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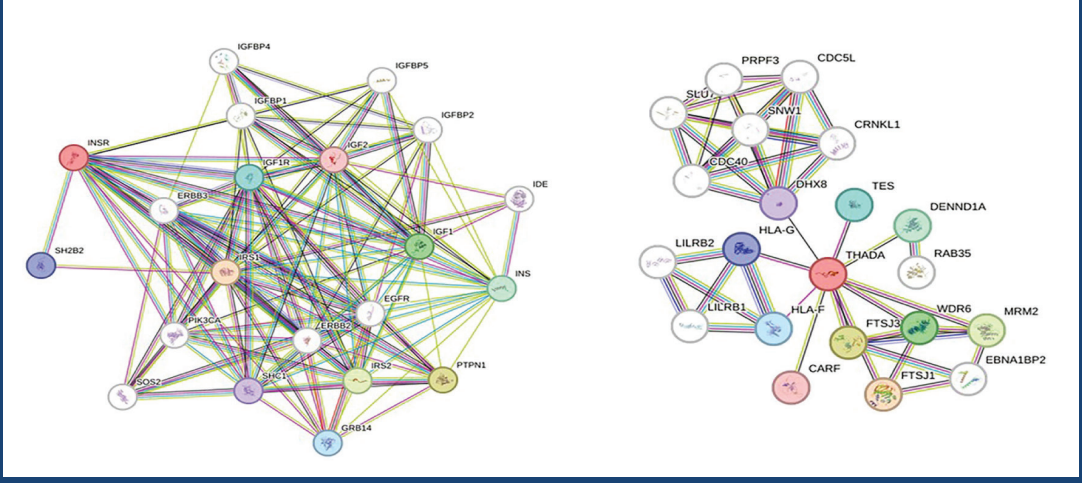

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Aims and Scope

Journal of the Turkish-German Gynecological Association is the official, open access publication of the Turkish-German Gynecological Education and Research Foundation and Turkish-German Gynecological Association and is published quarterly on March, June, September and December. The publication language of the journal is English. Manuscripts are reviewed in accordance with "double-blind peer review" process for both reviewers and authors.

The target audience of Journal of the Turkish-German Gynecological Association includes gynecologists and primary care physicians interested in gynecology practice. It publishes original works on all aspects of obstertrics and gynecology. The aim of Journal of the Turkish-German Gynecological Association is to publish high quality original research articles. In addition to research articles, reviews, editorials, letters to the editor, diagnostic puzzle are also published. Suggestions for new books are also welcomed. Journal of the Turkish-German Gynecological Association does not charge any fee for article submission or processing.

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The ''Journal of the Turkish-German Gynecological Association'' (EISSN 1309-0380; Abbreviated as "J Turk Ger Gynecol Assoc") is the official, open access publication of the Turkish-German Gynecological Education and Research Foundation and the Turkish-German Gynecological Association. Formerly named "ARTEMIS", the journal is published quarterly (March, June, September, December) in English and publishes original peer-reviewed articles, reviews, and commentaries in the fields of Gynecology, Gynecologic Oncology, Endocrinology & Reproductive Medicine and Obstetrics. Case reports are not accepted for publication. Reviews will be considered for publication only if they are prepared by authors who have at least three published manuscripts in international peer reviewed journals and these studies should be cited in the review. Otherwise only invited reviews will be considered for peer review from qualified experts in the area.

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

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

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

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All manuscripts should be accompanied by an abstract. A structured abstract is required with original articles and it should include the following subheadings: Objective, Material and Methods, Results and Conclusion. A structured abstract is not required with review articles. The abstract should be limited to 250 words for original articles and review articles.

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Address "Institutional Review Board" issues as stated above. State the generic names of the drugs with the name and country of the manufactures. Provide information on informed consent and ethics committee approval.

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Present the detailed findings supported with statistical methods. Figures and tables should supplement, not duplicate the text; presentation of data in either one or the other will suffice. Emphasize only your important observations; do not compare your observations with those of others. Such comparisons and comments are reserved for the discussion section.

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State the importance and significance of your findings but do not repeat the details given in the Results section. Limit your opinions to those strictly indicated by the facts in your report. Compare your finding with those of others. Provide information on the limitations and strenghts of the study. No new data are to be presented in this section.

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Book;

Kohler G; Egelkraut H. In Kohler G and Egelkraut H (edts).Munchener Funktionelle Entwicklungsdiagnostik im zweitem und drittem Lebensjahr. Handanweisung. Munchen: Uni Munchen, Institut fur Soziale Paediatrie und Jugendmedizin; 1984.

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Contents

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Editorial

Dear Colleagues,

It is my great pleasure to introduce the third issue of the "Journal of the Turkish-German Gynecological Association (J Turk Ger Gynecol Assoc)" in the publishing year of 2024. This issue is consisted of seven articles, and two reviews that we hope you will read with interest. Also you may have the oppurtunity to read the quiz. Here we share some of our favorite articles that were published in this issue of the journal.

Around the world, postpartum hemorrhage (PPH) continues to be a leading cause of maternal morbidity and mortality. For the treatment of PPH, the uterine artery and the hypogastric artery are clamped down. However, it is unclear how these devascularization procedures may affect ensuing pregnancies. You will have the opportunity to read an article comparing fertility and pregnancy outcomes after bilateral uterine artery ligation or bilateral hypogastric artery ligation for PPH.

Anti-Mullerian hormone (AMH)- a dimeric glycoprotein belonging to the transforming growth factor-beta family- is secreted by the granulosa cells of primary, preantral, and early antral follicles. AMH tends to decrease before FSH rises, making it a more sensitive indicator of ovarian reserve than FSH. You will also have the opportunity to read a review establishing reference values for AMH in Turkish girls.

Dear Esteemed Readers, Authors and Reviewers,

I'm proud to present the JTGGA's official Citescore value for 2023. Scopus data for 2023 indicate that JTGGA has a Citescore value of 2.4. When compared to our previously disclosed Citescore values of 1.7 in 2021 and 2.1 in 2022, this result shows a discernible improvement. This year, the JTGGA is placed 108/209 in the obstetrics and gynecology category with a Citescore value of 2.4. We truly value your confidence and support. We are moving forward quickly to continue growing and adding value to the field we work in.

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We are looking forward to receiving your valuable submissions, thank you in advance for your contributions.

Sincerely,

Prof. Cihat Ünlü, M.D.
Editor in Chief of *J Turk Ger Gynecol Assoc*
President of TGGF

Comparison of three umbilical entry sites for intraperitoneal access by the direct trocar insertion technique: a randomized pilot study

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Abstract

Objective: The most effective methods and entry sites for laparoscopic surgery remain a subject of ongoing investigation and discussion. The purpose of the study was to analyze and compare three umbilical entry sites for intraperitoneal access using the direct trocar insertion technique.

Material and Methods: A randomized pilot study was conducted between March 2021 and January 2023, involving women eligible for laparoscopic gynecological surgery. The women were allocated to one of three equally sized groups based on trocar entry points: subumbilical, supraumbilical, or umbilical. Success and failure rates of trocar entry, factors influencing success or failure, and early and late complications were systematically evaluated and compared across groups.

Results: A total of 243 patients, with a mean age of 32.93±8.33 years, were included in three groups of 81 each. Trocar entry success rates were 97.5%, 89.2%, and 89.5% in the supraumbilical, umbilical, and subumbilical groups, respectively ($p>0.05$). Failed trocar entry was significantly associated with age, gravidity, body mass index (BMI), waist circumference, hip circumference, and abdominal subcutaneous fat thickness ($p<0.001$). Regression analysis revealed that, in the subumbilical group, higher gravidity [odds ratios (OR): 0.390, 95% confidence interval (CI): 0.174-0.872, $p=0.022$) and greater abdominal subcutaneous fat thickness (OR: 0.090, 95% CI: 0.019-0.431, $p=0.03$) were associated with lower odds of successful trocar entry. In contrast, in the umbilical group, a higher waist circumference was associated with lower odds of successful trocar entry (OR: 0.673, 95% CI: 0.494-0.918, $p=0.012$). None of the covariates were significant in the supraumbilical group.

Conclusion: The study highlighted the importance of selecting the appropriate trocar entry site in laparoscopic gynecological surgery. Surgeons should consider factors such as age, gravidity, BMI, waist circumference, hip circumference, and abdominal subcutaneous fat thickness, as these factors significantly influence the success of trocar entry. (J Turk Ger Gynecol Assoc. 2024; 25: 116-23)

Keywords: Trocar insertion, laparoscopic surgery, complications, gynecologic surgery

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Introduction

Laparoscopy, a versatile diagnostic and surgical procedure employed in a variety of surgical specialties, has numerous advantages for patients, healthcare systems, and society at large. The recent years have witnessed a significant enhancement in the proficiency of gynecologists in conducting both fundamental and sophisticated endoscopic procedures (1). An extensive meta-analysis incorporating findings from 27 randomized controlled trials comparing laparoscopy and laparotomy for benign gynecological procedures reported a notable 40% reduction in the risk of minor complications associated with laparoscopy. However, the risk of major complications remains comparable between the two surgical approaches (2). Significantly, trocar insertion, serving as the primary entry point in laparoscopic procedures, continues to present substantial risks, contributing to 40% of laparoscopic complications and the majority of fatalities associated with this surgical approach (3,4). Life-threatening complications, including bowel and abdominal vessel perforations, underscore the critical nature of trocar insertion; 81% of trocar-related deaths are attributed to vascular injuries and 19% to intestinal injuries (5). Besides, minor complications, such as extraperitoneal insufflation, postoperative infection, subcutaneous emphysema, and trocar site hernia are also associated with laparoscopic entry (6,7). Despite the existence of various laparoscopic entry methods, including the Veress needle (VER), direct trocar insertion (DIR), and open (OP) techniques, there is no clear consensus regarding the optimal method (8-10). Indeed, each technique possesses its own set of advantages and disadvantages, and the choice of the entry method frequently depends on the surgeon's experience, training, and preference (3,8,9,11).

Clinicians do not concur about the optimal method of entering the peritoneal cavity (12,13). The ongoing debate about the methods and sites of initial entry underscores the need for further research in this field (14). The aim of the present study was to compare three umbilical entry sites, subumbilical, supraumbilical, and umbilical, for intraperitoneal access using the DIR technique.

Material and Methods

Study design and participants

This randomized pilot study was conducted at Afzalipour Hospital, affiliated with Kerman University of Medical Sciences, Kerman, Iran, from March 2021 and January 2023. Three umbilical entry sites (subumbilical, supraumbilical, umbilical) for intraperitoneal access in the DIR technique were compared. Women who could undergo gynecological laparoscopy, without a history of abdominal surgery, scars, contraindications for laparoscopic surgery, umbilical hernia, burns in the umbilical region, underlying heart or lung disease, gynecological malignancy, or the use of anticoagulant medication, were considered eligible. Women who were willing to participate in the study were included. Those requiring conversion to laparotomy, diagnosed with malignancy, transferred to an intensive care unit, or refusing further participation were excluded. The CONSORT flow diagram is shown in Figure 1.

Intervention and randomization of study participants

Prior to the intervention, the objectives of the study were explained to the patients in detail. They were also informed of the confidentiality of information and the voluntary nature of their participation. Informed consent was obtained from each participant. A total of 243 patients were randomly assigned to one of three equally sized groups: subumbilical (n=81), umbilical (n=81), or supraumbilical (n=81). Randomization was performed using a computer-generated random table block of size 6 (e.g., ABCABC, AABBCC, and all other possible restricted permutations).

