels of these autoantibodies were higher in patients with endometriosis from North America than in those from other regions (p=0.001). The study revealed a significant correlation between serum autoantibodies and susceptibility to endometriosis, highlighting autoimmunity as a potential cause.

Keywords: Autoantibody, endometriosis, meta-analysis, serum

Öz

Endometriozis kadınlarda sık görülen bir durumdur ve karın ağrısı, dismenore ve infertilite gibi komplikasyonlara neden olabilir. Bu hastalığın potansiyel nedenlerinden biri otoimmünitedir. Ancak otoimmünitenin rolüne ilişkin kanıtlar çelişkili ve belirsizdir. Bu çalışmanın amacı, endometriozis hastası kişilerde otoimmünite belirtisi olan otoantikorların mevcut olup olmadığını araştırmaktır. 14 Nisan 2023 tarihine kadar olan ilgili çalışmalar Scopus, PubMed, Web of Science, Embase ve Google Scholar'da sistematik olarak arama yapılarak belirlendi. Bu meta-analiz, serum otoantikorları ile endometriozis hastaları arasındaki ilişkiyi analiz eden insan popülasyonlarına ilişkin tüm nitelikli olgu-kontrol çalışmalarını içermektedir. Olasılık oranları ve %95 güven aralıkları hesaplanmıştır. Ayrıca heterojenlik ve yayın yanlılığı incelenmiş, bölge ve hedef antijenlere göre alt grup analizleri yapılmıştır. Bu yazıya 2.825 endometriozis hastasını 4.158 sağlıklı kontrolle karşılaştıran 41 çalışma dahil edilmiştir. Meta-analiz bulguları, serumda otoantikorların varlığı ile endometriozise karşı artan duyarlılık arasında anlamlı bir ilişki olduğunu göstermiştir (risk oranı: 4,242, güven aralığı %95: 3,824-4,706, p<0,001). Ayrıca endometriozis varlığı ile anti-nükleer antikorlar, B2 glikoprotein 1, CA125, karbonik anhidraz 1, kardiyolipin, endometrial, laminin-1, düz kas syntaxin antikorlarının serum seviyeleri arasında anlamlı bir korelasyon olduğu bulunmuştur. Daha ileri analizler sonucunda, Kuzey Amerika'daki endometriozisli hastalarda bu otoantikorların serum seviyelerinin diğer bölgelerdekilere göre daha yüksek olduğu bulunmuştur (p=0,001). Çalışma, serum otoantikorları ile endometriozise yatkınlık arasında anlamlı bir korelasyon olduğunu ortaya çıkarmış ve otoimmünitenin potansiyel bir neden olduğunu vurgulamıştır.

Anahtar Kelimeler: Otoantikor, endometriozis, meta-analiz, serum

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Introduction

Endometriosis is a prevalent medical condition characterized by endometrial tissue in extrauterine locations[1]. This misplaced tissue responds to hormones and can cause internal bleeding, inflammation, and fibrosis[2]. A definitive diagnosis of endometriosis is performed after biopsy by surgery; therefore, its exact prevalence is uncertain[3]. However, it is estimated to affect 10-15% of women of reproductive age[4]. People affected by this disease experience symptoms such as pelvic pain, dysmenorrhea, and infertility[5].

The exact etiology of this endometriosis remains unknown. However, previous research has shown that both genetic and environmental factors contribute to its development. Epigenetic abnormalities, anomalous estrogen production, retrograde menstruation, autoimmune responses, and allergic reactions are potential etiological factors[6,7].

Several studies have been conducted on the role of autoimmune responses as a potential etiological factor in endometriosis. They have demonstrated that endometriosis is correlated with persistent regional inflammation and autoantibodies. Their results showed that women with endometriosis display immune system abnormalities similar to those seen in autoimmune diseases[8,9]. This includes disrupted immune surveillance, abnormal T and B-cell functions, heightened humoral immune response with increased autoantibodies in the serum, and inflammatory tissue damage[9,10].

Although several studies have explored the connection between the presence of autoantibodies in the serum and the likelihood of developing endometriosis, the results have been inconsistent. The aim of this study was to analyze all relevant research and perform a meta-analysis to investigate this association.

Materials and Methods

Eligibility Criteria, Information Sources, and Search Strategy

This meta-analysis followed the Preferred Reporting Items for Systematic Reviews and Meta-analysis guidelines[11]. Various databases, including Scopus, PubMed, Web of Science, EMBASE, and 30 pages of Google Scholar, were searched to identify relevant studies. The search was not restricted by language and covered the period from April 2023. The references of the included studies were also hand-searched. The search strategies consisted of the terms "endometriosis", "autoantibodies", "serum" and their related synonyms.

Women diagnosed with endometriosis based on standard criteria confirmed by laparoscopic sampling and/or histological examination will participate in this review. This study will compare endometriosis patients with healthy women of the same age group.

Study Selection

This meta-analysis included all available case-control studies that met the following criteria: Analysis of the association between autoantibodies and endometriosis patients provides necessary information for a meta-analysis.

The exclusion criteria were as follows: Letters, editorials, abstracts, conference abstracts, or publications lacking adequate information; studies that used women with diseases other than endometriosis, particularly autoimmune diseases; and studies that explored the presence of autoantibodies in bodily fluids or tissues other than serum.

Data Extraction

Two reviewers independently extracted the pertinent data from the selected studies. If necessary, the investigators resolved the differences through discussion and consultation with a third-party. The information extracted was entered into an Excel form, including the last name of the first author, date and location of the study, number of participants in both the case and control groups, mean age of participants, method of diagnosing endometriosis, subclass of autoantibodies, sample type, method of evaluating autoantibodies, and the number or mean value and standard deviation of autoantibody-positive cases and controls.

Assessment of the Risk of Bias

The articles' quality was evaluated by two reviewers using the Newcastle-Ottawa Quality Assessment scale (NOS)[12], and any discrepancies were resolved by a third reviewer. Articles that achieved a NOS score of 7 or above were classified as high quality, whereas those with scores between 5 and 7 were moderate quality.

Data Synthesis

All data were statistically analyzed using Comprehensive meta-analysis software version 3.0 (Biostat, USA). To determine the odds ratios (ORS) and their corresponding 95% confidence intervals (CIS), the statistical analysis used the groups' sample size, mean serum autoantibody level, and standard deviation for both patients diagnosed with endometriosis and the healthy control group. Some studies presented continuous variables as median and quartile, which were converted to mean and standard deviation using the formula in the Cochrane Handbook[13,14]. The heterogeneity of the studies was assessed using Cochrane Q and I^2 statistics. The random-effects model was used to estimate the outcome data in cases where the Cochrane Q p-value was less than 0.1 and the I^2 value exceeded 50%, indicating the presence of statistical heterogeneity. Conversely, a fixed-effects model was employed in other instances. Subgroup analysis was performed to evaluate the impact of confounding variables on the outcomes of the meta-analysis. A sensitivity analysis was also conducted, in which each study was systematically excluded to assess the stability of the obtained results.

Results

Study Selection

After conducting a comprehensive search of the available resources, 1,053 studies were obtained. We then removed any duplicate sources and examined the titles and abstracts of 663 studies. Of these, 71 studies were selected for full-text review. Finally, after applying the inclusion and exclusion criteria, 41 studies were included in our meta-analysis. The process of literature screening and its outcomes are presented in Figure 1. The studies included in the analysis were assigned ratings ranging from zero to nine based on the Ottawa-Newcastle scale for case-control studies. As shown in Table 1, thirty-three studies were assessed as being of high quality, whereas eight studies were evaluated as being of moderate quality.

Study Characteristics

In line with the research methodology, we included a total of forty-one eligible case-control studies to investigate the correlation between the presence of autoantibodies and the risk of endometriosis. Eighteen studies were conducted in Europe[15-32], eleven in Asia[33-43], nine in North America[29,44-51], and four in South America[52-55]. The basic characteristics of the included studies are listed in Table 1 and Table 2. Table 3 shows the number of studies investigating each autoantibody.

Risk of Bias of the Included Studies

Funnel plots, Begg's rank correlation, and Egger's regression tests were used to examine the existence of publication bias. Figure 2 and Table 3 present the outcomes of publication bias. Based on the results of statistical tests and asymmetry analysis of funnel plots, there is a possible publication bias in the studies that investigated pooled and anti-laminin-1 autoantibodies (as shown in Table 3). However, in other autoantibody studies, although a few exhibits slight visual asymmetry, statistical tests do not indicate any significant publication bias. The funnel plot diagrams, which show the likelihood of publication bias in studies on autoantibodies, were modified using the trim and fill test. The modifications did not result in any significant changes in studies that investigated the total and anti-laminin-1 autoantibodies (data not shown).

Synthesis of the Results

The findings of the meta-analysis indicate a substantial correlation between the existence of autoantibodies and susceptibility to endometriosis (OR: 4.242, CI 95%: 3.824-4.706, p<0.001) (Figure 3). Furthermore, individuals with endometriosis exhibited significantly higher levels of anti-nuclear antibodies (anti-ANA) (OR: 5.862, CI 95%: 3.454-9.950, p<0.001), B2 glycoprotein 1 (OR: 4.542, CI 95%: 1.360-15.175, p=0.014), CA125 (OR: 3.602, CI 95%: 1.485-8.733, p=0.005), carbonic anhydrase 1 (OR: 6.860, CI 95%: 3.043-15.468, p<0.001), cardiolipin (OR: 6.806, CI 95%: 3.369-13.749, p<0.001), endometrial (OR: 11.793, CI 95%: 2.382-58.383, p=0.002), laminin-1 (OR: 6.340, CI 95%: 3.151-12.757, p<0.001), smooth muscle (OR: 8.041, CI 95%: 4.442-14.557, p<0.001), and syntaxin (OR: 3.815, CI 95%: 1.249-11.649, p=0.019) autoantibodies than healthy controls. Statistical analysis found no significant relationship between autoantibodies against enolase (OR: 2.067, CI 95%: 0.739-5.782, p=0.167) and PDIK1L (PDLIM1 interacting kinase 1 like) (OR: 1.434, CI 95%: 0.241-8.528, p=0.692) and susceptibility to endometriosis.

The relevant forest plots are presented in Figure 4. It is worth mentioning that the conducted studies[52,53,55] failed to detect anti-dsDNA autoantibodies in individuals suffering from endometriosis, indicating the absence of any correlation.

Heterogeneity Test and Subgroup Analysis

The I^2 test and Cochrane Q statistic showed heterogeneity among studies analyzing total, anti-CA125, anti-endometrial, anti-enolase, anti-PDIK1L, and anti-syntaxin autoantibodies. However, studies related to other autoantibodies showed no significant between-study heterogeneity, as shown in Table 3. To investigate the cause of heterogeneity, subgroup analysis was conducted based on the region. The findings revealed that patients with endometriosis from North America had notably higher levels of autoantibody titers than patients from other regions (p=0.001), and the region of living was identified as one of the sources of heterogeneity. In addition, subgroup analysis based on differences in target antigens of autoantibodies demonstrated this factor as a cause of heterogeneity in the study of pooled autoantibodies (p<0.001).