Coded envelopes were created by a nurse who was not involved in the research project. The envelopes were prepared using sequential numbers, and patients were then allocated to one of the three groups, based on the assignment in the coded envelopes. All laparoscopic surgeries were performed by the primary gynecologic surgeon (Gh.M.) under general anesthesia using Storz endoscopic tools (Karl Storz). All patients were placed in the supine position, and a vertical or transverse incision was appropriately widened (1.0-1.5 cm) to allow for trocar insertion. Access to the abdominal cavity was achieved using a direct trocar in all patients. For initial intraperitoneal access, incisions were made either subumbilically (1-2 cm below the lower transverse fold of the navel), at the umbilicus, or supraumbilically (1-2 cm above the upper transverse fold of the navel). The trocar entry sites for intraperitoneal access were randomized. A 10 mm trocar was inserted, and a pneumoperitoneum was achieved by carbon dioxide insufflation to a pressure of 12-14 mmHg. The surgical procedures were conducted using conventional laparoscopic instruments under vision with a rigid 0-degree, 10 mm telescope (Karl Storz Company, Germany). The success of trocar insertion was established with a telescope. The fascia was repaired using Vicryl 1 thread and skin closure performed using 0.3 nylon.

Outcomes, measurements, and follow-up

In each group success and failure rates of trocar entry, factors influencing success or failure, and early and late complications were systematically evaluated. Successful trocar entry was defined as the trocar entering the abdominal cavity after one or two attempts. More than two attempts or the need to switch to another entry technique was considered a failed entry.

Factors contributing to success or failure included gravidity,

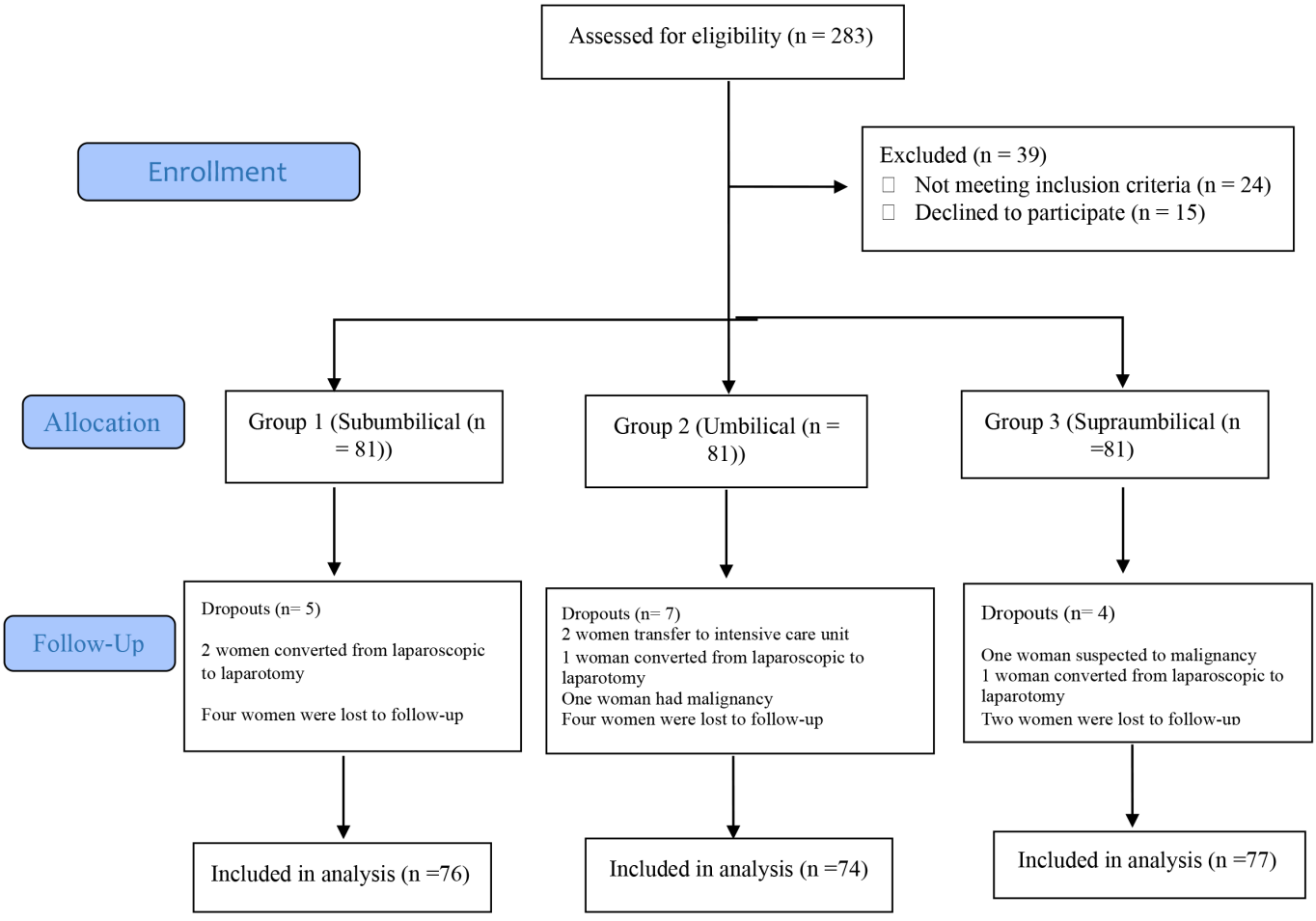

Figure 1. Diagram of the study

body mass index (BMI) in kg/m^2, waist and hip circumferences, and abdominal subcutaneous fat thickness. Postoperative complications were categorized as early (including damage to vessels and viscera, ileus, postoperative fever, and bleeding/hematoma) and late (including hernia, wound infection, hematoma, and pain at the operative site).

All outcomes were compared among groups, and baseline parameters such as age, gravidity, weight, height, BMI, waist and hip circumferences, and abdominal subcutaneous fat thickness were recorded prior to the intervention. Waist circumference was measured using a flexible non-elastic measuring tape, midway between the lowest costal margin and the anterior superior iliac spine at the end of normal expiration, while hip circumference was measured at the widest part of the hip in a standing position. Abdominal subcutaneous fat thickness was measured using a caliper (Holtain, Dyfed, UK) with 0.2 mm accuracy, following a standardized protocol (15). Abdominal subcutaneous fat thickness was measured on the right side of the body with the patient standing upright. The exact measuring sites were selected and found according to Eston and Reilly (16), with the horizontal fold raised 3 cm lateral and 1 cm inferior to the umbilicus.

Measurements were conducted by an experienced and trained nurse. The duration of laparoscopic surgery and indications were recorded at the end of the surgery. All participants were followed up for 6-8 weeks, and any complications were documented in the patients' files.

Ethical considerations

In all steps carried out in this study, the principles of the Declaration of Helsinki (ethical principles for medical research involving human subjects) and the Ethics Committee of Kerman University of Medical Sciences were followed. The study was approved by the ethics committee of the Afzalipour Hospital-Kerman University of Medical Sciences (approval number: IR.KMU.AH.REC.1400.168, date: 25.10.2021), and registered at the Iranian Registry of Clinical Trials (no:

IRCT20230307057644N1). The objectives of the trial were explained to women enrolling in the trial, and their written informed consent was obtained. The subjects were free to discontinue their participation at any time. All personal data were treated confidentially and only reported in collective form.

Statistical analysis

The statistical software program IBM SPSS Statistics for Windows, version 21.0 (IBM Corp., Armonk, NY, USA), was used for data analysis. Quantitative variables were described using means (standard deviation), and qualitative variables were described using frequencies (percentage). The chi-square test was employed for qualitative variables. Normal distribution of quantitative data was assessed using the one-sample Kolmogorov-Smirnov test. Student's t-test and ANOVA were used to examine intragroup differences. Logistic regression models utilizing the backward (Wald) method were employed to assess the association between successful trocar entry and entry site. Estimates were adjusted for age, gravidity, BMI, waist and hip circumferences, and abdominal subcutaneous fat thickness. Dependent variables were categorized as successful (1) or failed (0) trocar entry. Estimated logits and odds ratios (OR) with corresponding 95% confidence intervals (CI) 95% were reported, and the level of significance was set to 0.05.

Results

Of 283 eligible patients, 243 were included in the study (81 cases in each group). Five, seven, and four cases were excluded from the first, second, and third groups, respectively (Figure 1). The mean age of the patients was 32.93±8.3 years, with no significant differences in demographic or clinical characteristics between the three groups prior to the intervention (Table 1). Indications for surgery included hysterectomy, cystectomy, myomectomy, infertility, ectopic pregnancy, and diagnostic laparoscopy. Differences in the indications for surgery and the duration of surgery did not differ significantly between groups.

Failure and success rates of trocar entry into the abdomen in the three groups are presented in Table 2. As shown in Table 2, the overall success rate of entry was 92.9% while the success rates in the subumbilical, umbilical and supraumbilical groups were 89.5% (68/76), 89.2% (66/74), and 97.5% (75/77), respectively. The successful entry rate was higher in the supraumbilical group than in the umbilical and subumbilical groups, but the difference was not significant.

Age, gravidity, BMI, waist circumference, hip circumference,

Table 1. Comparison of demographic and clinical characteristics between groups prior to intervention

Variable		Subumbilical, (n=76)	Umbilical, (n=74)	Supraumbilical, (n=77)	p*
Age, years	Min.-max.	18-51	18-57	19-57	0.65
	Mean ± SD	32.8±7.87	33.6±8.6	32.4±8.5	
Gravidity, (n)	Min.-max.	0-5	0-5	0-5	0.25
	Mean ± SD	2.01±1.5	1.9±1.4	1.6±1.3	
BMI, kg/m^2	Min.-max.	18.2-43.7	17.5-47	18-47.5	0.3
	Mean ± SD	31.2±6.03	32.7±6.9	31.3±7.3	
Waist circumstance, cm	Min.-max.	69-130	68-128	67-130	0.5
	Mean ± SD	91.6±12.3	94±13.3	93.28±15.8	
Hip circumstance, cm	Min.-max.	85-142	85-149	82-150	0.3
	Mean ± SD	108.3±12.06	111.5±12.8	109.7±15.49	
Abdominal subcutaneous fat thickness, cm	Min.-max.	0.5-6.5	0.5-6	0.5-6.5	0.43
	Mean ± SD	3.25±1.34	3.55±1.38	3.47±1.57	

*Comparison of three groups prior to intervention by ANOVA, SD: Standard deviation, min.: Minimum, max.: Maximum, BMI: Body mass index

Table 2. Failure and success rates of trocar entry in the studied groups

			Subumbilical, (n=76)	Umbilical, (n=74)	Supraumbilical, (n=77)	Total	p*
			Number (%)				
Success and failure in trocar entry	Failed		8 (10.5)	8 (10.8)	2 (2.6)	18 (7.9)	0.1
	Successful	First entry	52 (68.4)	45 (60.8)	61 (79.2)	158 (69.6)	
		Second entry	16 (21.1)	21(28.4)	14 (18.2)	51 (22.5)	

*Chi-square (χ^2)

and abdominal subcutaneous fat thickness were systematically compared between successful and failed trocar entries in each of the three groups. As detailed in Table 3, patients with failed trocar entry across all groups exhibited a significantly older age, and higher gravidity, BMI, waist circumference, hip circumference, and abdominal subcutaneous fat thickness compared to those with successful trocar entry ($p<0.001$).

Logistic regression was used to analyze successful trocar entry, adjusting for age, gravidity, BMI, waist circumference, hip circumference, and abdominal subcutaneous fat thickness. In the subumbilical group, the regression results demonstrated that the odds of success entry decreased with higher gravidity (OR: 0.390, 95% CI: 0.174-0.872, $p=0.022$) and abdominal subcutaneous fat thickness (OR: 0.090, 95% CI: 0.019-0.431, $p=0.03$). The remaining covariates (age, BMI, waist circumference, and hip circumference) were not statistically significant. In the umbilical group, a greater waist circumference was associated with decreased odds of successful entry (OR: 0.673, 95% CI: 0.494-0.918, $p=0.012$). In the supraumbilical group, none of the covariates reached statistical significance. The results of the multiple logistic regression analysis of successful trocar entry are summarized in Table 4.

Complications included fever, bruising at the operation site, chronic pain, hematoma, and surgical site infection. As shown in Table 5, both early and late complications related to trocar entry tended to be lower in the supraumbilical and subumbilical groups than in the umbilical group. However, once again the differences were not statistically significant. A comparison of early and late complications of trocar entry into the abdomen among the three groups is provided in Table 5.

Discussion

The advancement of laparoscopic surgical techniques in recent decades has notably improved patient safety and reduced complications. In view of the fact that a significant number of laparoscopic complications occur during abdominal entry, the method of accessing the abdomen is of importance (13). The umbilicus is commonly selected as the site for primary trocar insertion because it offers the shortest distance between the skin and the anterior peritoneum, is cosmetically appealing, and its anatomic relation to vital retroperitoneal structures is well understood. The specific site of trocar entry has not been extensively evaluated (17), although a number of authors have analyzed and compared the outcomes of different entry techniques (14). To fill this gap, we undertook a comparison of three umbilical entry sites for intraperitoneal access using the DIR technique in laparoscopic gynecological surgery. Our findings revealed an overall failed trocar entry rate of 7.9%, which is consistent with previously reported rates of 7.8% to 35% (18,19). While we registered a higher success rate in the supraumbilical group than in the umbilical or subumbilical groups, the difference was not significant. Although we were unable to compare our findings with others due to the absence of specific studies on the optimal trocar entry site for laparoscopic gynecological surgery, Stanhiser et al. (17) reported theoretic modelling and suggested that supraumbilical primary port placement is superior to umbilical entry in laparoscopy. This is because supraumbilical entry provides a greater distance to retroperitoneal vessels compared to umbilical entry, potentially reducing the risk of vascular injury (17).

Table 3. Success and failure of trocar insertion according to the studied variables in the three groups

	Subumbilical, (n=76)		p†	Umbilical, (n=74)		p†	Supraumbilical, (n=77)		p†
	Successful, (n=68)	Failed, (n=8)		Successful, (n=66)	Failed, (n=8)		Successful, (n=75)	Failed, (n=2)	
	Mean ± SD								
Age, y	32.5±7.9	35.38±7.34	<0.001	33.08±8.8	38.13±4.6	<0.001	32.11±8.5	42.5±6.3	<0.001
Gravidity, (n)	1.91±1.5	2.87±1.4	<0.001	1.8±1.39	2.75±1.28	<0.001	1.62±1.4	2±0	<0.001
BMI, kg/m²	30.31±5.5	38.87±4	<0.001	31.64±6.38	42.01±3.37	<0.001	31.09±7.3	40.35±0.91	<0.001
Waist circumstance, cm	89.61±10.91	108.75±11.28	<0.001	91.43±11.63	115.12±6.49	<0.001	92.61±15.31	118.5±4.9	<0.001
Hip circumstance, cm	106.5±11.11	123.37±9.42	<0.001	109.25±11.28	130.87±7.73	<0.001	109.1±15.21	132.5±6.36	<0.001
Abdominal subcutaneous fat thickness, cm	3.03±1.2	5.11±0.9	<0.001	3.3±1.26	5.5±0.34	<0.001	3.4±1.5	5.95±0.35	<0.001

†Success and failure in each group compared by Student's t-test, BMI: Body mass index, MD: Mean differences, SD: Standard deviation

Compeau et al. (20) reviewed general surgical practices in Canada regarding laparoscopic entry, including 248 of 1000 members of the Canadian Association of General Surgeons. The findings showed that, in a virgin abdomen, the umbilical region was the primary port site, with 51.5% of surgeons favoring infraumbilical entry and 35.7% preferring supraumbilical entry. In addition, when peritoneal adhesions were known or suspected, the umbilical site remained the most popular choice for entry location (20). Şentürk et al. (21) evaluated the cosmetic outcomes of infraumbilical, supraumbilical, and transumbilical entry routes in laparoscopic surgery in 2018, and found no statistically significant differences in cosmetic results between the three groups.

We focused on the site of trocar entry and assessed success based on various factors: patients with failed trocar entry tended to be older, had higher gravidity, were more obese, and possessed a greater waist and hip circumference, as well as increased abdominal subcutaneous fat thickness. In the study of Warchałowski et al. (22), factors such as age, gender, neurological disease, and diabetes were predictor variables of laparoscopic surgery success and failure. In contrast, Tam et al. (23) found no statistically significant difference in patient characteristics (age and BMI) and the success of the surgery.

Subgroup analysis further identified associations between specific factors and trocar entry success or failure within each entry site group. In the subumbilical trocar entry group, higher

Table 4. Binary logistic regression of the effects of variables on successful or failed entry

Groups	Predictors	B	S.E.	OR	95% CI	Wald	p
	Model						
Subumbilical	Age	-0.089	0.081	0.915	0.780-1.073	1.200	0.273
	Gravidity	-0.942	0.411	0.390	0.174-0.872	5.261	0.022
	BMI	-0.072	0.220	0.931	0.605-1.431	0.107	0.743
	Waist circumstance	0.063	0.189	1.066	0.735-1.54	0.113	0.737
	Hip circumstance	-0.132	0.130	0.876	0.679-1.130	1.039	0.308
	Abdominal subcutaneous fat thickness	-2.409	0.799	0.090	0.019-0.431	9.082	0.003
Umbilical	Age	-0.214	0.113	0.808	0.647-1.008	3.565	0.059
	Gravidity	0.194	0.869	1.215	0.221-6.665	0.05	0.823
	BMI	-0.169	0.32	0.845	0.451-1.582	0.279	0.598
	Waist circumstance	-0.396	0.158	0.673	0.494-0.918	6.267	0.012
	Hip circumstance	0.325	0.343	1.384	0.706-2.713	0.894	0.344
	Abdominal subcutaneous fat thickness	-3.005	2.668	0.05	0-9.242	1.269	0.26
Supraumbilical	Age	-0.124	0.079	0.884	0.757-1.032	2.451	0.117
	Gravidity	1.899	1.754	6.679	0.215-207.92	1.172	0.279
	BMI	-11.011	334.183	0	0-4.73	0.001	0.974
	Waist circumstance	-0.14	0.081	0.869	0.742-1.018	3.013	0.083
	Hip circumstance	0.534	0.54	1.706	0.593-4.91	0.98	0.322
	Abdominal subcutaneous fat thickness	-191.306	4581.642	0	0-8.08	0.002	0.967

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

Table 5. Early and late complications of trocar entry into the abdomen in the three groups

		Subumbilical, (n=76)	Umbilical, (n=74)	Supraumbilical, (n=77)	p*
		Number (%)			
Early postoperative complications	Fever	3 (3.94)	10 (13.51)	2 (2.59)	0.099
	Bruising at the operation site	5 (6.57)	6 (8.1)	7 (9.09)	
Late postoperative complications	Chronic pain	3 (3.94)	4 (5.4)	6 (7.8)	
	Hematoma	0	1 (1.35)	1 (1.29	
	Surgical site infection	5 (6.57)	4 (5.4)	4 (5.19)	
Total		16 (21.05)	24 (32.43)	20 (25.97)	

*Chi-square (χ^2)

gravidity and abdominal subcutaneous fat thickness were associated with failed trocar entry. In the umbilical group, a greater waist circumference was a significant predictor of failed entry. However, in the supraumbilical group, none of the covariates achieved statistical significance. This finding is consistent with the published literature, which supports different trocar insertion locations based on various factors (17,24). In gynecological surgery, when dealing with cases involving large uteri or pelvic masses, the use of a primary umbilical port is severely limited due to its close proximity to the uterus or pelvic mass. This limitation reduces operative exposure and significantly complicates instrument manipulation as well as the overall surgical procedures (17). In cases of large fibroid uteri during myomectomy or hysterectomy (25), adnexal masses requiring cystectomy or oophorectomy (26), and surgery in pregnant patients, the use of a supraumbilical port has proven beneficial (27,28). These ports provide better exposure and easier instrument manipulation, thereby facilitating minimally invasive procedures and avoiding injury to the pregnant uterus (17). In addition, Hurd et al. (24) found that the position of the umbilicus relative to major vessels varied significantly with BMI. In women of normal weight the umbilicus was 0.4 cm caudal to the aortic bifurcation, while in overweight and obese women it was 2.4 and 2.9 cm caudal, respectively (24). These anatomical differences highlight the need for alternative entry locations and techniques in obese patients in order to reduce surgical risks.

An inherent challenge in trocar entry is the possibility of delayed recognition of injury, potentially necessitating abdominal repair (29). We registered postoperative complications such as fever, bruising at the operation site, chronic pain, hematoma, and surgical site infection. Notably, complications associated with trocar entry tended to be less frequent in the supraumbilical and subumbilical groups compared to the umbilical group, but the differences were not significant. In line with our findings, a study conducted by Jategaonkar et al. (30) involving 2,300 patients suggests that trocar entry through the umbilicus is safe due to the thinness of the umbilicus. Conversely, Stanhiser et al. (17) reported that primary supraumbilical port placement is safer than entry through the umbilicus in laparoscopy, probably because the distance to retroperitoneal vessels was greater when the supraumbilical entry site was used.

Study limitations

The principal strength of the present study is its randomized clinical design to evaluate factors influencing the success and complications of trocar entry sites in laparoscopic gynecological surgery. The study effectively mitigated confounding variables, ensuring comparability between the three groups and enhancing the validity of the results. Despite the valuable insights provided by the investigation, its inherent limitations are worthy of mention. Prime among these is the relatively small sample size. Future studies should prioritize the design and execution of high-quality, long-term clinical studies featuring larger sample sizes. This approach will be essential for a comprehensive exploration of life-threatening issues, thereby substantiating and extending the findings of the current study.