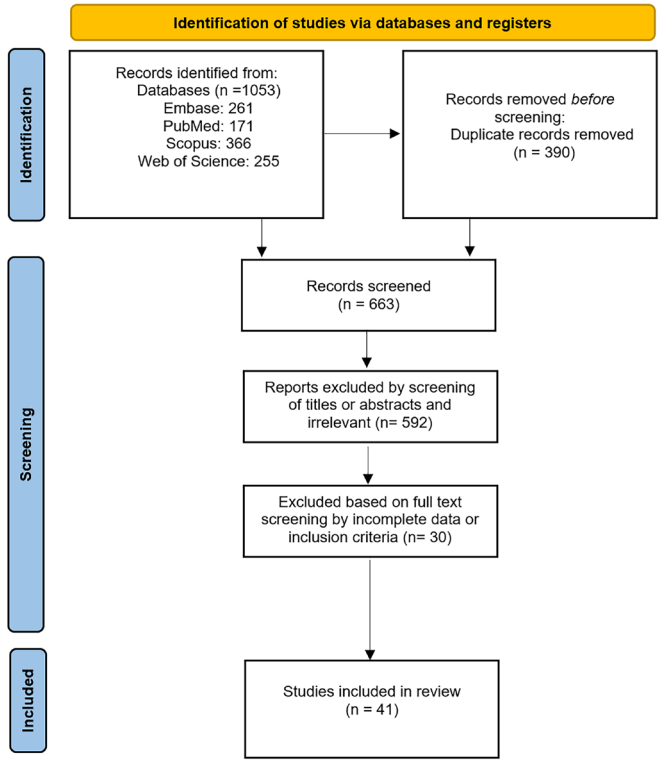

Figure 1. The flow chart of literature search and study selection

Table 1. Basic characteristics of the included studies

First author	Year	Country	Ethnicity	Endometriosis diagnosis protocol	Mean age or age range case	Mean age or age range control	NOS score
Mathur et al.[44]	1982	USA	North American	Laparoscoy and biopsy or laparotomy	26-37	26-37	6
Taylor et al.[15]	1991	UK	European	Laparoscoy and biopsy or laparotomy	33.5	31.6	6
Gorai et al.[33]	1993	Japan	Asian	Laparoscopy or laparotomy	21-46	20-46	7
Fernández-Shaw et al.[16]	1993	UK	European	Laparoscopy	-	-	7
Kiechle et al.[45]	1994	USA	North American	Laparoscoy	34.8	32.6	7
Kim et al.[34]	1995	Korea	Asian	Laparoscopy or laparotomy	30-40	-	6
Aoki et al.[46]	1995	USA	North American	Laparoscopy	33	33	7
Nip et al.[17]	1995	UK	European	Laparoscopy	34	34	8
Odukoya et al.[18]	1995	UK	European	Laparoscopy and histology	33	33	7
Pillai et al.[50]	1996	USA	North American	Laparoscopy	-	25-35	6
Hatayama et al.[35]	1996	Japan	Asian	Laparoscopy or laparotomy	35.3	35.3	6
D'Cruz et al.[47]	1996	USA	North American	Laparoscopy	<45	<45	7
Fernández-Shaw et al.[19]	1996	UK	European	Laparoscopy	-	-	7
Kim et al.[36]	1997	Korea	Asian	Laparoscopy	32	32	7
Kaider et al.[48]	1999	USA	North American	Laparoscopy	-	-	8
Shanti et al.[51]	1999	USA	North American	Histologically	18-45	-	7
Szczepańska et al.[25]	2001	Poland	European	Laparoscopy	29.0	-	7
Reimand et al.[26]	2001	Estonia	European	Laparoscopy and histology	27.5	31.0	8
Ulcová-Gallová et al.[32]	2002	Czech Republic	European	Laparoscopy	35.6	35.6	6
Pasoto et al.[52]	2002	Brazil	South American	Laparoscopy	18-40	18-40	7
Inagaki et al.[43]	2003	Israel	Asian	Laparoscopy and laparotomy	33.7	29.6	7

Table 1. continued

First author	Year	Country	Ethnicity	Endometriosis diagnosis protocol	Mean age or age range case	Mean age or age range control	NOS score
Pasoto et al.[53]	2005	Brazil	South American	Laparoscopy or laparotomy and histological	18-40	18-40	7
Haller et al.[27]	2006	Estonia	European	Laparoscopy	33.0±5.5	-	7
Randall et al.[49]	2007	USA	North American	Laparoscopy	31.8±6.5	31.8±6.5	7
Haller et al.[28]	2007	Estonia	European	Laparoscopy	34.0±4.8	44.9±10.7	8
Caccavo et al.[20]	2007	Italy	European	Laparoscopy	34.4±4.2	33.3±3.1	7
Aguiar et al.[54]	2009	Brazil	South American	laparoscopy and histology	29.2±5.6	27.9±4.5	8
Nabeta et al.[37]	2009	Japan	Asian	Laparoscopy	34.7±7.6	35.0±3.7	6
Nabeta et al.[38]	2010	Japan	Asian	laparosccopy or laparotomy	35.2±8.0	34.2±9.3	7
Inagaki et al.[41]	2011	Japan	Asian	Laparoscopy or laparotomy and histology	31.9	29.6	9
Caccavo et al.[21]	2011	Italy	European	laparoscopy and histology	34.8±3.7	-	7
Nabeta et al.[39]	2011	Japan	Asian	Laparoscopy or laparotomy	35.2±8.0	34.2±9.3	7
Gajbhiye et al.[42]	2012	India	Asian	Laparoscopy and histology	31.2±4.9	28.9±5.3	8
Ozhan et al.[22]	2014	Turkey	European	Laparoscopy or laparotomy	32.33±7.01	34.20±6.88	7
Gajbhiye et al.[40]	2017	India	Asian	Laparoscopy and histology	32.6±6.3	32.13±6.03	8
Yu-Rice et al.[29]	2017	USA & Germany	North American and European	-	28-70	18-65	7
Ek et al.[23]	2019	Sweden	European	Laparoscopy	38.0	42.5	7
Toullec et al.[30]	2020	French	European	Histologically	33.00±5.365	-	7
Artymuk et al.[31]	2021	Russia	European	Laparoscopy and histology	31.6±4.8	31.8±6.5	6
Svensson et al.[24]	2022	Sweden	European	Laparoscopy	32.0-43.0	33-53	7
Vilas Boas et al.[55]	2022	Brazil	South American	Laparoscopy and histology	37.2±7.1	37.8±5.6	8

NOS: Newcastle-Ottawa Quality Assessment scale

Table 2. The fundamental details of the selected studies about the evaluation of autoantibodies

First author	Year	Case number	Control number	Ig type	Sample type	Method	The investigated autoantibody
Mathur et al.[44]	1982	13	15	IgG	Serum	Passive haemagglutination	Endometrial Ovary Granulosa Theca
Taylor et al.[15]	1991	71	109	IgG, IgM and IgA	Serum	IF, WB, Counterimmunoelectrophoresis and Double immunodiffusion	Cardiolipin ANA Lupus anti-coagulant Smooth muscle RO (SS-A) La (SS-B)
Gorai et al.[33]	1993	18	27	-	Serum	WB	Endometrial
Fernández-Shaw et al.[16]	1993	13	7	IgG	Serum	IHC	Endometrial Endothelial
Kiechle et al.[45]	1994	23	17	IgG	Serum	WB	Carbonic anhydrase I
Kim et al.[34]	1995	33	20	IgG	Serum	IHC and WB	Endometrial
Aoki et al.[46]	1995	64	97	IgG	Serum	ELISA	Cardiolipin B2 Glycoprotein 1 Antiphospholipid antigens (PA, PG, PE, PI, PS)
Nip et al.[17]	1995	20	20	IgG and IgM	Serum	ELISA and IF	Cardiolipin ANA Smooth muscle
Odukoya et al.[18]	1995	55	43	IgG	Serum	ELISA	Endometrial Ovary Thyroid Skeletal muscle
Pillai et al.[50]	1996	46	18	IgG	Serum	Passive hemagglutination	Endometrial Transferrin Alpha 2-Heremans Schmidt (HS) Glycoprotein
Hatayama et al.[35]	1996	20	20	IgG	Serum	ELISA	Endometrial
D'Cruz et al.[47]	1996	319	100	IgG	Serum	ELISA	Carbonic anhydrase I and II
Fernandéz-Shaw et al.[19]	1996	51	23	IgG	Serum	ELISA	Endometrial
Kim et al.[36]	1997	42	87	IgG	Serum	ELISA	Cardiolipin ANA Lupus anti-coagulant
Kaider et al.[48]	1999	23	105	IgG, IgM and IgA	Serum	ELISA	Antiphospholipid antigens (PL) ANA Thyroid
Shanti et al.[51]	1999	40	16	IgG	Serum	ELISA	Malondialdehyde-modified Oxidized low-density lipoprotein Lipid peroxide-modified
Szczepańska et al.[25]	2001	50	20	IgG	Serum	ELISA	Zona pellucida

Table 2. continued

First author	Year	Case number	Control number	Ig type	Sample type	Method	The investigated autoantibody
Reimand et al.[26]	2001	38	392	IgG	Serum	IF and ELISA	B2 Glycoprotein 1 Carbonic anhydrase I ANA Smooth muscle Parietal cell Thyroid microsomal Mitochondrial
Ulcová-Gallová et al.[32]	2002	323	101	IgG	Serum	ELISA	Cardiolipin B2 Glycoprotein 1 Antiphospholipid antigens (PA, PG, PE, PI, PS) Zona pellucida
Pasoto et al.[52]	2002	39	18	-	Serum	IF	ANA RO/LA RNP/Sm dsNDA
Inagaki et al.[43]	2003	42	39	IgG	Serum	ELISA	Laminin-1
Pasoto et al.[53]	2005	45	21	IgG and IgM	Serum	IF and ELISA	Cardiolipin Carbonic anhydrase I ANA RO (SS-A) dsDNA Histon Smooth muscle U1RNP
Haller et al.[27]	2006	12	56	IgG	Serum	IF and ELISA	ANA Ovary Smooth muscle Parietal cell Thyroid microsomal Mitochondrial B2 Glycoprotein I Cardiolipin
Randall et al.[49]	2007	278	249	IgG	Serum	IF	Endometrial
Haller et al.[28]	2007	12	85	IgG, IgA and IgM	Serum	ELISA and IF and ImmunoCAP technology	FSH ANA Ovary Smooth muscle Parietal cell Thyroid microsomal Mitochondrial B2 Glycoprotein I Cardiolipin
Caccavo et al.[20]	2007	18	16	IgG and IgM	Serum	ELISA	Cardiolipin
Aguiar et al.[54]	2009	120	1500	IgA	Serum	ELISA and IF	Human tissue transglutaminase Endomysium
Nabeta et al.[37]	2009	65	70	IgG	Serum	ELISA	CA125 Enolase
Nabeta et al.[38]	2010	69	82	IgG	Serum	ELISA	CA125 PDIK1L

Table 2. continued

First author	Year	Case number	Control number	Ig type	Sample type	Method	The investigated autoantibody
Inagaki et al.[41]	2011	45	39	IgG	Serum	ELISA	Laminin-1
Caccavo et al.[21]	2011	35	50	IgG	Serum	ELISA	Laminin-1
Nabeta et al.[39]	2011	69	82	IgG	Serum	ELISA	Syntaxin
Gajbhiye et al.[42]	2012	50	27	IgG	Serum	WB and ELISA	Tropomyosin 3 Stomatin-like protein 2 Tropomodulin 3
Ozhan et al.[22]	2014	60	20	-	Serum	ELISA	Laminin-1 Endometrial CA125 Syntaxin PDIK1L Enolase
Gajbhiye et al.[40]	2017	133	104	-	Serum	ELISA	CA125 CA19-9 Syntaxin PDIK1L Enolase Tropomyosin 3a Tropomyosin 3b Tropomyosin 3c Tropomyosin 3d Stomatin-like protein 2a Stomatin-like protein 2b Stomatin-like protein 2c Tropomodulin 3a Tropomodulin 3b Tropomodulin 3c Tropomodulin 3d
Yu-Rice et al.[29]	2017	18	30	IgG	Serum	ELISA	Selenium binding protein 1
Ek et al.[23]	2019	100	100	IgG	Serum	ELISA	LH LH receptor GnRH1 GnRH1 receptor Matrix metalloproteinase-9 Tenascin-C
Toullec et al.[30]	2020	106	92	IgG	Serum	ELISA	Granulocyte-macrophage colony stimulating factor
Artymuk et al.[31]	2021	100	100	IgG	Serum	ELISA	Estrogen Progesterone
Svensson et al.[24]	2022	53	50	IgG	Serum	ELISA	LH LH receptor FSH FSH receptor TSH TSH receptor HCG
Vilas Boas et al.[55]	2022	94	91	IgG	Serum	IF and ELISA	ANA ENA dsDNA

Ig: Immunoglobulin, IF: Immunofluorescence, WB: Western-Blot, ELISA: Enzyme-Linked Immunosorbent Assay, IHC: Immunohistochemistry, FSH: Follicle-stimulating hormone, LH: Luteinizing hormone, PDIK1L: PDLIM1 interacting kinase 1 like, ANA: Anti-nuclear antibodies, TSH: Thyroid-stimulating hormone, HCG: Human chorionic gonadotropin, CA125: Carcinoembryonic antigen 125, CA19-9: Carcinoembryonic antigen 19-9, PA: Antiphospholipid antigen PA, PE: Antiphospholipid antigen PE, PG: Antiphospholipid antigen PG, PI: Antiphospholipid antigen PI, PL: Antiphospholipid antigen PL, PS: Antiphospholipid antigen PS, RO: A kind of extractable nuclear antigens (RO/SSA)

Table 3. The findings related to the analysis of heterogeneity and publication bias of the conducted studies

Variables (autoantibodies)	Number of included studies	I^2 (%)	Q-test's p-value	P-value of Begg's test	P-value of Egger's test
All of the autoantibodies	41	84.47	<0.001	0.130	0.05
Anti-ANA	8	0.00	0.859	0.804	0.406
Anti-B2 glycoprotein 1	3	0.00	0.578	0.601	0.169
Anti-CA125	4	75.25	0.007	0.174	0.415
Anti-carbonic anhydrase I	4	36.39	0.194	1.000	0.663
Anti-cardiolipin	7	1.51	0.413	0.880	0.746
Anti-endometrial	9	93.07	<0.001	0.251	0.788
Anti-enolase	3	73.63	0.023	0.601	0.394
Anti-laminin-1	4	23.43	0.270	0.308	0.036
Anti-PDIK1L	3	92.49	<0.001	0.296	0.125
Anti-smooth muscle	3	0.00	0.620	0.601	0.652
Anti-syntaxin	3	79.70	0.007	0.601	0.940

ANA: Anti-nuclear antibodies, CA125: Carcinoembryonic antigen 125, PDIK1L: PDLIM1 interacting kinase 1 like

Sensitivity Analysis

Sensitivity analyses were conducted to evaluate the potential impact of a single study on the overall effect of autoantibodies in endometriosis. The sensitivity analyses indicated that upon exclusion of each study, the general conclusions remained substantially unchanged. These analyses consistently exhibited the robustness of the meta-analysis outcomes.