Conclusion

The present investigation is a significant contribution to laparoscopic gynecological surgery in that it provides a comprehensive evaluation of the impact of trocar entry site on surgical success rates and postoperative complications. Our findings emphasize the importance of tailoring entry site selection based on patient-specific factors, such as gravidity, abdominal subcutaneous fat thickness, and waist circumference, for the purpose of optimizing surgical outcomes. Patients with higher gravidity and abdominal subcutaneous fat thickness exhibited increased odds of failed trocar entry, particularly in the subumbilical entry group. A greater waist circumference was also associated with a greater likelihood of failed entry in the umbilical group. These insights underscore the need for surgeons to carefully consider patient characteristics when determining the most suitable trocar entry site. While our study sheds light on the impact of the trocar entry site, further research will be needed to refine entry techniques and assess their impact on surgical outcomes. Continued investigation in this field will contribute to the ongoing enhancement of laparoscopic gynecological surgery, ultimately leading to better patient care and improved outcomes.

Ethics Committee Approval: *The study was approved by the Ethics Committee of the Afzalipour Hospital-Kerman University of Medical Sciences (approval number: IR.KMU.AH.REC.1400.168, date: 25.10.2021).*

Informed Consent: *Informed consent was obtained from each participant.*

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

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The effect of the distance between mesh and the urethra on sexual function in patients who underwent transobturator tape

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Abstract

Objective: To evaluate the effect of mesh-urethra distance on sexual function in continent patients who underwent transobturator tape (TOT) surgery due to isolated stress urinary incontinence (SUI).

Material and Methods: Continent patients who had undergone TOT surgery for SUI were eligible. Objective treatment for SUI was defined as the absence of urine leakage during a stress test. Translabial perineal ultrasound was performed six months after surgery. The successful surgical group was split into two subgroups based on the distance from the posterior of the urethra at the bladder neck to the nearest proximal edge of the tape: <5 mm and >5 mm. In addition to these, band percentile, the descent of bladder neck and urethra length measured by perineal ultrasound, pubo-urethral distance, urethral thickness, detrusor thickness, cystocele descent, rectal descent, and uterine descent were evaluated. Preoperative and postoperative results of the standardized and internationally valid incontinence questionnaires Incontinence Questionnaire Urinary Incontinence Short Form and Female Sexual Function Index (FSFI) were compared between groups.

Results: Eighty-two patients were included. The postoperative FSFI scores for the >5 mm group were significantly lower than those of the <5 mm group, including the postoperative FSFI average, all subscales except lubrication, and average change scores due to the operation ($p<0.001$). There was no statistically significant relationship between the percentile occupied and postoperative FSFI score ($p=0.553$), and the FSFI preoperative-postoperative difference was not significant ($p=0.905$).

Conclusion: Sexual functions are more affected in patients with a mesh-urethra distance >5 mm as measured by perineal ultrasound. (J Turk Ger Gynecol Assoc. 2024; 25: 124-31)

Keywords: Transobturator tape, transperineal ultrasound, female stress incontinence, sexualiy

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Introduction

Urinary incontinence is a common pathological condition observed among women. Stress urinary incontinence (SUI), which is responsible for 52-65% of cases of urinary leakage, occurs in women aged 30 to 60 years (1). SUI, which negatively affects women's quality of life, can impact their physical activities and emotional, psychosocial, and even sexual lives. Severe incontinence can lead to decreased libido and vaginal dryness in women (2). This reality affects not only the sexual function of women with SUI but also their relationships with their partners, potentially leading to an overall worse sexual experience and consequently a decrease in quality of life (3).

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Thus, a better understanding of the sexual functions of women with SUI is important (4).

The loss of connective tissue and pelvic floor muscle support leads to inadequate coaptation of the urethra in stress urinary physiopathology, causing incontinence. This loss of support results in urethral hypermobility, leading to downward movement of the bladder neck (5). Evaluating bladder neck mobility is part of an SUI assessment. However, traditional diagnostic methods, including medical history, physical examination, urinary incontinence surveys, urine analysis, and Q-tip tests, have specific limitations (6). Therefore, transperineal ultrasonography has become increasingly common for evaluating the bladder neck, especially proximal urethral mobility. Pelvic floor ultrasound is considered the best imaging technique for assessing the position and condition of mesh placement (7). Furthermore, by creating a hyperechoic image with ultrasound, tapes and materials can be easily visualized, providing good correlation with surgical exploration and facilitating the assessment of postoperative complications. Pelvic floor ultrasound is a useful tool for visualizing slings, assessing urethral mobility, and evaluating post-surgical changes after anti-incontinence surgery (8).

In recent years, mid-urethral sling (MUS) surgery using synthetic meshes that provide the necessary urethral support have emerged as an effective treatment method for SUI. The widespread preference for MUS surgery is due to its minimally invasive nature, high success rate, and relatively low complication rate. Despite these advantages, surgical outcomes after MUS may not always be successful. Perioperative complications, inadequate tension of the mesh, or incorrect mesh placement can lead to treatment failure (9). The aim of the present study was to evaluate the significance of the mesh-urethra distance on sexual function using perineal ultrasound in continent patients who underwent TOT surgery due to isolated SUI.

Material and Methods

Between January 2020 and January 2022, patients aged 18 to 45 years who underwent MUS surgery due to isolated SUI were included in the study. All patients who underwent TOT surgery were included in routine postoperative follow-up, including clinic visits on postoperative day seven and at six months. On the seventh day after surgery, patients underwent postoperative care, stress cough test, and residual urine volume measurement. At the 6-month follow-up, 1-hour pad test and perineal ultrasound, were added to the previously used tests. Objective results were evaluated with cough stress test and 1-hour pad test. SUI treatment was defined as no urine leakage during the cough stress test and a pad weight of less than 2 g during the 1-hour pad test at the follow-up visit. Healing was defined as a decrease of more than 50% in urine weight in the 1-hour pad test and a positive result in the cough stress test. Failure was defined as less than a 50% decrease in the 1-hour pad test and a positive result in the cough stress test. Subjective cure was defined as follows: A woman was urinary continent if her total score on the International Consultation on Incontinence Questionnaire-Urinary Incontinence-short form (ICIQ-UI-SF) was 0 and she answered “never” to “When does urine leak?”. Patients who achieved objective and subjective cures after surgery were identified as urinary continent, and only patients who did not experience postoperative urinary leakage were included in the study.

The study was approved by the University of Health Sciences Turkey, İstanbul Prof. Dr. Cemil Taşcıoğlu City Hospital Local Ethics Committee (approval number: 27, date: 23.01.2023) and registered with the National Clinical Trials Registry under NCT06211894. Written consent was obtained from each participant. The study design was conducted in accordance with the Declaration of Helsinki. Patients who experienced surgical failure in incontinence surgery, who had undergone previous vaginal surgery, who had a history of hysterectomy, who underwent additional surgeries during the same session, who had pelvic organ prolapse, who had received radiation therapy, who had a diagnosis of malignancy, who were menopausal, who received external hormone treatment, who were sexually inactive, whose partners had erectile dysfunction, who did not attend postoperative follow-up appointments, who became postoperatively pregnant, and who had undergone non-synthetic mesh (autologous fascia) surgery for SUI were excluded from the study.

Sexual dysfunction is briefly defined as a persistent or recurrent disorder in sexual desire or response. In this context, women with sexual dysfunction due to hormonal reasons or vasculogenic factors (such as diabetes mellitus, peripheral arterial disease, smoking), neurogenic factors (spinal cord injuries, multiple sclerosis, disc herniation, peripheral neuropathy), myogenic factors, pelvic surgery, medications, and psychogenic factors were also excluded from the study.

All the patients received intravenous antibacterial prophylaxis (cefazolin, 2 g) at the beginning of surgery, while no vaginal preparation was necessary the day before the surgery. Transobturator approach was performed as described by Delorme (10) in 2001 using a curved tunneler inserted from the outside entrance point to adjust the tape without any tension. Prolene light mesh (condensed monofilament non-absorbable polypropylene) was used as mesh material. A piece of polypropylene mesh (1.0-1.2 cm in width 20 cm in length) was cut by the surgeon from condensed monofilament non-absorbable polypropylene (TAHA Prolene Polypropylene mesh, Altaylar Bilim, Turkey). All patients who underwent routine TOT

procedures were operated on by specialized urogynecologists at a single center, and all surgical operations were performed by the same surgical team. Except for cases with complications related to bladder injury, routine cystoscopic examination was not performed. Perioperative and postoperative complication incidence, febrile morbidity, analgesic requirements, and postoperative hospital stay were recorded. The remaining urine volume was evaluated after Foley catheter removal. Difficulty in urinating was defined as a residual urine volume of 150 mL or more, controlled by catheterization after urination.

Preoperative and postoperative 6-month follow-ups included perineal ultrasound using an abdominal probe, often employing a Siemens Acuson X 300 ultrasonography device, to assess the position and functionality of the sling at rest. Patients were examined with a probe placed on the sagittal plane of the labia minora while in a semi-recumbent position, with the bladder filled to 200-300 mL. The methodology followed during the transperineal pelvic floor ultrasound consisted of capturing three volume measurements for each patient: at rest, during the Valsalva maneuver (minimum of 6 seconds) and at maximum contraction. The ultrasound parameters included are described in Figure 1.

Images were acquired in the median sagittal plane, encompassing views of the symphysis pubis, bladder, urethra, vagina, and anal canal. The suburethral sling was identified as a hyperechoic structure, and the sling's position was measured relative to the urethra and symphysis pubis both at rest and during the Valsalva maneuver.

Patients who achieved continence after surgery were split into two subgroups based on the distance from the posterior of the urethra at the bladder neck to the nearest proximal edge of the tape: <5 mm and >5 mm. The position of the sling along the urethra was measured as a percentage of urethral length and is referred to as the sling percentile. This measurement was calculated as follows: the proximal urethral length (distance from the sling's proximal point to the bladder neck) divided by the total urethral length (distance from the bladder neck to the external urethral meatus) in the sagittal plane, where the bladder neck and the external urethral meatus represent 0% and 100% of urethral length, respectively. Additionally, perineal ultrasound was used to evaluate various parameters including bladder descent, pubo-urethral distance, urethral thickness, detrusor thickness, cystocele descent, rectal descent, and uterine descent.

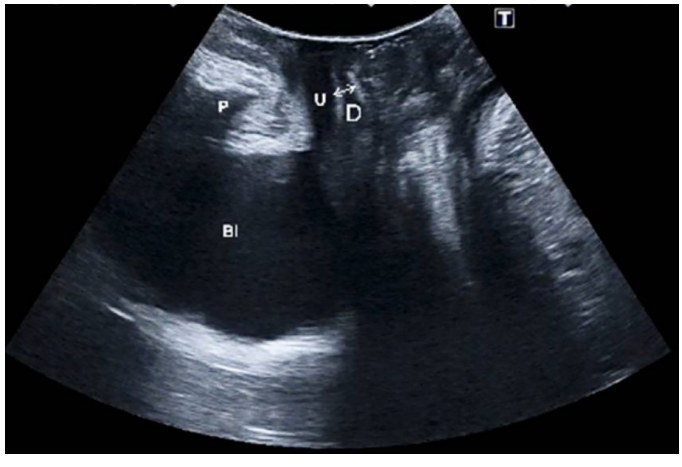

Figure 1. The ultrasound parameters
P: Symphysis pubis, U: Urethra, BL: Bladder, D: Urethra mesh between distance

In addition, questionnaires were administered preoperatively and at the 6-month postoperative follow-up. The FSFI is a Likert-type scale consisting of 19 items that assess sexual dysfunction in women. The Turkish version of the questionnaire was previously validated (11). The scale consists of six separate sections: desire; arousal; lubrication; orgasm; satisfaction; and pain. The overall score of the scale ranges from a minimum of 2 to a maximum of 36, with a higher score indicating better function. A score of 26.55 or below indicated the existence of sexual dysfunction in this study.