Discussion

Endometriosis is a significant health issue that affects women, but its exact cause remains unknown[56]. Several theories have been proposed to explain its origin, including autoimmunity[57]. However, studies investigating the development of autoantibodies in patients with autoimmune conditions have produced conflicting results. Therefore, the research collected and analyze the current body of information on this topic. The findings of our study indicate that the serum of individuals diagnosed with endometriosis has significantly higher levels of autoantibodies than that of healthy controls.

Studies have shown an increase in both the activity and quantity of B-cells in the serum of individuals with endometriosis[58,59]. The increase in B-cell activity leads to an elevated production of antibodies, which is consistent with the findings of the current study. Research has shown that immune cells, particularly B-cells, demonstrate an increase within lesions associated with endometriosis[60]. In addition, the secretion of cytokines responsible for activating B-cells, such as B lymphocyte-stimulators, also experiences an elevation within these specific regions[59]. These findings validate the results of our study.

Endometriosis relies on estrogen for its development[61] and estrogen has been identified as one of the potential mechanisms that increase the quantity and functionality of B-cells in individuals with endometriosis[62]. Research suggests that estrogen initiates a genetic program that alters the survival and activation of B-cells, leading to a shift in the naive immune system toward autoreactivity[62]. The exact cause of autoantibody formation in endometriosis is not well understood, but it is thought to be related to cellular damage and inflammation that occur in endometriosis-associated lesions. Abnormal exposure of self-antigens from damaged cells to the immune system triggers an autoimmune response, leading to the formation of autoantibodies that target cell-derived antigens[63].

This study suggests that differences in the target antigens of autoantibodies and continent of residence may contribute to the observed heterogeneity. The results of the subgroup analysis show that North American patients with endometriosis have higher levels of autoantibodies. Differences in genetic or environmental factors may explain this disparity. Additionally, according to the literature, autoimmune antibody manifestation varies among North American ethnic groups. Bruner et al.[64] discovered that African Americans with systemic lupus erythematosus exhibit significantly higher levels of anti-ANA autoantibody expression than other North American races. It is important to consider factors beyond ethnicity that can contribute to heterogeneity, such as sampling error, use of varied laboratory tests, body mass index, and age. It is crucial to study these factors in depth in future research efforts.

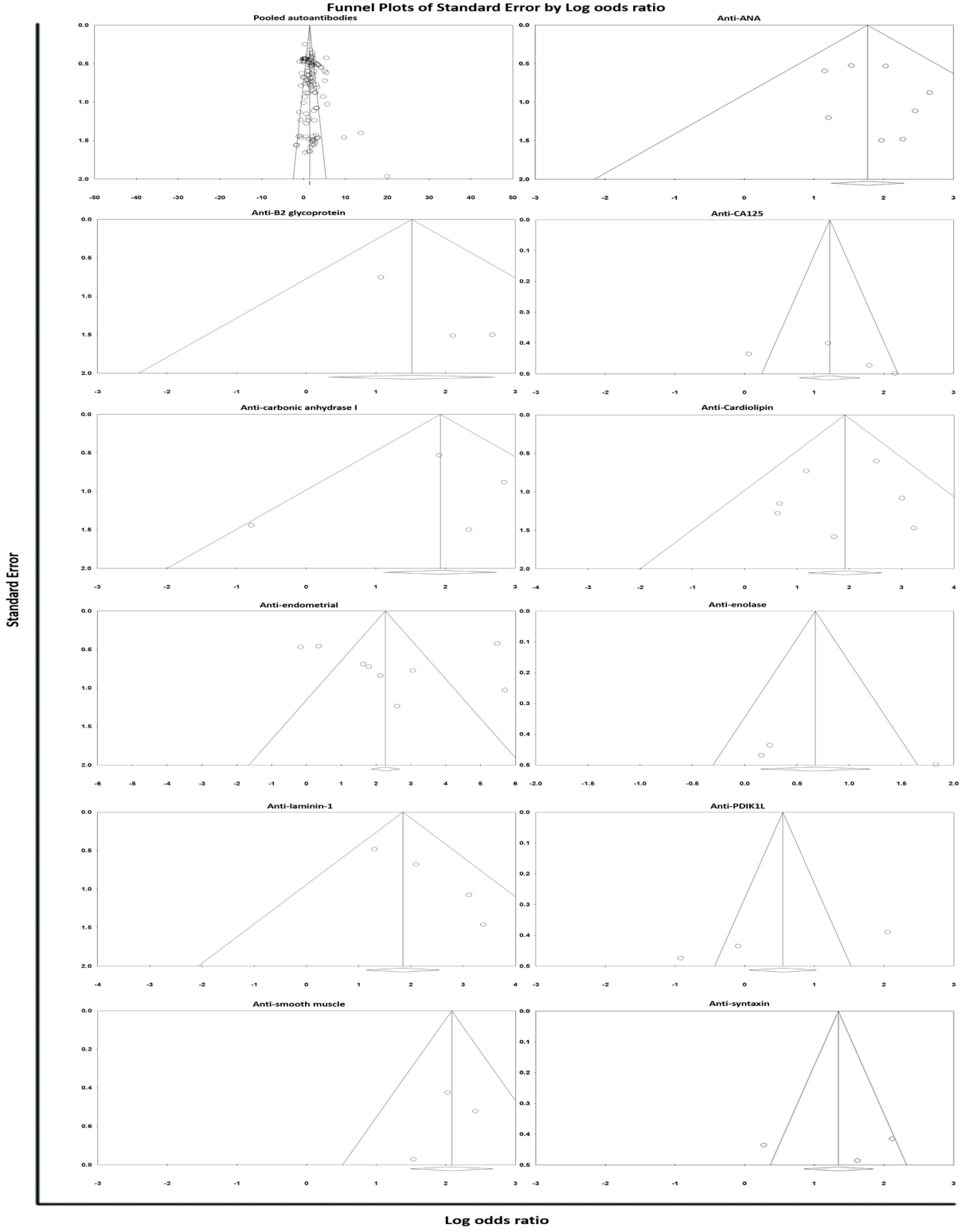

Figure 2. Begg's Funnel Plots of the studies that examined the correlation between serum anti-ANA, anti-B2 glycoprotein 1, anti-CA125, anti-carbonic anhydrase 1, anti-cardiolipin, anti-endometrial, anti-enolase, anti-laminin, anti-PDIK1L, anti-smooth muscle, anti-syntaxin, pooled autoantibodies for the identification of publication bias

ANA: Anti-nuclear antibodies, CA125: Carcinoembryonic antigen 125, PDIK1L: PDLIM1 interacting kinase 1 like

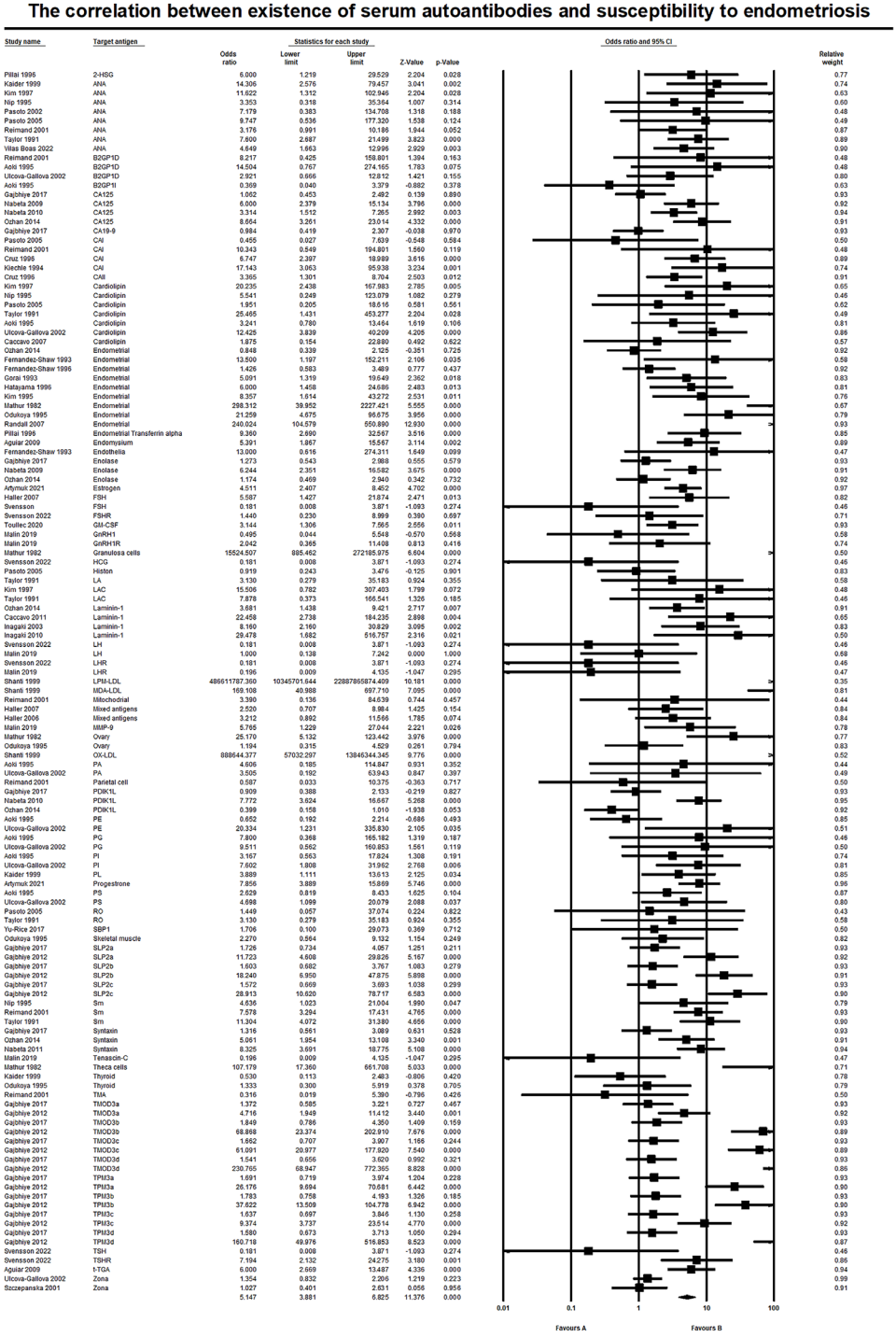

Figure 3. Forest plot investigating the link between serum autoantibodies and endometriosis risk