The ICIQ-UI SF was used to assess the severity of urinary incontinence at the 6-month follow-up (12). The ICIQ-UI SF score ranges between 0 and 21 and represents the weighted sum of three factors related to urinary incontinence: urinary incontinence frequency ("How often do you experience urine leakage?" rated from 0 = never to 5 = all the time); quantity of leakage ("How much urine do you usually leak?" rated from 0 = none to 6 = a large amount); and interference with daily life (rated from 0 = not at all to 10 = a great deal). Higher scores indicate more severe urinary incontinence. Secondary outcomes included no incontinence (defined as responses indicating "never" or "none" to ICIQ-UI SF frequency or quantity items) and improvement in urinary incontinence (indicated by a reduction in ICIQ-UI SF score of ≥3 points). The Turkish version of the questionnaire was validated (13).

Statistical analysis

SPSS, version 15.0 for Windows was used for statistical analysis (IBM inc., Armonk, NY, USA). Descriptive statistics encompassed the mean, standard deviation, minimum, maximum, and median values for numerical and categorical variables. When numerical variables did not adhere to the normal distribution assumption, independent two-group comparisons were performed using the Mann-Whitney U test. Relationships between numerical and ordinal variables were explored via Spearman's correlation analysis due to the unmet parametric test condition. A significance level of $p<0.05$ was adopted for the alpha.

Results

Eighty-two patients were included in the study and were divided into two groups based on the distance between the mesh and the urethra. There was a significant difference between these two defined groups (p<0.001). There were no differences observed between the two groups in terms of age, gravidity, parity, normal birth, and body mass index. In the perineal ultrasound of all patients in both groups, the preoperative urethral rotation angle was greater than 45°, and the preoperative retrovesical angle was greater than 140°. No differences were observed between the defined groups in terms of sling percentiles. The findings are summarized in Table 1.

There were no significant differences between the two groups in terms of preoperative urethral thickness, descent of the bladder neck, detrusor thickness, cystocele, rectocele, or uterine descent. Preoperative ICIQ-SF scores for questions 3-5 indicated moderate severity, and there was no difference between the two groups. Postoperatively, ICIQ-SF scores improved in both groups, with no differences observed between the groups. The mean pubo-urethral distance of patients in the <5 mm group was statistically significantly higher compared to patients in the >5 mm group (p=0.031). Postoperatively, detrusor thickness was statistically thicker in the group with a band distance >5 mm (p=0.030). The findings are summarized in Table 2.

No differences were observed between the groups in terms of preoperative FSFI scores. The postoperative FSFI scores for the >5 mm group were significantly lower than those of the <5 mm group, including the postoperative FSFI average, all subscales except lubrication, and average change scores due to the operation (p<0.001 for all). The FSFI pre- to post-operative changes are summarized in Table 3.

The distance to the urethra was found to be significantly and negatively correlated with patients' postop FSFI levels (p<0.001) and FSFI preop-postop difference (p<0.001). There was no significant relationship found between the placement percentile and postop FSFI levels (p=0.553) or the FSFI preop-postop difference (p=0.905). Relationships between Mesh-Urethra Distance and Percentile ΔFSFI are summarized in Table 4.

Relationship between FSFI Preop-Postop Difference and Mesh-Urethra Distance is shown in Figure 2. The cut-off value for postop FSFI level when the distance to the urethra was >5 mm was ≤28.4 in the assessment, with 100% sensitivity and 97.6% specificity. The receiver operating characteristic curve is shown in Figure 3.

Table 1. Demographic data of patients and perineal ultrasound findings

	Distance between the mesh and the urethra		
	<5 mm	**>5 mm**	**p[#]**
	Mean ± SD Min.-Max. (median)	**Mean ± SD Min.-max. (median)**	
Distance to urethra, mm	3.60±0.56 2.7-5.0 (3.6)	5.82±0.35 5.2-7.0 (5.8)	<0.001
Age, years	39.59±3.38 33.0-46.0 (40.0)	39.17±2.90 34.0-45.0 (40.0)	0.595
Gravidity, (n)	2.98±1.04 1.0-5.0 (3.0)	3.61±1.45 1.0-8.0 (3.0)	0.054
Parity, (n)	2.46±0.78 1.0-4.0 (2.0)	2.61±0.97 1.0-5.0 (3.0)	0.620
Spontaneous vaginal delivery, (n)	2.27±0.63 1.0-3.0 (2.0)	2.49±0.87 1.0-5.0 (2.0)	0.291
Body mass index, kg/m^2	25.14±3.18 18.3-31.2 (25.0)	24.81±2.06 18.3-29.4 (24.6)	0.666
Tobacco user, n (%)	16 (39.0)	13 (31.7)	0.488[ε]
Menopause, n (%)	0 (0%)	0 (0%)	-
Sling percentile placement	66.46±5.62 55.0-80.0 (65.0)	65.37±5.41 60.0-80.0 (65.0)	0.274
Preoperative retrovesical angle >140°, n (%)	41 (100)	41 (100)	-
Preoperative urethral rotation angle >45°, n (%)	41 (100)	41 (100)	-

[#]Mann-Whitney U test, [ε]chi-squared test, min.: Minimum, max.: Maximum, SD: Standard deviation

Table 2. Pre- and postoperative perineal ultrasound findings and ICIQ-SF scores

	Distance between the mesh and the urethra				
	<5 mm		>5 mm		p#
	Mean ± SD	Min.-max. (median)	Mean ± SD	Min.-max. (median)	
Preoperative					
Descent of bladder neck	31.63±3.47	26-40 (31)	31.15±2.74	28-42 (31)	0.667
Pubo-urethral distance	14.37±1.68	11-18 (14)	13.61±1.53	12-18 (13)	0.031
Urethral thickness	4.83±0.77	4-7 (5)	4.44±0.67	3-5 (5)	0.054
Detrusor thickness	3.54±0.60	3-5 (3)	3.76±0.80	2-5 (4)	0.139
Cystocele descent	7.20±1.29	4-10 (7)	7.15±1.15	5-10 (7)	0.728
Rectum descent	6.98±1.68	4-10 (7)	6.90±1.41	4-10 (7)	0.713
Uterine descent	6.07±1.71	4-12 (6)	6.20±1.36	4-9 (6)	0.465
ICIQ-SF question 6	3.06±0.17	3.0-3.5 (3.0)	3.06±0.17	3.0-3.5 (3.0)	1.000
ICIQ questions 3-5 total score	12.39±2.77	9.0-18.0 (12.0)	11.54±2.09	9.0-15.0 (11.0)	0.226
Standing stress test, n (%)	41 (100.0)		41 (100.0)		-
Supine stress test, n (%)	38 (92.7)		37 (90.2)		1.000£
Q tip test >30°, n (%)	41 (100.0)		41 (100.0)		-
Postoperative					
Descent of bladder neck	16.24±2.49	10-21 (16)	16.27±2.07	12-20 (16)	0.978
Pubo-urethral distance	5.85±1.24	4-8 (6)	6.15±1.30	4-8 (6)	0.316
Urethral thickness	3.93±0.57	3-5 (4)	4.15±0.69	3-5 (4)	0.113
Detrusor thickness	3.02±0.65	2-4 (3)	3.34±0.69	2-4 (3)	0.030
Cystocele descent	6.61±1.46	4-10 (7)	6.85±1.17	5-9 (7)	0.527
Rectum descent	7.00±1.77	4-10 (7)	6.88±1.31	5-10 (7)	0.637
Uterine descent	6.02±1.51	4-10 (6)	6.15±1.33	4-10 (6)	0.484
ICIQ-SF question 6	1±0	1-1 (1)	1±0	1-1 (1)	1.000
ICIQ questions 3-5 total score	1.71±1.97	0-4 (0)	1.20±1.79	0-4 (0)	0.205
Standing stress test, n (%)	0 (0)		0 (0)		-
Supine stress test, n (%)	0 (0)		0 (0)		-
Q tip test >30°, n (%)	0 (0)		0 (0)		-

#Mann-Whitney U test, £Chi-squared test, ICIQ-SF: Incontinence Questionnaire-Short Form, min.: Minimum, max.: Maximum, SD: Standard deviation

Discussion

In the present study, improvement in sexual function was observed after TOT. However, in the group with a mesh-urethra distance >5 mm, sexual function scores were mostly significantly lower compared to the <5 mm group. In recent years, the use of ultrasound has increased in urogynecological fields, such as urinary incontinence and pelvic organ prolapse due to its non-invasive, dynamic nature and easy accessibility. In a recent study focusing on SUI, changes in the proximal urethra and retrovesical angle were observed to be potentially correlated with clinical symptoms in perineal ultrasound assessments (6). Another study involving SUI and a control group found that perineal ultrasound revealed higher urethral rotation, bladder neck descent, and posterior urethrovesical angle in patients compared to the control group, both at rest and during the Valsalva maneuver (14). The same study showed that patients with higher maximum urethral closure pressure exhibited greater rotation angle and urethrovesical junction movement during urodynamic testing. In the present study, both groups of stress incontinent patients had preoperative retrovesical angles >140° and urethral rotation angles >45°. While imaging plays a limited role in evaluating mild pelvic prolapse cases with a single pelvic compartment, translabial perineal ultrasound allows for the assessment of multiple compartments (15). Beyond pelvic organ prolapse, a recent review highlighted the significant potential of translabial perineal ultrasound in investigating urinary and anal incontinence, complications following sling-mesh surgery, and pelvic floor disorders (16).

Table 3. Preoperative-postoperative FSFI scores

	Distance between the mesh and the urethra				
	<5 mm		>5 mm		p#
	Mean ± SD	Min.-max. (median)	Mean ± SD	Min.-max. (median)	
Preoperative FSFI					
Total score	19.53±1.47	15.1-23.1 (19.6)	19.96± 1.27	17.5-23.1 (20.1)	0.158*
Desire	2.37±0.50	1.2-3.6 (2.4)	2.30±0.54	1.2-3.6 (2.4)	0.574
Arousal	4.26±0.81	2.9-5.7 (3.8)	4.45±0.78	2.9-5.7 (4.5)	0.236
Lubrication	3.72±0.33	2.7-4.5 (3.6)	3.83±0.31	3.0-4.5 (3.9)	0.154
Orgasm	2.10±0.36	1.2-2.8 (2.0)	2.20±0.36	1.2-2.8 (2.0)	0.247
Satisfaction	3.05±0.31	2.0-3.6 (3.2)	3.10±0.28	2.4-3.6 (3.2)	0.565
Pain	4.03±0.33	3.6-4.8 (4.0)	4.09±0.29	3.6-4.8 (4.0)	0.285
Postoperative FSFI					
Total score	31.46±1.43	25.2-34.2 (31.6)	26.40±1.07	24.6-28.4 (26.4)	<0.001
Desire	4.98±0.57	3.6-6.0 (4.8)	3.67±0.33	3.0-4.8 (3.6)	<0.001
Arousal	8.27±0.64	6.3-9.2 (8.6)	6.80±0.57	5.7-7.6 (7.0)	<0.001
Lubrication	4.17±0.26	3.6-4.8 (4.2)	4.08±0.33	3.6-5.1 (3.9)	0.100
Orgasm	4.56±0.45	2.8-5.2 (4.8)	3.66±0.39	2.8-4.4 (3.6)	<0.001
Satisfaction	4.13±0.35	3.2-4.8 (4.0)	3.51±0.34	3.2-4.4 (3.6)	<0.001
Pain	5.37±0.38	4.8-6.0 (5.2)	4.68±0.58	4.0-5.6 (4.8)	<0.001
ΔFSFI	11.93±1.54	6.2-14.9 (12.1)	6.44±1.19	4.1-8.9 (6.5)	<0.001*