2-HSG: 2-Heremans Schmidt Glycoprotein, ANA: Anti-nuclear antibodies, B2GP1D: B2 glycoprotein 1 dependent, B2GP1I: B2 glycoprotein 1 independent, CA125: Carcinoembryonic antigen 125, CA19-9: Carcinoembryonic antigen 19-9, CAI, Carbonic anhydrase 1, CAII: Carbonic anhydrase II, FSH: Follicle-stimulating hormone, GM-CSF: Granulocyte-macrophage colony-stimulating factor, GnRH1: Gonadotropin releasing hormone 1, GnRH1R: Gonadotropin releasing hormone 1 receptor, HCG: Human chorionic gonadotropin, LA: One kind of neutrophile antigens (LA/SSB), LAC: Lupus anti-coagulant, LH: Luteinizing hormone, LHR: Luteinizing hormone receptor, LPM-LDL: Lipid peroxide-modified LDL, DMA-LDL: Malondialdehyde-modified LDL, MMP-9: Matrix metallopeptidase-9, OX-LDL: Oxidized low-density lipoprotein, PA: Antiphospholipid antigen PA, PDIK1L: PDLIM1 interacting kinase 1 like, PE: Antiphospholipid antigen PE, PG: Antiphospholipid antigen PG, PI: Antiphospholipid antigen PI, PL: Antiphospholipid antigen PL, PS: Antiphospholipid antigen PS, RO: A kind of extractable nuclear antigens (RO/SSA), SBP1: Selenium binding protein 1, SLP2a,b,c: Stomatin-like protein 2a,b,c ,Sm: Smooth muscle, TMA:, TMOD3a,b,c,d: Tropomodulin 3a,b,c,d, TPM3a,b,c,d: Tropomyosin 3a,b,c,d, TSH: Thyroid-stimulating hormone, TSHR: Thyroid-stimulating hormone receptor, t-TGA: Thyroglobulin

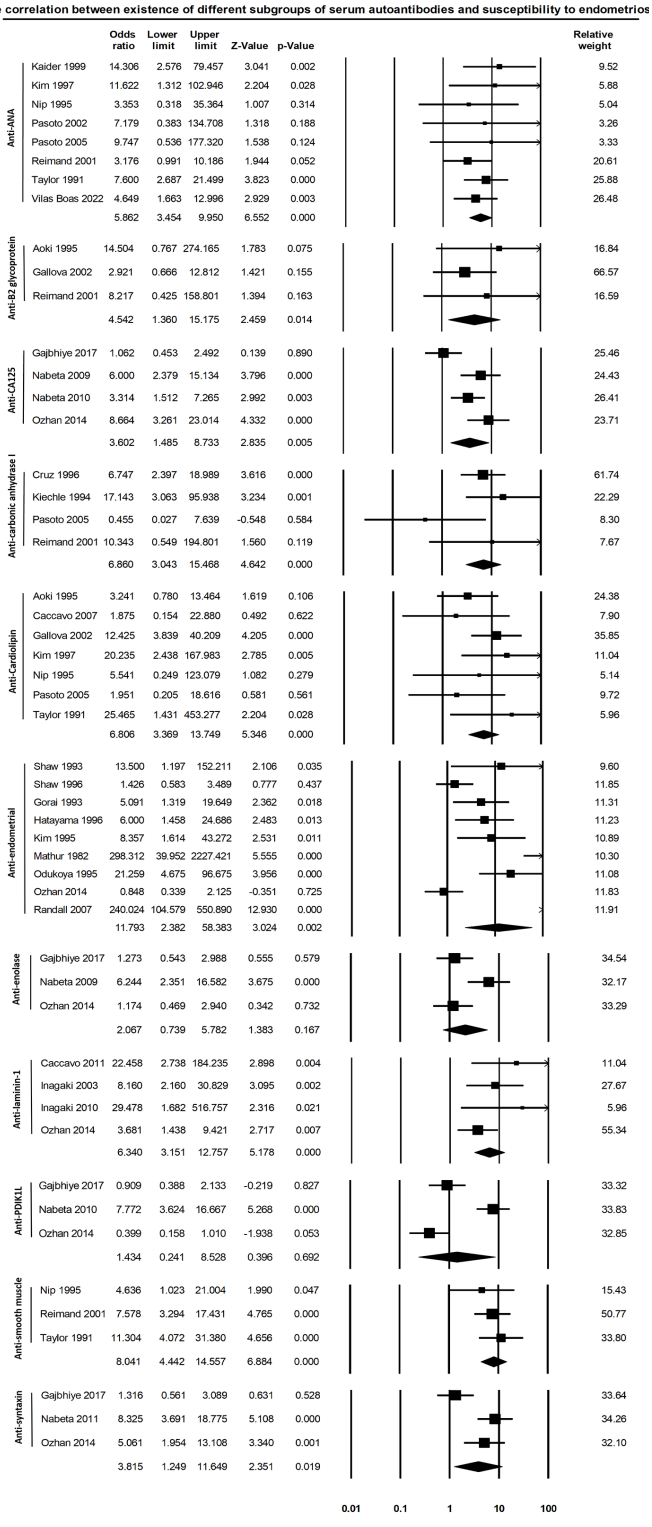

Figure 4. Forest plots exploring the link between serum anti-ANA, anti-B2 glycoprotein 1, anti-CA125, anti-carbonic anhydrase 1, anti-cardiolipin, anti-endometrial, anti-enolase, anti-laminin, anti-PDIK1L, anti-smooth muscle and anti-syntaxin autoantibodies and risk of endometriosis

ANA: Anti-nuclear antibodies, CA125: Carcinoembryonic antigen 125, PDIK1L: PDLIM1 interacting kinase 1 like

This meta-analysis is the most comprehensive study that systematically reviews and analyzes the relationship between autoantibodies and endometriosis, synthesizing over three decades of research. In addition, this study included a large sample size of 2,731 patients diagnosed with endometriosis and 4,067 healthy controls, potentially providing a definitive outcome with high accuracy and minimal bias for the general population.

It is important to note that there are limitations to this study. The laboratory data were measured in different centers using various methods and detection kits, which may have resulted in inconsistencies. Furthermore, the use of varying cutoff points across investigations could lead to inconsistencies in the outcome. In addition, the limited and insufficient data regarding the participants in the selected studies hindered our ability to examine other factors that may have an impact on heterogeneity, such as age and BMI. Therefore, future research should focus on exploring this aspect in more detail.

Conclusion

In conclusion, our meta-analysis revealed that patients diagnosed with endometriosis exhibit a greater prevalence of autoantibodies than healthy individuals. Based on the findings of this study, it is likely that autoimmune reactions are associated with the progression of endometriosis. However, further studies are required to determine the mechanism underlying autoantibody production in endometriosis. Future research should also investigate autoantibody levels during different phases of endometriosis.

Ethics

Authorship Contributions

Design: S.F., M.H.M., Data Collection or Processing: R.H.M., N.A., Analysis or Interpretation: S.F., R.H.M., N.A., M.H.M., Literature Search: M.H.M., Writing: S.F., R.H.M., N.A., M.H.M.

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Effects of bazedoxifene on endometriosis in experimental animal models: A systematic review and meta-analysis

Deneysel hayvan modellerinde bazedoksifenin endometriozis üzerine etkileri: Sistematik bir derleme ve meta-analiz

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Abstract

Endometriosis is a prevalent condition in women that causes pelvic pain and fertility issues due to the growth of endometrial tissue outside the uterus during menstrual cycles. Steroid hormones play a crucial role in the development and growth of endometriosis lesions; therefore, researchers have investigated several effective drugs that target hormones for treating this disease. One such drug is bazedoxifene, but despite several animal studies, there has yet to be a comprehensive evaluation of their combined results. A systematic search was conducted across several databases (Embase, PubMed, Scopus, and Web of Sciences) to identify studies investigating the effectiveness of bazedoxifene in animal models of endometriosis. Meta-analysis was performed using the size of endometriosis implants before and after drug administration in the case and control groups, along with the p-value of the associations. Begg's and Egger's tests were used to assess publication bias. This study included four eligible studies consisting of 45 endometrial animal models and 35 control subjects. The meta-analysis showed that bazedoxifene significantly reduced the size of endometriosis implants in animal models compared with the control group (odds ratio: 0.122, 95% confidence interval: 0.050-0.298, p<0.001). Detailed investigation determined that there was no significant heterogeneity between the studies (I^2=38.81, and p-value of the Q test=0.179). However, according to Egger's test, the study showed publication bias (p=0.035). This study found that bazedoxifene is a promising treatment option for endometriosis in animal models. However, more research on animals and humans is required to confirm these results.

Keywords: Animal model, bazedoxifene, endometriosis, meta-analysis

Öz

Endometriozis, adet döngüleri sırasında uterus dışında endometrial dokunun büyümesi nedeniyle kadınlarda yaygın bir durumdur ve pelvik ağrı ve doğurganlık sorunlarına neden olur. Steroid hormonlar, endometriozis lezyonlarının gelişiminde ve büyümesinde önemli bir rol oynadığından, araştırmacılar bu hastalığın tedavisinde hormonlara hedef olan birçok etkili ilacı araştırmışlardır. Bazedoksifen gibi bir ilaç da bunlardan biridir, ancak birkaç hayvan çalışmasına rağmen, bunların birleşik sonuçlarının kapsamlı bir değerlendirmesi henüz yapılmamıştır. Endometriozis hayvan modellerinde bazedoksifenin etkinliğini araştıran çalışmaları belirlemek için Embase, PubMed, Scopus ve Web of Sciences gibi birkaç veritabanında sistemik bir arama yapıldı. Meta-analiz, olgu ve kontrol gruplarında ilaç uygulamasından önce ve sonra endometriozis implantlarının boyutunu, ilişkilerin p-değeri ile birlikte kullanarak gerçekleştirildi. Yayın yanlılığını değerlendirmek için Begg ve Egger testleri kullanıldı. Bu araştırma, 45 endometrial hayvan modeli ve 35 kontrol grubundan oluşan dört uygun çalışmayı içermektedir. Meta-analiz, bazedoksifenin endometriozis implantlarının boyutunu kontrol grubuna kıyasla önemli ölçüde azalttığını göstermektedir (risk oranı: 0,122, %95 güven aralığı: 0,050-0,298, p<0,001). Detaylı inceleme, çalışmalar arasında anlamlı bir heterojenlik olmadığını belirlemiştir (I^2=38,81 ve Q testinin p-değeri=0,179). Ancak, Egger'ın testine göre, çalışma yayın yanlılığı göstermiştir (p=0,035).

PRECIS: An investigation was conducted to analyze the effects of bazedoxifene on endometriosis animal models by a comprehensive review of the existing literature.

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Bu çalışma, bazedoksifenin endometriozis için hayvan modellerinde umut verici bir tedavi seçeneği olduğunu bulmuştur. Bununla birlikte, bu sonuçları doğrulamak için hayvanlar ve insanlar üzerinde daha fazla araştırmaya ihtiyaç vardır.

Anahtar Kelimeler: Hayvan modeli, bazedoksifen, endometriozis, meta-analiz

Introduction

Endometriosis is the presence of endometrial glands and stroma-like lesions outside the uterus[1]. It is a long-lasting and debilitating condition linked to pelvic pain and infertility[2]. Approximately 10% of women in their reproductive years experience endometriosis, a condition that can be challenging to diagnose because of its complexity and diverse symptoms[3,4]. Several theories have been proposed regarding the etiology of endometriosis lesions. Some of them refer to the role of steroids and their receptors in controlling cells within abnormal endometrial lesions in endometriosis[5]. Research has revealed that ectopic endometrial tissues can produce estrogen and display variations in the expression of estrogen receptors, enzymes, and molecular pathways associated with hormones. These molecular changes play a crucial role in the formation and growth of endometriosis lesions[6].

Over time, numerous treatment choices have emerged for managing endometriosis. The primary aims of these treatments were to reduce pain and inhibit hormonally active endometriotic tissue[7]. These options encompass nonsteroidal anti-inflammatory drugs and various hormonal therapies such as combined oral contraceptives, progesterone-only contraceptives, gonadotropin-releasing hormone agonists, aromatase inhibitors, and danazol[8]. Although these therapies have shown considerable success, they come with undesired side effects due to hormonal suppression and require vigilant monitoring[8,9]. One of the newly proposed hormone-effective drugs for treating endometriosis is bazedoxifene[7].

Bazedoxifene, a third-generation selective estrogen receptor modulator (SERM), is used in combination with conjugated estrogens to treat vasomotor symptoms related to menopause and prevent postmenopausal osteoporosis[10]. SERMs have a unique ability to function as both activators and inhibitors. They can activate estrogenic effects in specific tissues, such as the bone and liver, while inhibiting estrogenic actions in other areas, like the uterus and breast[11]. Bazedoxifene did not stimulate the endometrium in clinical trials, indicating its exceptional endometrial safety[12].