*Student's t-test, #Mann-Whitney U test, FSFI: Female Sexual Function Index, min.: Minimum, max.: Maximum, SD: Standard deviation

Table 4. Relationship between mesh-urethra distance and percentile ΔFSFI

	Distance to the urethra		Percentile placement	
	r	p	r	p
Sling percentile placement	-0.193	0.082		
Postoperative FSFI	-0.729	<0.001	0.067	0.553
ΔFSFI	-0.726	<0.001	0.013	0.905

FSFI: Female Sexual Function Index

In recent years, the widespread adoption of midurethral slings in patients with SUI has been attributed to the natural structure of the sling. In a prospective study involving Tension-free vaginal tape, TOT, and single-incision sling procedures that investigated the angle between the mesh arms and their position relative to the urethra, retropubic slings were found to have a more frequent midurethral placement compared to other types of slings (17). However, in the same study, sonographic measurements were not found to be to correlated with urinary symptoms at three years after surgery. In a similar study involving patients who underwent TOT and were evaluated with transperineal ultrasonography, women with incontinence six months after surgery showed discrepancies in the movement of the urethra with the sling compared to continent women, along with asymmetry between the mesh arms, bladder neck descent, and varied sling positions (18).

In another study using the symphysis pubis as a reference point, successful TOT outcomes demonstrated that the sling position on ultrasound became more caudal than the symphysis pubis during the Valsalva maneuver, yet it still remained within the midurethra (19). A recent study using perineal ultrasonography revealed that in cases of postoperative urinary retention, the mesh tended to be situated more proximally rather than within the midurethra, with obstructive slings positioned <10 mm from the bladder neck (20). An ultrasound study conducted a decade after TOT procedures found a cut-off of 5 mm for the distance between the sling and urethra, and urinary incontinence in women was found to be associated with mesh-urethra distances >5 mm (21). That study also indicated that the sling position was not significantly associated with overactive bladder symptoms.

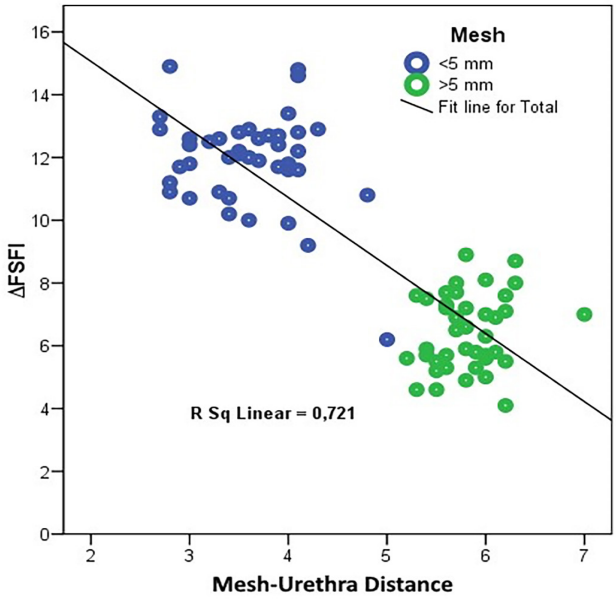

Figure 2. Relationship between FSFI preoperative-postoperative difference and mesh-urethra distance
FSFI: Female Sexual Function Index

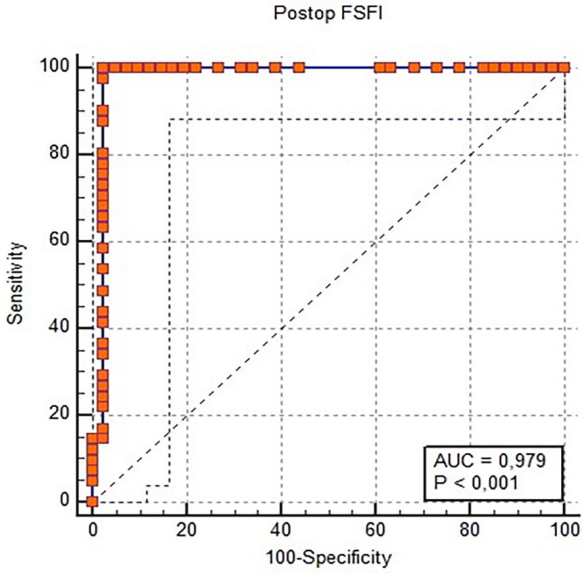

Figure 3. ROC curve
FSFI: Female Sexual Function Index, ROC: Receiver operating characteristic, AUC: Area under the curve

For patients with SUI who receive TOT procedures, postoperative sexual function can significantly improve (1). In the present study, we observed an increase in postoperative FSFI scores in both groups. In our literature review, the combination of perineal ultrasonography, TOT, and sexual functions stands out as being unique. We believe that, in patients with a mesh-urethra distance of >5 mm, the mesh being closer to the anterior vaginal wall's erogenous zone might have a greater impact on sexual functions. In the present study, we observed no difference in terms of the position on the sling percentile among the continent patients in the groups, and we also found that in terms of sexual functions, the distance between the mesh and the urethra was more influential than the sling percentile where the mesh was positioned.

The strengths of this study stem from its rigorous follow-up and comprehensive clinical approach, including ultrasound measurements. The fact that follow-up examinations were not conducted by the operating surgeon helped to minimize bias. Subjective data were collected using validated questionnaires.

Study limitations

The limitation of the study was its small patient cohort. Although the inclusion of only continent patients in our study was seen as a limiting factor, both preoperative and postoperative low FSFI scores are expected in women with postoperative incontinence, which could lead to confusion in the results. Therefore, we believed that evaluating sexual functions and the relationship with perineal ultrasound in patients who achieved both objective and subjective cures would be more realistic and in line with norms. Additionally, the pathology underlying sexual dysfunction may be multifactorial and linked to etiological factors. While we found that mesh position affected FSFI scores in our study, reaching a definitive conclusion on this matter would require support from larger cohorts and multicenter data.

Conclusion

Sexual functions are more adversely affected in patients with a mesh-urethra distance >5 mm as observed via perineal ultrasound.

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Ethics Committee Approval: *The study was approved by the University of Health Sciences Turkey, İstanbul Prof. Dr. Cemil Taşcıoğlu City Hospital Local Ethics Committee (approval number: 27, date: 23.01.2023).*

Informed Consent: *Written consent was obtained from each participant.*

Author Contributions: *Surgical and Medical Practices: O.D.; Concept: O.D.; Design: F.Ş.; Data Collection or Processing: F.Ş., O.D.; Analysis or Interpretation: F.Ş., O.D.; Literature Search: F.Ş., O.D.; Writing: F.Ş.*

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

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Pregnancy outcomes and fertility after ligation of uterine artery only and hypogastric artery only in postpartum hemorrhage

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Abstract

Objective: To determine and compare pregnancy outcomes after bilateral uterine artery ligation (BUAL) or bilateral hypogastric artery ligation (BHAL) for postpartum hemorrhage (PPH).

Material and Methods: This retrospective cross-sectional study was conducted from January 2010 to June 2018 at a tertiary referral hospital. Patients who had undergone arterial ligation for PPH were included in the study. Patients who had undergone BUAL and BHAL were compared with a control group in terms of fertility and pregnancy outcomes.

Results: A total of 156 patients were included, of whom 47 underwent BUAl, 59 underwent BHAL and 50 were in the control group. There was no significant difference between the groups in subsequent pregnancies in terms of the incidence of miscarriage, fetal growth restriction, preeclampsia, primary cesarean deliveries, and infertility ($p>0.05$). There was a significant difference between all groups in gestational age at birth and birthweight. Preterm birth was observed in 32.2% of patients in the BHAL group, and this rate was significantly higher than in the BUAL (12.8%) and control (6%) groups ($p=0.001$).

Conclusion: PPH is a life-threatening obstetric problem. The effects of interventions performed to reduce pelvic blood flow in patients may lead to persistent problems, such as preterm birth and low birth weight in the next pregnancy. However, these interventions do not appear to affect the risk of miscarriage. In subsequent pregnancies of patients who received BHAL, special attention should be paid to preterm birth. (J Turk Ger Gynecol Assoc. 2024; 25: 132-7)

Keywords: Hypogastric artery, ligation, postpartum hemorrhage, uterine artery

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Introduction

Postpartum hemorrhage (PPH) remains a major cause of maternal morbidity and mortality throughout the world, including in industrialized countries. Severe PPH of more than 1000 mL occurs in about 1% of births (1). The cause of around 90% of all cases is uterine atony. In patients who do not respond to drug treatment and intrauterine balloon tamponade, surgical vascular ligations, such as bilateral uterine artery ligation (BUAL), gradual uterine devascularization, and/or bilateral hypogastric artery ligation (BHAL), and uterine

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compression sutures are effective alternatives to hysterectomy (2). Theoretically, all of these procedures, except hysterectomy, offer the advantage of preserving fertility.

The hypogastric artery (internal iliac artery) is a branch of the common iliac artery and is the main blood supply to the pelvic structures. There are a large number of small vessels, collateral circulation, and variations in the pelvic vasculature (3,4). The role of BHAL in controlling severe pelvic bleeding was described by Kelly (5) in 1893 during a Wertheim operation. This procedure has become a life-saving procedure for the control of PPH in subsequent years. In massive pelvic bleeding, bilateral ligation of the hypogastric artery reduces pelvic arterial blood flow by 49% and pulse pressure by 85% (6). After bilateral ligation of the hypogastric artery, blood supply to the pelvis is maintained without necrosis by the collateral circulation, consisting of profundal femoral artery, superior gluteal artery, obturator artery, and ovarian artery. It has been shown that in patients who have undergone postpartum BHAL, the collateral circulation generally functions well and the complication rate is significantly lower than in oncologic patients. The relatively young age of the patients and the low rate of atherosclerotic vascular disease explain this difference (7). However, there are studies showing that uterine and ovarian blood flow decreases in the early postoperative period (8,9). It is unclear how this situation affects subsequent fertility and pregnancy.

BUAL is a relatively safe, simple and fast surgical procedure compared to BHAL. The surgical success rate is over 90%. ACOG endorsed its use in postpartum haemorrhage in 2006 (10). Recanalization of uterine arteries has been demonstrated radiologically after uterine artery embolization for uterine fibroids in the sixth month after surgery (11). However, uterine artery flow patterns have been shown to change significantly in patients who have undergone BUAL (12). Studies investigating the effects of uterine artery occlusion on subsequent fertility and pregnancy outcomes have provided limited data, and most of them relate to uterine artery embolization for the treatment of fibroids (13-15).

Although there are published case series investigating pregnancies after BHAL and BUAL, we found no study comparing fertility and pregnancy outcomes after BHAL and BUAL. The aim of the present study was to determine and compare fertility and pregnancy outcomes after BHAL and BUAL for postpartum haemorrhage.