Various studies have explored the impact of bazedoxifene on endometriosis in animal models, but the findings of these studies have yet to be compiled and analyzed in a comprehensive study. Therefore, we have decided to systematically analyze animal studies in this research to better understand the effect of bazedoxifene on endometriosis.

Materials and Methods

Search Strategy

Studies were identified through a comprehensive literature search of Embase, PubMed, Scopus, and Web of Sciences (up to February 16, 2024) using the keywords "Endometriosis" and "Bazedoxifene" along with their respective synonyms. Furthermore, the reference lists of the articles that were retrieved were manually examined to discover any other pertinent studies. There were no language restrictions imposed.

Study Selection and Data Extraction

The research papers were evaluated by two reviewers (RHM and NA), and only those that met the inclusion and exclusion criteria were selected. In cases of disagreement, a third- party (SF) was consulted for resolution. Our main inclusion criteria were case-control studies that investigated the effect of bazedoxifene on endometriosis in animal models. In our study, the following exclusion criteria were employed: 1) research without a control group; 2) studies conducted on humans or *in vitro*; 3) letters, editorials, abstracts, conference abstracts, and publications with inadequate information. The information gathered was entered into an Excel spreadsheet that contained the surname of the first author, the study's location and date, the total number of cases, the number of controls, the race of the involved animals, the age of the cases and controls, the method of drug administration, the dosage of bazedoxifene administered, the experimental plan, the average size of endometriosis implants before and after drug administration in both case and control animals, and the calculated p-values for the associations. The key features of the selected studies are outlined in Table 1.

Assessing the Risk of Bias

Two authors (RHM and NA) assessed the quality of each case–control study using the CAMARADES 10-item quality checklist[13]. A third reviewer (SF) was assigned the responsibility of addressing any inconsistencies that may have arisen. This checklist includes criteria such as 1) publication in a peer-reviewed journal, 2) control of temperature, 3) random allocation to groups, 4) allocation concealment, 5) blinded assessment of outcome, 6) use of an anesthetic without intrinsic neuroprotective activity, 7) use of comorbid animals, 8) sample size calculation, 9) compliance with animal welfare regulations, and 10) a statement of potential conflicts of interest. A quality score of a maximum of 10 points was assigned to each study, and a median quality score for the included studies was then determined.

Statistical Asnalysis

The CMA 3.0 software (Biostat, USA) was used to conduct statistical analyses. Odds ratios (ORs) were calculated, along with 95% confidence intervals (CIs), to evaluate the effect of bazedoxifene on endometriosis by considering the size of the endometrial implants before and after the treatment in both the case and control groups, as well as the calculated p values. The statistical heterogeneity among studies was assessed using Cochran's Q test and the I^2 statistic. The random-effects model was used to combine the data in cases of heterogeneity, whereas the fixed-effect model was employed when there was no heterogeneity. To determine publication bias, a funnel plot was created and Egger's regression asymmetry test was applied. A sensitivity analysis was conducted to assess the dependability of the combined findings.

Ethics Statement

The purpose of this research is to analyze previously published data, and there are no ethical concerns associated with this.

Results

Study Design and Description of the Included Studies and Risk of Bias Assessment

After conducting a comprehensive literature search, 85 studies were identified. After removing duplicates, 51 titles and abstracts were reviewed. Of these, 19 studies were chosen for a thorough evaluation of their full texts. Finally, after applying the inclusion and exclusion criteria, four studies were deemed suitable for inclusion in our meta-analysis. The details of the literature screening process are shown in Figure 1. The selected studies provided information on 45 animal models with endometriosis and 35 animals in the control group. These studies have reported estimates regarding the association between the impact of bazedoxifene and endometriosis. The quality of the studies was evaluated using the CAMARADES 10-item quality checklist, as described in the methods section, and the corresponding scores are displayed in Table 1. The median quality score of the included studies was 6.5 (range 5 to 8).

Main Analysis

The studies conducted by Kulak et al.[14], Naqvi et al.[15], Sakr et al.[16], and Lyu et al.[17] revealed that the mean size of endometriosis implants in the bazodexifene treatment groups was 21, 8.8, 8.7, and 24 mm^3, whereas the control groups had mean sizes of 60, 19.6, 31, and 48 mm^3, respectively. The meta-analysis of these findings showed that bazedoxifene causes a significant reduction in the size of endometriosis implants in animal models compared with the control group (OR: 0.122, CI 95%: 0.050-0.298, p<0.001). This suggests that bazedoxifene may have valuable therapeutic properties for treating endometriosis. Figure 2 displays the relevant forest plot.

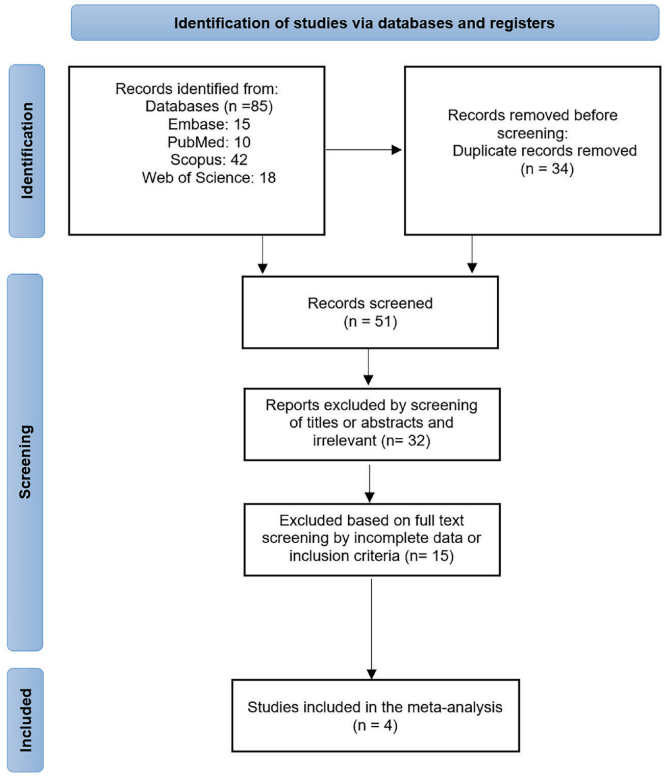

Figure 1. Flowchart for selection of studies

Table 1. Basic characteristics of the included studies

First author	Year	Country	Number of included cases	Number of included controls	Type of animal model	Age of the included animals (weeks)	Injected dose of bazedoxifene (mg/kg per day)	Rout of drug administration	CAMARADES quality assessment Score
Kulak et al.[14]	2011	USA	10	10	CD1 female mice	8	3	Intraperitoneal	6
Naqvi et al.[15]	2014	USA	20	10	CD1 female mice	8	1, 2, 3, 5	Intraperitoneal	5
Sakr et al.[16]	2014	USA	5	5	Female C57BL/6 mice	8-10	3	Intraperitoneal	8
Lyu et al.[17]	2015	China	10	10	Female rats	8-10	-	Intraperitoneal	7

Heterogeneity Analysis

Cochran's Q test and the I^2 statistic were employed to assess heterogeneity. The I-squared test resulted in a value of 38.81, and the p-value for the Q test was 0.179. Based on these findings, it can be concluded that there was no significant heterogeneity among the studies. Therefore, a fixed-effects model was used to analyze the data.

Publication Bias

A funnel plot was drawn to check for potential publication bias (Figure 3). The funnel plot of the included studies displays some asymmetry, which could be due to the small number of studies analyzed. Furthermore, while the Begg and Mazumdar rank correlation test did not show statistically significant results for publication bias (p=0.308), the Egger's test yielded significant findings (p=0.035). These findings indicate that some studies do not have been published because they did not achieve the desired outcomes.

Sensitivity Analysis

To assess the strength of the estimated combined effect size, we conducted a sensitivity analysis by systematically excluding one study at a time and reevaluating the combined effect size based on the remaining studies. The findings revealed that the combined effect remained consistent, suggesting that the outcomes were not influenced by any individual study. This outcome confirmed the validity of our findings.

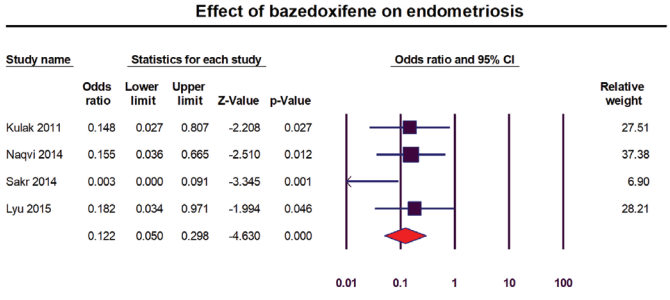

Figure 2. Meta-analysis to investigate the effect of badoxifene on the size of endometriosis graft lesions in animal models

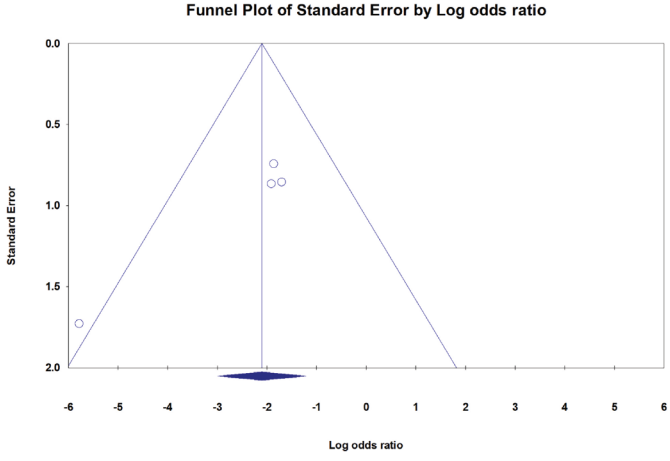

Figure 3. Begg's funnel plots for the studies that evaluated the effects of bazedoxifene on endometriosis

Discussion

Endometriosis is a complicated and common women's disease that is difficult to treat because of various factors affecting its progression[18]. Bazedoxifene is a new treatment option for endometriosis that has demonstrated promising results in animal studies and several case reports of human patients. This study aims to collect and analyze data on the effects of bazedoxifene on animal models of endometriosis.

Our study showed that the size of endometriosis implants in animals treated with bazedoxifene was significantly reduced compared with the control group. The meta-analysis results of the included studies consistent with their findings and validated their conclusions. Kulak et al.[14], Naqvi et al.[15], and Sakr et al.[16] in mice models, as well as Lyu et al.[17] in rat models, all reported that bazedoxifene can decrease the size of endometrial implants.

Some studies have attempted to uncover the underlying mechanism of action of bazedoxifene in treating endometriosis. Kulak et al.[14] found that bazedoxifene's ability to reduce estrogen-mediated cell proliferation is the reason for its effectiveness in treating endometriosis. This is evidenced by the decreased expression levels of estrogen receptor and proliferating cell nuclear antigen. Nevi et al.[15] discovered that bazedoxifene reduces the expression of estrogen receptor 1 without affecting the expression of progesterone receptors. Sakr et al.[16] reported that bazedoxifene reduced stem cell recruitment and restored endometrial engraftment. Furthermore, Hou et al.[19] showed that VEGF, VEGFR2, and COX-2 expression levels were significantly lower in endometriosis animal models treated with bazedoxifene than in the untreated control group. This suggests that bazedoxifene effectively reduces the vascularization and expansion of endometriosis lesions.

Limited studies have investigated the effects of bazedoxifene on endometriosis in humans. Flores et al.[20] reported that a combination therapy of bazedoxifene and conjugated estrogens relieved pelvic pain in a patient with stage III endometriosis. In a separate study, Hill et al.[21] treated three patients with endometriosis who did not respond to traditional drug therapy by administering a combination of bazedoxifene, conjugated estrogens, and leuprolide. The findings of these studies align with the results obtained from our research, suggesting that bazedoxifene may reduce endometriosis lesions and stop pelvic pain and bleeding in these patients.

This study is the first systematic review and meta-analysis that specifically examines the impact of bazedoxifene on endometriosis in animal models. In addition, the studies included in this research were highly homogeneous, which enhanced the accuracy and reliability of the investigation results. Our study has some limitations that we should mention. First,

limited studies were available to conduct this research, and repeating this investigation with more available studies would be beneficial. Furthermore, the studies we analyzed in our research only focused on the effect of bazedoxifene in reducing the size of endometriosis implants in animal models. However, to determine the complete efficacy of bazedoxifene in treating endometriosis, it is essential for future studies to also evaluate its ability to alleviate other symptoms, such as pelvic pain and bleeding.

Conclusion

In summary, the findings of this research suggest that bazedoxifene shows promising clinical effectiveness in treating endometriosis in animal models, indicating its potential as a future treatment option for this condition. However, further research involving both animals and humans is imperative to validate these results.

Ethics

Authorship Contributions

Design: S.F., M.H.M., Data Collection or Processing: R.H.M., N.A., Analysis or Interpretation: S.F., R.H.M., N.A., M.H.M., Literature Search: M.H.M., Writing: S.F., R.H.M., N.A., M.H.M.

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

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Maternal occupational exposure to asthmogenic during pregnancy and the future risk of asthma in children: A meta-analysis

Annenin hamilelik sırasında astmojenlere mesleki maruziyeti ve çocuklarda astım riski: Bir meta-analiz

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Abstract

The association between maternal occupational exposure during pregnancy and the subsequent development of asthma in their children has always been a matter of debate, and the results of cohort studies on this issue have been controversial. The current study is the first systematic review and meta-analysis aimed at evaluating the risk of developing subsequent asthma in children based on maternal occupational exposure during the gestation period. To retrieve eligible studies, an advanced literature search was performed up to August 10, 2023 from the following databases: PubMed, Scopus, and Google Scholars. The title and abstract of related articles were screened; hence, the full texts were reviewed. Data extraction was conducted; hence, the included articles were analyzed to assess the mention association. From a total of 10 cohort studies with a total record of 5372, it was found that there is no significant relationship between occupational exposure to asthmogenic during pregnancy and later asthma in children. The pooled odds ratio of asthmatic children in patients with maternal occupational exposure to asthmogenic during pregnancy was 1.03 (95% confidence interval, 0.97-1.09) I^2= 13% p=0.62. It was concluded that there is no significant association between maternal occupational exposure and future asthma in children. However, future large-scale studies are required to support these results.

Keywords: Maternal occupational exposure, asthma, pregnancy, systematic review, meta-analysis

Öz

Hamilelik sırasındaki mesleki maruziyet ile daha sonra çocuklarda astım gelişimi arasındaki ilişki her zaman bir tartışma konusu olmuştur ve bu konuyla ilgili kohort çalışmalarının sonuçları tartışmalı olmuştur. Bildiğimiz kadarıyla bu, gebelik döneminde annenin mesleki maruziyetine bağlı olarak çocuklarda sonradan astım gelişme riskini değerlendirmeyi amaçlayan ilk sistematik derleme ve meta-analizdir. Uygun çalışmalara ulaşmak için 10 Ağustos 2023 tarihine kadar aşağıdaki veritabanlarından ileri düzeyde bir literatür taraması yapıldı: PubMed, Scopus ve Google Scholars. İlgili makalelerin başlığı ve özeti taranarak buradaki tam metinler incelenmiştir. Veri çıkarma gerçekleştirildi, dolayısıyla söz konusu ilişkiyi değerlendirmek için analizin yapıldığı makaleler dahil edildi. Toplam kaydı 5372 olan toplam 10 kohort çalışmasından, annenin hamilelik sırasında astmojenlere mesleki maruziyeti ile daha sonra

PRECIS: Investigating maternal occupational exposure during pregnancy and childhood asthma risk, our meta-analysis found no significant association, suggesting further research is needed for conclusive evidence.

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çocuklarda astım arasında anlamlı bir ilişki olmadığı bulunmuştur. Hamilelik sırasında annenin mesleki olarak astmojenlere maruz kaldığı hastalardaki astımlı çocukların toplu olasılık oranı 1,03 idi [%95 güven aralığı, 0,97-1,09] I^2= %13 p=0,62. Annenin mesleki maruziyeti ile çocuklarda gelecekteki astım arasında anlamlı bir ilişki olmadığı sonucuna varıldı. Ancak bu sonuçların desteklenmesi için gelecekte geniş ölçekli çalışmalara ihtiyaç duyulmaktadır.

Anahtar Kelimeler: Annenin mesleki maruziyeti, astım, gebelik, sistematik inceleme, meta-analiz

Introduction

As stated by the World Health Organization, non-communicable diseases (NCD) claim the lives of 41 million individuals annually, accounting for 74% of global mortality[1]. Asthma, a prevalent NCD, imposes a substantial burden of morbidity and mortality. It is projected to impact almost 262 million individuals in 2019, resulting in 455,000 fatalities worldwide. Furthermore, it is the primary chronic disease among children on a global scale[2]. There are over 300 million people worldwide who are affected by asthma, and each day 1,000 people die because of asthma[3,4]. Moreover, young adults with asthma are 20 times more prone to develop chronic obstructive pulmonary disease[5].

The apparent epidemic of asthma and allergies recently seems to follow in the footsteps of economic development, the process of production, consumption, and urbanization[6]. Numerous studies have shed light on the connection between being exposed to various domestic and industrial ambient pollutants and experiencing bronchial wheezing[7]. Occupational exposures are responsible for 5-25% of all asthma cases among adult workers[8-10] by inducing the mechanism of immunoglobulin E (IgE)-mediated responses[11]. Promisingly, this number can be prevented if occupational exposures are obviated[12].

Potential risk factors have been identified for predisposing children to para-occupational asthma via their parents, such as stress[13], secondhand tobacco smoking[14], asbestos, pesticides, organic solvents, and mold[15-18]. Organic solvents are volatile lipophilic compounds that can cross the placenta[19] and lead to a shift in the balance between (Th1) and (Th2) in offspring's umbilical cord blood[20]. They appear to play a key role in the development of childhood asthma[21]. Studies indicate that persistent maternal exposure to organic pollutants, including organochlorine pesticides and polychlorinated biphenyls (PCB), is linked to a higher likelihood of respiratory symptoms and asthma in infants[22].

Several studies have shown that certain parenting occupations are linked with a higher risk of respiratory conditions in children[23-25]. In contrast, Christensen et al.[23] reported no notable link between maternal job exposure and childhood asthma. For the first time, this study represents a systematic review/meta-analysis focusing on assessing the likelihood of children developing asthma later due to maternal occupational exposure during pregnancy.

Materials and Methods

In this meta-analysis, our objective was to investigate the influence of maternal occupational exposure to cosmogenic agents during pregnancy on the subsequent occurrence of asthma in their offspring. Our methodology adheres to the Preferred Reporting Items for Systematic Reviews and Meta-analyses[26] guidelines. The research protocol for this review has been registered on the Open Science Framework.

Literature Search

To retrieve eligible studies,an advanced literature search was performed up to August 10, 2023 from the following databases:Pubmed,Scopus,and Google scholar. The search strategy included three main keywords. One subgroup encompassed terms associated with pregnancy, whereas the other two subgroups encompassed terms related to asthma and occupational exposures. The subgroups were linked together using the "AND" operator, with no limitations on date, publication type, or language. The search strategy was modified on the basis of the query format for each database. Our search strategy methodology is summarized in Table 1. To minimize the risk of missing relevant articles, we investigated

Table 1. Search strategy for systematic review through PubMed and Scopus

Search engine	Search strategy	Additional filtres
PubMed	#1: (pregnancy [tiab] OR pregnant[tiab] OR maternal[tiab]) #2: (asthma[tiab]) #3: (exposure[tiab] OR "occupational exposure" [tiab] OR asthmogen*[tiab] OR pollen*[tiab] OR aerosol*[tiab] OR solvent*[tiab] OR insecticide*[tiab] OR fungicide*[tiab] OR pesticide*[tiab] OR chemical*[tiab] OR mite*[tiab] OR latex*[tiab] OR dust*[tiab] OR antibiotic*[tiab] OR animal dander[tiab]) #4: #1 AND #2 AND #3	English, August 10th,2023
Scopus	(pregnant* OR pregnancy OR maternal) AND (asthma) AND ("occupational exposure" OR exposure* asthmogen* OR aerosol* OR antibiotic* OR pollen* OR solvent* OR chemical OR insecticide* OR fungicide* OR pesticide* OR mite* OR "animal dander" OR dust* OR latex OR "grass pollen")	English, August 10th,2023

the reference lists of relevant systematic reviews and included studies that were assessable in our analysis. The procedure was performed by two reviewers, with any discrepancies being resolved through discussion between the reviewers.

Criteria for Selecting Studies

To be eligible for inclusion in this meta-analysis, studies must adhere to the following criteria:

1. The studies involved pregnant women and their children, with a focus on maternal occupational exposure during pregnancy and its potential impact on childhood asthma.
2. Studies should have evaluated maternal occupational exposure to asthmogenic or related environmental agents at work during pregnancy
3. The primary outcome of this study was the development of asthma or wheezing in children, which was typically investigated at several follow-up points throughout childhood.
4. Definitions of asthma were provided based on the study design

Studies including pregnant women with preexisting pathologic conditions or those focused on outcomes unrelated to asthma were excluded. In addition, case reports, review articles, papers in languages other than English, and animal studies were excluded from the review.

Data Extraction and Study Quality Assessment

Two independent reviewers conducted an initial assessment of each study's title and abstract to assess whether it met the inclusion criteria for this meta-analysis. Articules that did not meet our predetermined criteria were excluded. Subsequently, the full texts of the remaining studies were further evaluated, and those meeting the eligibility criteria were included in the data extraction process. Next, data extraction was conducted in four distinct categories, comprising the following information:

1. Study Details (i.e. authors, location, year of publication, and study type)
2. Patient-Specific Factors (i.e. eligibility criteria for women included in the study and gestational age)
3. Study Design (i.e. the number of participants, sampling method and duration, and the definition of asthma)
4. Outcomes (i.e. the asthma rate and concentrations of exposures).

The two reviewers mentioned earlier used critical appraisal checklists specifically created for cohort, case-control, and analytical cross-sectional studies, as outlined by the Joanna Briggs Institute. If there were any discrepancies, a third author was involved in the assessment.

Statistical Analysis

We performed data analysis using STATA 13.1 software developed by StataCorp LP in College Station, TX. The outcomes are displayed as combined odd ratios (ORs) with a 95% confidence interval (CI), illustrated in a forest plot. The presence of heterogeneity among the qualified studies was evaluated using the I^2 statistic, and in cases of significant heterogeneity (I^2 >50%), a random-effects model was employed. In addition, we investigated the potential for publication bias by visually examining funnel plot symmetry and conducting Egger's regression analysis.

Result

Study Selection and Characteristics

The search yielded 5372 records. The screening of titles and abstracts resulted in 50 potentially eligible studies. After reviewing the final full texts, nine studies and 121.710 patients remained as our final result, in which maternal occupational exposure to asthmogenic was measured and met the inclusion criteria (Figure 1). The studies included in this analysis were published from 2006 to 2021 and were conducted in Denmark, the USA, Canada, and the United Kingdom. All studies were cohort studies with varying follow-up durations ranging from 12 to 84 months. The mean age of the patients ranged from 5 to 30 years. The follow-up duration of the cohort studies varied from 4 to 7 years. The details of these studies can be found in Table 2.

The analysis results suggest no statistically significant association between maternal occupational exposure to asthmogenic during pregnancy and the development of asthma in children.