Material and Methods

This retrospective, cross-sectional study was performed in a tertiary center. Ethical approval was obtained from the University of Health Sciences Turkey, Etlik Zübeyde Hanım Woman's Health Training and Research Hospital Local Ethics Committee (approval number: 04, date: 16.03.2022).

Sociodemographic and clinical data were obtained from the medical records of the participants. The following data were collected: age, parity, previous miscarriage, smoking, assisted reproduction, previous cesarean delivery, indication for vascular ligation, fertility status, and feto-maternal outcomes such as preterm birth, fetal growth restriction (FGR), preeclampsia, low birth weight, and presence of fetal anomaly in the subsequent pregnancy.

Based on the surgical approach, the groups were defined as group 1 (BUAL), group 2 (BHAL), and group 3 (controls). Then, these data were compared among the three groups. All patients received fundal massage, bimanual compression, standard administration of oxytocin (40 IU in 500 mL intravenously) and methyl ergonovine maleate (0.20-0.40 mg, intramuscularly) before the surgical procedure. For severe PPH that did not respond to these measures, BHAL or BUAL was administered. Administration of BHAL or BUAL depended on the surgeon's judgment and experience. The control group consisted of patients with PPH who responded to standard medical therapy. Only patients who underwent cesarean section were included in the study. Patients younger than 18 years and patients with other causes of PPH, such as coagulation disorders or retained placenta, were excluded. The study did not include patients who had been treated with balloon tamponade, B-lynch suture, or other hemostatic sutures or who had undergone hysterectomy. In addition, patients with gestational diabetes, preeclampsia, hypertension, and other chronic conditions were excluded from the study because they are at risk for adverse obstetric events such as intrauterine growth retardation and preterm delivery.

Statistical analysis

All statistical analyzes were performed using the RStudio integrated development environment for statistical computing (Affero General Public License v3; released 2011. RStudio for Linux, version v2021.09.4+403.pro3 Ghost Orchid; September 19, 2022; developed by Posit, PBC.). Variables were examined using visual (histogram, probability plots) and analytic methods (Kolmogrov-Simirnov/Shapiro-Wilk test) to determine whether or not they were normally distributed. Descriptive analyzes were performed using means and standard deviations for normally distributed variables. The One-Way method ANOVA was used to compare these parameters between groups. Levenes test was used to assess homogeneity of variance. Descriptive analyzes using medians and quartiles (Q1-Q3) were performed for numerical data that were not normally distributed. Kruskal-Wallis tests were performed to compare these parameters between groups.

The Mann-Whitney U test was performed to test the significance of pairwise differences, using the Bonferroni

correction to adjust for multiple comparisons. Descriptive analyzes were performed for categorical variables using frequency and percentage. Relationships between categorical variables were analyzed using the chi-square test or Fisher's exact test (when the assumptions of the chi-square test did not apply because of low expected cell counts). An overall 5% type 1 error level was used to infer statistical significance. A p-value of less than 0.05 was considered to show a statistically significant result.

Results

Between January 2010 and June 2018, 162 patients were analyzed who underwent 77 (47.5%) BHAL and 85 (52.5%) BUAL due to PPH. Patients' records for five years postpartum were reviewed and their birth histories were evaluated. Of these initial 162, 26 patients from the BUAL group and 22 patients from the BHAL group used a contraceptive method and did not want another pregnancy. Three patients from the BUAL group and two patients from the BHAL group became menopausal. One patient from the BUAL group and two patients from the BHAL group had infertility from unknown causes. In total, 47 patients who received BHAL and 59 patients who received BUAL became pregnant again after delivery and 106 patients who became pregnant after these procedures and a control group of 50 women who had not undergone any of these procedures in their previous pregnancy were included for analysis. The demographic and clinical characteristics of the patients are shown in Table 1. There were no differences between groups in age, parity, smoking status, and use of assisted reproductive techniques in current pregnancies.

The BUAL group and the BHAL group had significantly more previous miscarriages than the control group (63.8%, 69.5%, and 42%, respectively; p=0.010). In addition, the BUAL group and the BHAL group were significantly more likely to have a previous cesarean delivery than the control group (36.2%, 39%, and 10%, respectively; p=0.001). The most common indication for arterial ligation in both groups was uterine atony (66% in the BHAL group and 45.8% in the BUAL group). In addition, a significantly higher spectrum of placenta accreta was observed in the BHAL group than in the BUAL group (p=0.037).

The subsequent pregnancy outcomes of the patients are shown in Table 2. There was no significant difference between groups in the incidence of miscarriage, FGR, preeclampsia, primary cesarean deliveries, and fetal anomalies in subsequent pregnancies. While the median gestational age at delivery was 39 weeks in the control group, it was 38 in the BUAL group and 37 weeks in the BHAL group. There was a significant difference between all groups in gestational age at delivery and birth weight. Preterm birth was observed in 32.2% of patients in the BHAL group, and this rate was significantly higher than in the BUAL (12.8%) and control (6%) groups (p=0.001). The percentage of low birth weight children was 42.4% in BHAL group and was significantly more likely than in BUAL group (21.3%) and control group (10%) (p=0.001).

Table 1. Sociodemographic and clinical characteristics

	BUAL, (n=47)	**BHAL, (n=59)**	**Control, (n=50)**	**p**
Age (years)	**30 (26-34)**	**30 (26-34)**	**28 (26-31.25)**	**0.363**
Parity (n, %)				
1	17 (36.2%)	18 (30.5%)	14 (28%)	0.556
2	19 (40.4%)	23 (39%)	26 (52%)	
≥3	11 (23.4%)	18 (30.5%)	10 (20%)	
Previous miscarriage (n, %)				
No	17 (36.2%)	18 (30.5%)	29 (58%)	**0.010***
Yes	30 (63.8%)	41 (69.5%)	21 (42%)	
Smoking (n, %)	9 (19.1%)	15 (25.4%)	6 (12%)	0.208
ART (n, %)	0	3 (5.1%)	2 (4%)	0.311
PCD (n, %)	17 (36.2%)	23 (39%)	5 (10%)	**0.002****
Indication for vessel ligation (n, %)				
Uterine atony	31 (66%)	27 (45.8%)	N/A	0.06
PAS	7 (14.9%)	24 (40.7%)	N/A	**0.007**
EUI/UL	5 (10.6%)	4 (6.8 %)	N/A	0.506
PH	4 (8.5%)	4 (6.8%)	N/A	>0.05

P<0.05 means there is significantly statistical difference between groups. Data are given as median (Q1-Q3) or n (%). *: The difference between the BHAL vs. controls is significant, **: The difference between the BUAL vs. controls and BHAL vs. controls is significant, BUAL: Bilateral uterine artery ligation, BHAL: Bilateral hypogastric ligation, ART: Assisted reproductive technology, PCD: Previous cesarean section, PAS: Placenta accreata spectrum, EUI/UL: Extended uterine incision/uterine laceration, PH: Pelvic hematoma

Table 3 shows the fertility results. Although one patient from the BUAL group and two patients from the BHAL group stopped using a contraceptive method within 5 years of their procedures, they did not become pregnant again. There was no significant difference between the groups in terms of infertility ($p>0.05$).

Discussion

PPH is an important cause of morbidity and mortality that seriously threatens maternal health. The main cause is uterine atony, but the number of placental invasion disorders is steadily increasing. Postpartum atony is considered an emergency, and if bleeding persists despite initial measures, surgical intervention is initiated (16).

The hypogastric artery, which plays an important role in blood supply to the pelvis, and its branch, the uterine artery, are both subject to ligation for the treatment of PPH (17). In theory, these treatments reduce uterine blood flow, control bleeding, and prevent hysterectomy. However, the effects of these devascularization treatments on subsequent pregnancies are not clearly known. The literature contains information on arterial ligation, which is used to reduce blood flow, particularly in the treatment of uterine fibroids. Torre et al. (18) found no loss of ovarian function in any of the patients. In the present study, no significant difference in infertility was found between groups undergoing arterial ligation. Pregnancy did not occur in two patients with BHAL and one patient with BUAL, although they tried to become pregnant for at least five years after the procedures. However, these patients did not want to receive infertility treatment during this time.

Karlsen et al. (19), in a meta-analysis of 988 patients from 17 studies, found that the miscarriage rate in pregnancies after uterine artery embolization for fibroid treatment was 34%. In the present study the miscarriage rate in patients who underwent arterial ligation was 14.9% in the BUAL group and 13.6% in the BHAL group. Chen et al. (20) studied the subsequent pregnancies of 423 women who had undergone uterine artery ligation, and 7 of 17 (24%) pregnancies ended in miscarriage. These authors suggested that this procedure should be avoided in patients who wish to become pregnant again in the future (20). In the present study, a total of 106 patients were treated with BHAL or BUAL, and miscarriage occurred in 15 pregnant women (14%). No significant difference was found between the patients who received BHAL and BUAL and the control group in terms of miscarriage rate. According to a systematic review, the risk of miscarriage is 15.3% (95% confidence interval: 12.5-18.7) of all recognized pregnancies (21). Considering the similar result in our study, it appears that BHAL and BUAL do not cause a risk of miscarriage that is different from the general risk in society. Although the number of abortions in previous pregnancies was significantly higher in the BHAL and BUAL groups compared with the control group, the number of abortions in postoperative pregnancies showed no difference. In light of this information, we believe that contrary to the studies in the literature, it is not necessary to avoid the BHAL and BUAL procedures to avoid miscarriage in subsequent pregnancies. This suggests that the possible reduction of blood flow in the uterus may not responsible for miscarriages in early pregnancy, but may come into play in late pregnancy.