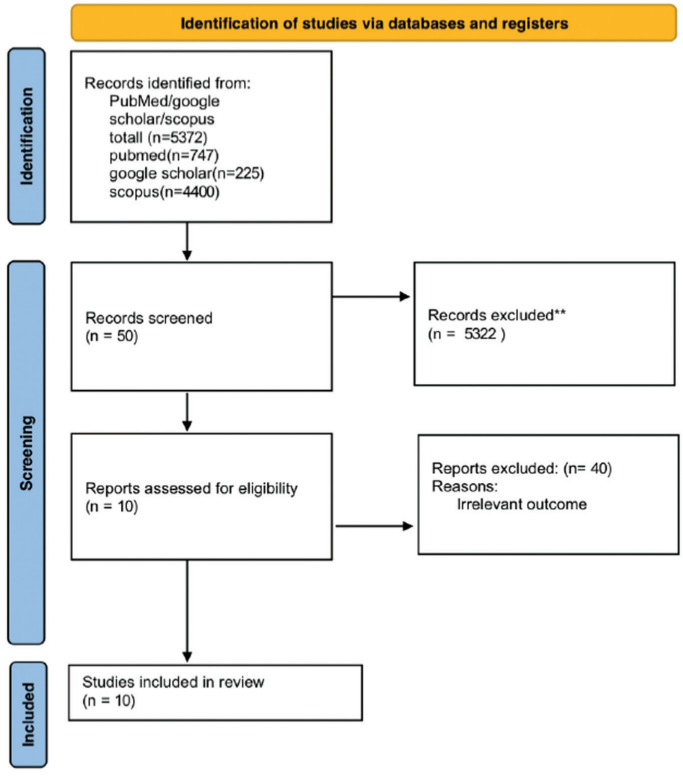

Figure 1. PRISMA diagram of current systematic review and meta-analysis

PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-analyses

Meta-analysis

The combined odds ratio for asthmatic children in patients with maternal occupational exposure to asthmogenic during pregnancy was 1.03 (95% CI, 0.97-1.09). The meta-analysis showed moderate statistical heterogeneity with an I^2 of 13%. Figure 2 illustrates the forest plot of the meta-analysis. Sensitivity analyses did not yield significantly different results from the overall analysis.

Publication Bias

Examination of the funnel plot (Figure 3) did not reveal any indication of publication bias because the graph appeared relatively symmetrical. Furthermore, Egger's regression test did not show evidence of publication bias (p=0.62).

Discussion

This meta-analysis of nine cohort studies explored the association between maternal occupational exposure to asthmogenic during pregnancy and the risk of asthma in children. These findings did not provide significant evidence of a connection between maternal occupational exposure to asthmogenic during pregnancy and the risk of asthma in children.

Jøhnk et al.[27] did not find a correlation between prenatal exposure to phthalates and asthma in children. This could be attributed to the lower maternal exposure levels. In addition, the older age of the mothers in the study and their non-smoking status could be contributing factors to the lower prevalence of asthma (7.4%) in these children.

Christensen et al.[28] found a positive borderline association between maternal occupational exposure to low molecular weight/irritant agents OR = 1.11, 95% CI = (1.01,1.23)] and heavy molecular weight allergens [OR = 1.12, 95% CI = (0.85,1.47)] and asthma in their children.

Christensen et al.[23] found no association between mothers' occupational exposures during pregnancy and asthma in their 7-year-old children. Exposure to low molecular weight agents

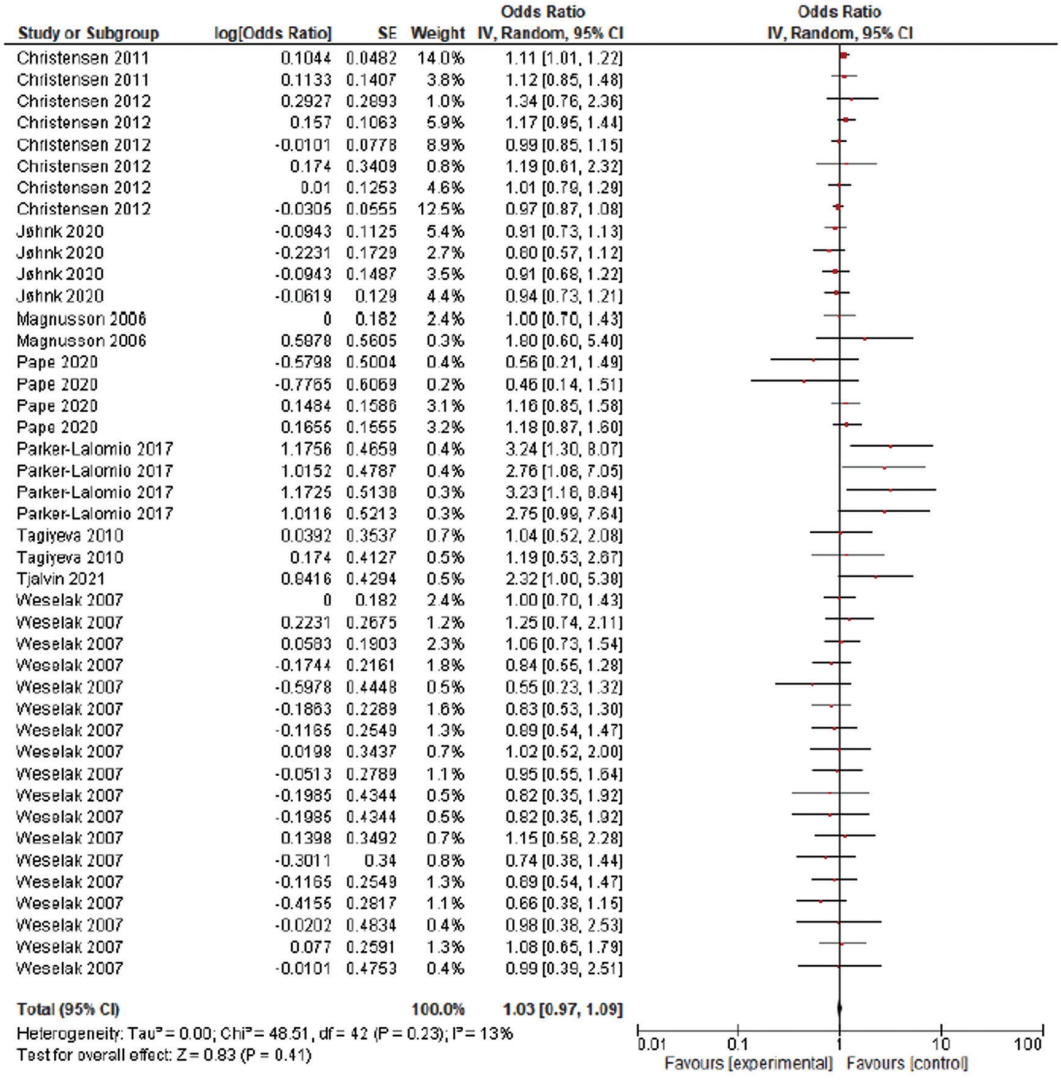

Figure 2. Forest plot shows any significant association between maternal occupational exposure and future asthma in children

Table 2. Characteristics of included studies

Author (ref) (year)	Country	Study design	Follow-up duration	Participants (n)	Occupational exposure	Mean age of children	Asthma diagnosis
Christensen et al.[28] (2011)	Denmark	Cohort study	Last 12 months	Include 45658 children and their mothers.	Maternal occupational exposure/high molecular weight (HMW) Low molecular weight/irritant (LMW)/mixed exposure/student and reference (office workers)/ farmers	7 years old	Used DISC (Danish) International Standard Classification of Occupations) codes.
Christensen et al.[23] (2013)	Denmark	Cohort study	18 months	A total of 100,418 pregnancies were enrolled, but only 41,724 mother/child pairs were eligible for analysis in 7-year-old children with an increased likelihood of asthma.	Prenatal maternal occupational exposure/low molecular weight agents early in the child*s life may predispose them to asthma/latex and biocides/fungicides/high molecular weight agents/ farmers/students/mixed HMW and LMW agents/Unclassifiable/ Reference	7 years old	Used validated core questions on asthma from the International Study of Asthma and Allergies in Childhood and Asthma Job Exposure Matrix (JEM)18 based on known risk factors for occupational asthma.
Jøhnk et al.[27] (2020)	Denmark	Cohort study	5 years	870 pregnant women provided a fasting spot urine sample for analysis of 12 phthalate metabolites. Finally, 552 mother-child pairs with measurements of phthalate metabolites and information about asthma, eczema, and rhinitis were included.	Prenatal phthalate exposure/ Prenatal exposure to DiNP and DEHP	5 years	Used a questionnaire based on the International Study of Asthma and Allergies in Childhood (ISAAC).
Magnusson et al.[16] (2006)	Denmark	Prospective cohort study	14-18 years	7844 children (4045 boys/3798 girls) from 6418 mothers.	Maternal occupational exposure to organic solvents	16	Based on parental report of a physician's diagnosis via ISAAC questionnaire and hospitalization data
Pape et al.[30] (2020)	Denmark	Two-generation cohort study	Onset of asthma at 0-15	3985 adult offspring ≥18 years of age participating in the (RHINESS) with 2931 of their parents participating in the ECRHS II/RHINE II.	Paternal or maternal occupational exposure to: 1. Microorganisms (molds, endotoxin). 2. Pesticides (herbicides, insecticides, fungicides). 3. Allergens (animals, flour, house dust mites, storage mites, plant mites, enzymes, latex, fish/shellfish). 4. Reactive chemicals (high-level chemical disinfectants, isocyanates, acrylates, epoxy resins, persulfates/henna, aliphatic amines, bleach).	30	RHINESSA questionnaire

Table 2. Continued

Author (ref) (year)	Country	Study design	Follow-up duration	Participants (n)	Occupational exposure	Mean age of children	Asthma diagnosis
Tagiyeva et al.[24] (2010)	United Kingdom	Cohort study	0-91 months	13971	Paternal or maternal occupational exposure to wood, diisocyanate, flour, glues/resins, animals, solders, enzymes, biocides/fungicides, foods, latex and dyes.		Postal questionnaires and clinical assessments (Serum total IgE and allergen skin-prick testing).
Tjalvin et al.[31] (2022)	USA	Cohort study	2 years	Out of a total of 3318 children, 1307 had mothers who had worked for at least 6 months in jobs that involved exposure to indoor cleaning agents. Meanwhile, 150 children had mothers who were exposed to these agents only before conception, while 610 children had mothers who were exposed both before conception and after. Lastly, in 470 children, the mother's exposure to indoor cleaning agents started after the children were born.	Maternal occupational exposure to indoor cleaning agents (cleaning products/detergents and disinfectants).	10 years old	Used occupational health tools include ISCO (International Standard Classification of Occupations), JEM (Job-Exposure Matrix), OAsJEM (Occupational Asthma-specific Job-Exposure Matrix), RHINE (Respiratory Health in Northern Europe), and RHINESSA (Respiratory Health In Northern Europe, Spain, and Australia).
Parker-Lalomio et al.(32) (2018)	USA	Retrospective Cohort study		800	Polychlorinated biphenyls (PCBs)		Phone interview
Weselak et al. (2005)	Canada	Cross-sectional cohort study		3405 children of farm couple (the age of wife was at most 44 years) couples were eligible for inclusion if they were married or living as married, living near-round on a farm operation. Including family-run farms.	Farm couple exposures to any pesticides Fungicides Insecticides Herbicides Other Pesticides Phenoxy Triazine Thiocarbamate Organo-phosphates Dicamba Glyphosate 2,4-DB 2,4-D MCPA Atrazine Cyanazine Carbaryl Captan		Questionnaires were filled based on prior doctor's visit.

during the postnatal period [OR = 1.13, 95% CI = (0.99, 1.29)] or a combination of prenatal and postnatal exposure [OR = 1.34, 95% CI = (1.19, 1.51)] was linked to a higher prevalence of asthma in children[23].

Magnusson et al.[16] found little association between occupational exposure of mothers to organic solvents and asthma in children. Allergens with high molecular weight can contribute to the development of asthma through immune mechanisms mediated by IgE. In contrast, allergens with low weight, in addition to the above mechanism, may exert a role in asthma development through immunological reactions without IgE or even non-immunological reactions[16]. Sensitivity to allergens can occur in the uterus without the mother's sensitivity[29].

Pape et al.[30] found a correlation between maternal exposure to allergens and reactive chemicals before and after pregnancy and the development of early-onset asthma in children. Interestingly, no such association was found in men, specifically in relation to their occupational exposures. One possible explanation for this difference could be the increased vulnerability of women's reproductive cells compared with men's.

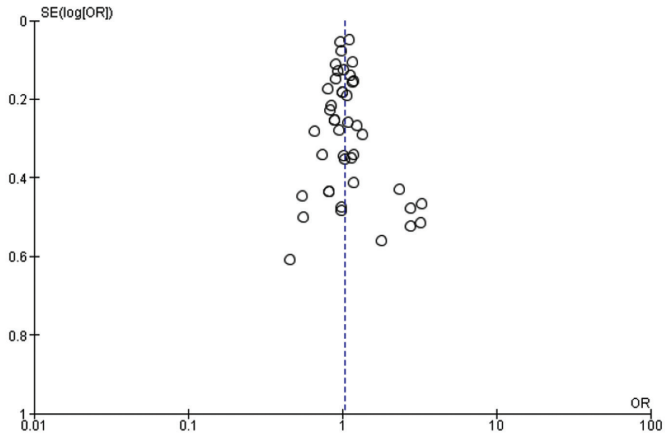

Figure 3. Funnel plot shows a symmetrical pattern indicating no publication of bias

Another study by Tjalvin et al.[31] demonstrated that Occupational exposure of mothers to indoor cleaning products before and during pregnancy was linked to a higher likelihood of asthma in children [OR = 1.56, 95% CI = (1.05-2.31)]. At the same time, exposure after birth was unrelated to asthma outcomes [OR =1.13, 95% CI = (0.71, 1.80)].

A strong link between occupational exposure to PCBs and childhood asthma was reported [OR = 3.24, 95% CI = (1.30, 8.09)] in the study by Parker-Lalomio et al.[32].

Tagiyeva et al.[24] research revealed that exposure to latex and biocides/fungicides in the workplace during pregnancy can increase the chances of childhood wheezing and asthma. The likelihood of childhood wheezing and asthma is further amplified when there are elevated levels of exposure to latex, biocide/fungicide, or a combination of both, with odds ratios of 1.26, 1.22, and 1.22 [95% CI = (1.07, 1.50), (1.02, 2.05), and (1.03, 1.43)], respectively.

Children's asthma was confirmed in two ways: diagnosis by a doctor or by reporting children's wheezing episodes by parents. Inaccurate recall of wheezing episodes and differences in access to medical care could bias the results. In addition, some studies did not have access to the family history of allergic diseases. In addition, due to the possibility of mothers suffering from allergic diseases avoiding certain specific occupations, it can lead to incorrect occurrence of negative results.

Conclusion

The current meta-analysis did not reveal any statistically significant association between maternal occupational exposure during pregnancy and the likelihood of asthma in children. Additional cohort and cross-sectional studies are necessary to determine the precise relationship between exposure and asthmogenic during pregnancy and asthma risk in children.

Ethics

Authorship Contributions

Design: P.S.E., M.A.B., Z.M.T., H.P., M.M., B.G., F.S., A.I.P., N.D., M.P., Data Collection or Processing: P.S.E., M.A.B., Z.M.T., H.P., M.M., B.G., F.S., A.I.P., N.D., M.P., Analysis or Interpretation: P.S.E., M.A.B., Z.M.T., H.P., M.M., B.G., F.S., A.I.P., N.D., M.P., Literature Search: P.S.E., M.A.B., Z.M.T., H.P., M.M., B.G., F.S., A.I.P., N.D., M.P., Writing: P.S.E., M.A.B., Z.M.T., H.P., M.M., B.G., F.S., A.I.P., N.D., M.P.

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

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Letter to the Editor / Editöre Mektup

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Critical review of the SHAPE trial

SHAPE çalışmasının eleştirel incelemesi

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Keywords: Cervical cancer, type 1 hysterectomy, radical hysterectomy

Anahtar Kelimeler: Serviks kanseri, tip 1 histerektomi, radikal histerektomi

Dear Editor,

An international randomized trial comparing radical hysterectomy (RH) and *vs*. simple hysterectomy (SH) in patients with low-risk early-stage cervical cancer was recently published in NEJM by Plante et al.[1], and we read it with great interest. Although the literature excludes any relevant randomized trials, early-stage cervical cancers are traditionally treated using the RH technique pioneered by Ernst Wertheim and others >100 years ago[2]. Currently, efforts to reduce the complications and morbidity associated with RH and improve patient quality of life post-surgery are ongoing; therefore, we appreciate the SHAPE Trial researchers' efforts to improve our knowledge of this topic and improve patient outcomes. The SHAPE Trial researchers performed a non-inferiority trial that included 130 centers in 12 countries and compared SH and RH in patients with low-risk cervical cancer (lesions ≤2 cm with limited stromal invasion). They noted that, "SH was not inferior to RH with respect to the 3-year incidence of pelvic recurrence and was associated with a lower risk of urinary incontinence or retention"[1]. each year, almost 600,000 cases of cervical cancer occur worldwide, of which nearly 80% occur in undeveloped or developing countries. Although the researchers wrote that their results cannot be generalized to developing countries, as practicing gynecologic oncologists from a developing country, we also think that some patients with cervical tumors <2 cm might benefit from and urgently need less radical surgery, especially in low resource settings in which there is limited or no screening or radiotherapy facilities, operative infrastructure, or trained gynecologic oncologists. In contrast to what was written, we strongly believe that clinicians in developing and undeveloped countries can make good use of the SHAPE Trial findings; however, before we can reach a definitive conclusion, we have some criticisms and concerns about the Trial, as detailed below, that we think must be addressed.

- The study was conducted at 130 centers; however, we would like to definitively know if a central pathology and imaging review was performed.
- A contentious issue for us is that laparoscopic surgery was performed in both the SH and RH groups. debate about the use of laparoscopic/robotic surgery in patients with cervical carcinoma is ongoing[3]. Although the LACC Trial reported that the oncological outcome was the worst in patients who underwent laparoscopic/robotic surgery for cervical cancer, the SHAPE Trial routinely used laparoscopic/robotic surgery. We believe that this non-inferiority study should have compared abdominal SH and abdominal RH and excluded minimally invasive techniques to yield more definitive findings. In addition, the SHAPE Trial researchers did not provide the number of patients in the SH and RH groups who underwent laparoscopic and/or robotic surgery and did not mention if there were any differences in overall and disease-free survival between the two groups. Also missing from their report are the number of patients in each group who underwent full pelvic lymphadenectomy, sentinel lymphadenectomy, and no lymphadenectomy, as well as clearly stated outcomes. Consequently, we think that the researchers' use of both abdominal and laparoscopic/robotic SH and RH might be among the important confounding factors related to their findings; therefore, in general, we think

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that the study population was too heterogeneous to reach a definitive conclusion. For example, some patients underwent laparoscopic/robotic SH, some underwent RH, and some underwent sentinel lymphadenectomy and some did not. The study also lacks clarity regarding the use of any uterine manipulators. Finally, the 12.5% pelvic recurrence rate at 3 years in the RH group versus 0% in the SH group from Asia was an especially interesting finding, whereas the pelvic recurrence rate at 3 years in the RH group from North America was 0%. These concerns lead us to question the validity of the study's findings, which require further clarification.

- Although the SHAPE Trial was originally planned as a superiority trial, due to the lack of events during follow-up, it was changed to a non-inferiority trial. It is well known that the primary weakness of non-inferiority trials, as compared with superiority trials, is that deviations from the protocol can result in false rejection of the null hypothesis that the experimental treatment is inferior[4]. The study's CONSORT flow diagram shows that 10% of the patients in the SH group violated the study protocol, versus 12.9% in the RH group. Furthermore, although 9.1% of the SH group and 8.9% of the RH group received adjuvant treatment, in our routine clinical practice, when we perform SH in patients who meet the same criteria used in the SHAPE Trial, the majority of patients do not require any adjuvant treatment, especially when the patients are carefully selected. Despite there being a similar adjuvant treatment rate in the SH and RH groups, when we look at the causes of death in Table 3, the hazard ratio (HR) for disease recurrence was 1.54 based on intention-to-treat analysis, versus 1.19 based on protocol analysis. Furthermore, the HR for extrapelvic recurrence was 3.82 based on the intention-to-treat analysis, versus 2.03 based on the protocol analysis. Interestingly, the HR for death was 0.79 although the 95% CI included 1 based on protocol analysis. How can SH be associated with fewer deaths than RH despite both groups having similar adjuvant treatment rates and higher extrapelvic recurrence rates? These findings suggest that the addition of radiotherapy to the pelvis compensates for the ineffectiveness of SH but does not prevent extrapelvic recurrence beyond the radiotherapy area. In our routine clinical practice, we generally do not administer radiotherapy or any adjuvant treatment in patients that have tumors <2 cm with no LVSI and limited depth of cervical stromal invasion. Furthermore, we generally prefer using RH to avoid unnecessary radiotherapy. The SHAPE Trial did show that adjuvant treatment had a greater sparing effect than SH, which indicates that there might have been patient selection bias.

- On the other hand, Table S2 shows that the number of patients with surgical margin positivity, tumor size >2 cm, and positive metastatic lymph nodes was higher in the RH group. Although the number of patients with poor prognostic factors was higher in the RH group, the use of adjuvant treatment was slightly more common in the SH group (9.2% *vs.* 8.4%), which might explain the similarity of the pelvic recurrence rate in both groups and the higher extrapelvic recurrence rate in the SH group, as emphasized above.

- In addition, the SUCCOR study[5] observed that preoperative LEEP or conization has a positive effect on survival following RH. Table 2 of the SHAPE Trial shows that preoperative LEEP or conization was performed in 84% of the SH group patients versus 76% of the RH group patients. All these factors (protocol violations, preoperative LEEP, and adjuvant treatment) can increase the effectiveness of SH and require additional clarification.

Another important drawback of the SHAPE Trial is the lack of adequate long-term survival data. We believe that in the absence of sufficient long-term overall and disease-free survival data, we cannot sacrifice the oncological safety associated with RH and switch to SH in cervical cancer patients to have a lower urinary incontinence rate; until many of the SHAPE Trial issues are addressed, we believe that doing so based on the SHAPE Trial findings should be carefully interpreted.

To conclude, as did the SHAPE Trial researchers, we also believe that some patients with low volume and small tumors might benefit from reducing surgical radicality; however, this should have been proven by comparing abdominal SH and abdominal RH in the absence of confounding factors such as preoperative LEEP/conization, adjuvant radiotherapy/chemotherapy, laparoscopy, and uterine manipulation.

Sincerely yours,

Ethics

Authorship Contributions

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

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Letter to the Editor / Editöre Mektup

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Is tracheal stenosis more common and developed earlier in intubated pregnant patients?

Entübe gebe hastalarda trakeal stenoz daha sık ve daha erken gelişir mi?

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Dear Editor,

The most common factor in the development of tracheal stenosis (TS), in addition to tube size, cuff pressure, and multiple intubation attempts, is prolonged intubation[1]. The incidence of TS after endotracheal intubation in the intensive care unit is 6-21%, but only 1-2% of cases are symptomatic[2]. The symptoms become obvious only when the tracheal lumen is reduced by 50-75%.

Although the frequency of post-intubation TS in pregnant patients in need of coronavirus disease 2019 (COVID-19)-related intubation is unclear, we can say that TS was more common in pregnant patients. During the COVID-19 pandemic, 14 pregnant patients required invasive ventilation. Four patients developed TS. Only one patient was in the second trimester, others were in the third trimester. The average ventilation time in COVID-19 patients was 17 days, and re-intubation has a high incidence in COVID-19[3]. The duration of intubation was similar to that reported in the literature, which was 10 days, and two patients were reintubate (Table 1).

TS was diagnosed in the first patient 2 weeks after discharge, in the second patient 2 months after discharge, in the third patient 3 days later, and in the fourth patient during hospitalization. The symptoms of two patients improved with endoscopic dilation, but one patient required resection-anastomosis surgery. In another patient, despite endoscopic dilation, tracheostomy was performed because of coma.

During pregnancy, elevation of estrogen levels increases transforming growth factor beta-1, which promotes the deposition of collagens and finally fibrosis. Especially in the third trimester, estrogen causes airway edema[4,5]. We postulate that when the physiological changes causing airway edema during pregnancy are combined with prolonged intubation, impaired immunity, obesity, and prone position, the development of TS may be facilitated. Complications of the prone position may occur earlier and more frequently in pregnant patients due to physiological changes.

It is debatable whether TS will occur earlier and more frequently in pregnant patients or not. However, we would like to emphasize the importance of considering TS in pregnant patients, especially those with symptoms, even if the intubation duration is very short.

Table 1. Patients' data of intubated patients (number=14)

	TS (+) (n=4)	TS (-) (n=10)
Age (years)	32±5.35	31±5.20
Gestational week (days)	216±36.61	212±33.99
Duration of intubation (days)	12±5.47	11.4±8.57
Pron position (yes)	4 (100)	8 (80%)
Reentubation (yes)	3 (75%)	1 (10%)

TS: Tracheal stenosis, Continuous data are presented as mean ± standard deviation, categorical data by frequencies and percentages

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