Table 2. Subsequent pregnancy outcomes

	BUAL, (n=47)	BHAL, (n=59)	Control, (n=50)	p
Miscarriage (n, %)	7 (14.9%)	8 (13.6%)	4 (8%)	0.537
Gestational age at delivery (weeks)	38 (37-39)	37 (35-37)	39 (38.25- 40)	**<0.001***
Preterm birth (<37 weeks) (n, %)	6 (12.8%)	19 (32.2 %)	3 (6%)	**0.001****
Fetal growth restriction (n, %)	5 (10.6%)	8 (13.6%)	4 (8%)	0.648
Preeclampsia (n, %)	2 (4.3%)	2 (3.4%)	3 (6%)	0.803
Postpartum hemorrhage (n, %)	1 (2.1%)	6 (10.2%)	0	**0.019*****
Birthweight (g)	3100 (2900-3330)	2600 (2220-3100)	3365 (3105-3600)	**<0.001***
Low birthweight infant (<2500 g) (n, %)	10 (21.3%)	25 (42.4%)	5 (10%)	**0.001****
Fetal anomaly (n, %)	1 (2.1%)	2 (3.4%)	0	0.585

Data are given as median (Q1-Q3) or n (%). *: The difference between all groups are significant, **: The difference between the BUAL vs. BHAL and BHAL vs. controls is significant, ***: The difference between the BHAL vs. controls is significant, BUAL: Bilateral uterine artery ligation, BHAL: Bilateral hypogastric ligation

Table 3. Infertility outcome

	BUAL, (n=48)	BHAL, (n=61)	p
Infertility (n, %)	1 (2%)	2 (3%)	>0.05

BUAL: Bilateral uterine artery ligation, BHAL: Bilateral hypogastric ligation

It is known that the blood flow to the uterus gradually increases during pregnancy and the fetus benefits from this flow through the placenta. There is little information in the literature on the outcome of pregnancy after surgery that reduces blood supply to the pelvis in humans. Although Morikawa and Takamizawa (22) reported a fetus with low gestational age in their case report of bilateral UAL, Mengert et al. (23) reported a term and uneventful pregnancy after uterine artery ligation in their series of three cases. In addition, in animal studies in which uterine artery ligation was performed during pregnancy, fetuses have been shown to miscarry, and preterm labor, intrauterine growth restriction, and neural development abnormalities occur. This study highlighted that uterine artery ligation causes placental insufficiency and leads to developmental delay in fetuses (24). We hypothesized that placental insufficiency may also occur in pregnancies after arterial ligation. However, in our study, there was no significant difference between the groups in terms of FGR. Therefore, it may be that the collateral circulation also prevents placental insufficiency in subsequent pregnancies. In addition, there was no difference between groups in terms of rates of preeclampsia in our study. As there was no significant difference between groups in rates of FGR, abortion, and preeclampsia suggests that the BHAL and BUAL procedures do not pose a problem in either overall uterine perfusion or microvascular function. Chang et al. (25) found that uterine artery occlusion for the treatment of fibroids increased the risk of preterm delivery, miscarriage, and FGR due to decreased uterine and placental perfusion as a result of decreased blood flow to the myometrium and endometrium. In the present study, we found that of these risks, only the incidence of preterm birth, which also affects birth weight, was increased but only in the BHAL group. This suggests that ligation of the hypogastric artery may affect gestational week in subsequent pregnancies. This may be due to more proximal obliteration of the vascular tree, which may be anastomosed and suggests that proximal artery occlusion contributes to preterm labor by preventing healthy function of the existing anastomoses. We suggest that the physiology of preterm birth is not fully understood and that possible local inflammatory reactions after ligation may increase the risk of preterm birth.

We hypothesize that the reason why atony indication was more frequent in the BUAL group was because of easier technical applicability. However, there was no difference between the BUAL and BHAL groups. The fact that the placenta accreta spectrum produces more indications for BHAL may support the idea that this is due to surgeon preference. It is known that women with a history of previous atony are at higher risk for PPH in their next pregnancies (26). Consistent with the literature, we found that there was a higher PPH rate in the BHAL group compared with the BUAL group in subsequent pregnancies. We suggest that, in view of these data, surgeons prefer BHAL for the control of severe bleeding and that more PPH may be observed in the patients' next pregnancies because there is a higher predisposition to PPH in pregnancies in which BHAL was used. None of the women in the control group had PPH in a subsequent pregnancy.

For indications requiring arterial ligation, the patient's desire to become pregnant again should be clearly understood and she should be informed about possible complications of subsequent pregnancies. In addition, in this context, the patients' informed consent should be obtained before the procedure. Arterial ligation should be performed taking into account the possibility of preterm labor in subsequent pregnancy.

More comprehensive studies are needed. To this end, investigators should be supported and a surgical procedure should be established to address subsequent pregnancies with regard to the management of PPH. Thus, it can be ensured that legal responsibilities are clearly laid out.

Study limitations

Our study was a retrospective study and it can not clearly reveal the difference in the weeks of birth between the groups and the indication of delivery. When current patient data are categorized by birth indications, there is not enough patient data to reliably understand the effect of homogeneous distribution and the results of subsequent pregnancies. The number of patients was too small to draw conclusions about infertility after these procedures. However, to the best of our knowledge, this study is the most comprehensive study in the literature investigating pregnancy outcomes following arterial ligation for PPH and this is also the strength of our study.

Conclusion

PPH is one of the peripartum causes of maternal mortality. In cases where medical treatment is inadequate, arterial ligation is performed to reduce blood supply to the pelvis through surgery. However, the results of the present study suggest that later pregnancies of patients who have undergone arterial ligation should be carefully monitored for preterm labor.

Ethics Committee Approval: *Ethical approval was obtained from the University of Health Sciences Turkey, Etlik Zübeyde Hanım Woman's Health Training and Research Hospital Local Ethics Committee (approval number: 04, date: 16.03.2022).*

Informed Consent: *Retrospective study.*

Author Contributions: *Surgical and Medical Practices: Ç.Ö., C.Ç., C.T.İ., Y.E.Ü.; Concept: B.T.Ç., B.B., G.A., G.K., E.S.; Design: B.T.Ç., Ç.Ö., M.B., S.S.; Data Collection or Processing: B.T.Ç.,*

M.B., C.O.U., E.S.; Analysis or Interpretation: C.Ç., C.T.İ., Y.E.Ü.; Literature Search: B.T.Ç., Ç.Ö., Z.Ş., S.S., M.B., C.O.U.; Writing: B.B., C.Ç., C.T.İ., Y.E.Ü.

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Serum anti-Mullerian hormone levels in Turkish girls aged 18 and younger for ovarian reserve determination

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Abstract

Objective: Our aim was to show that anti-Mullerian hormone (AMH) may be used as a quantitative marker of ovarian reserve in Turkish girls aged 18 years and younger and establish the reference values for AMH in Turkish girls.

Material and Methods: This retrospective study included girls between 8-18 years old, without premature ovarian failure or without genetic factors resulting in ovarian dysgenesis. Blood specimens were collected after overnight fasting early in the morning during the early follicular phase. Measurement of serum levels of gonadotropins and AMH was done. Mean serum AMH levels of different age groups and best fitting curve representing AMH percentiles (10^{th}, 25^{th}, 50^{th}, 75^{th}, 90^{th}) were calculated.

Results: In total 785 girls with a mean age of 16.16±1.90 years were included, divided into seven age groups. The mean serum AMH level for the total cohort was 5.20±4.19 ng/mL. There was a significant difference between the mean values of AMH in age groups as follows: ≤12 and 17-≤18 (p=0.011). The best fitting curves for AMH percentiles were 4^{th} order polynomial functions. There was a significant correlation between AMH and age and follicle stimulating hormone levels (r=0.148, p<0.001 and r=-0.092, p=0.010).

Conclusion: Our results reflect the real-life data for serum AMH values in Turkish girls. Our nomogram may be useful for counseling adolescents about their ovarian reserve and diagnosing other gynecological diseases. A longitudinal study is necessary for improving the predictive value of AMH values in girls aged 18 and younger. (J Turk Ger Gynecol Assoc. 2024; 25: 138-43)

Keywords: Anti-Mullerian hormone, ovarian reserve, adolescent, nomograms

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Introduction

Anti-Mullerian hormone, (AMH), is a dimeric glycoprotein and a member of transforming growth factor-beta family, produced by the granulosa cells of primary, preantral and early antral follicles (1). It affects ovarian folliculogenesis by inhibiting the recruitment of primordial follicles. Moreover, it reduces the sensitivity of antral follicles to follicle-stimulating hormone (FSH) and inhibits FSH-stimulated estradiol production and aromatase expression (2). It has been assumed that after the 36^{th} week of gestation, the number of fetal primordial follicles decline with age until a critical level. However, the increased rate of primordial follicle recruitment overcomes the decline in total primordial follicle and serum AMH levels increase until the age of 14 years (3). This confirms that AMH levels are not regulated by gonadotropins but also that AMH level is not totally independent from gonadotropins (4).

The rate of follicle decline, in other words ovarian reserve, is influenced by age, genetic and environmental factors (1). Currently, basal FSH, antral follicle count (AFC) and AMH are frequently used in clinical practice for assessing ovarian reserve. FSH, due to the unknown variation pattern and AMH, due to its interindividual differences in random measurements may not give accurate results for patients during childhood and adolescence (5,6). AMH may serve better than AFC as an ovarian reserve marker in children and adolescents due to the difficulty in assessing AFC by transabdominal ultrasound in non-sexually active females. Although reference levels of AMH, are used for the evaluation of ovarian reserve in infertility

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patients; the physiological role and the clinical use of AMH in adolescence and childhood is less often discussed (7-9). Low or undetectable AMH levels are already used for the diagnosis of premature ovarian failure in pediatric patient for example for Turner syndrome and in girls on chemotherapy. However, low AMH levels due to partial gonadotropin dependency in hypogonadotropic hypogonadism patients are not reliable for the diagnosis of ovarian failure (10). In addition to that, in contrast to adult patients, the expected high levels of AMH in polycystic ovary syndrome (PCOS) are not necessarily observed in adolescents with PCOS (11-13). Thus, the aim of this study was to show that AMH may be used for assessing ovarian reserve and to establish reference values for AMH in Turkish girls.

Material and Methods

Study design and patient selection

This retrospective study was performed in the gynecology outpatient clinic of a faculty, from 2010 to 2020. AMH levels were obtained from all girls aged between 8-18 years who attended our clinic with a complaint unrelated to ovarian function, such as abdominal pain, suprapubic pain, or heavy menstruation. The data was extracted from the electronic records. Patients diagnosed with premature ovarian failure or with other genetic factors resulting in ovarian dysgenesis were excluded. Patients who had surgery that may result in a decrease in ovarian function, such as ovarian cystectomy and who were using oral contraception during the examination were excluded.

All procedures performed in studies involving human participants were in accordance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Approval was granted by the İstanbul University-Cerrahpaşa, Cerrahpaşa Faculty of Medicine Clinical Research Ethics Committee (approval number: 52825153-604.01.01-134000, date: 12.10.2020).

Hormone assays

Blood specimens were collected after overnight fasting early in the morning from the antecubital vein during the early follicular phase (3^{rd}-5^{th} day of the cycle), if after menarche. Measurement of serum levels of gonadotropins, lutenizing hormone (LH) and FSH, was done at the biochemistry laboratory of our faculty.

Serum levels of AMH were measured using the DSL-10-14400 Active Mullerian Inhibiting Substance/AMH ELISA kit (Diagnostic Systems Laboratories, Webster, TX, USA) before 2015 and the Elecsys AMH Plus test on the Cobas-E electrochemiluminescence immunoassay platform (Roche Diagnostics GmbH, Mannheim, Germany) after 2015. Serum AMH values are reported as ng/mL and the assay range was 0.01-23 ng/mL. The intra- and inter-assay variation coefficients were <8% and <12%, respectively. Basal fasting FSH, LH and E_2 were measured by electrochemiluminescence method (Cobas 8000 systems, Roche Diagnostics GmbH, Mannheim, Germany).

Statistical analysis

Statistical analysis was performed by SPSS version 21 for Mac (IBM Inc., Armonk, NY, USA). Demographic characteristics are reported with descriptive statistics. Continuous variables are expressed as mean ± standard deviation. Statistical comparisons of the means of more than two groups were carried out according to ANOVA, where appropriate. A $p<0.05$ was accepted to be statistically significant. The 10^{th}, 25^{th}, 50^{th}, 75^{th} and 90^{th} percentiles of AMH by age were calculated and graphed according to the best fitting curve in Microsoft Excel, version 16.53 for Mac 21. A fourth order polynomial function was chosen since it was the best fitting curve for AMH percentiles with highest R^2 values. The correlation between age, FSH and AMH was calculated by Pearson's correlation coefficient.

Data availability statement

The data associated with the paper are not publicly available but are available from the corresponding author on reasonable request.

Results

In total 785 Turkish girls with mean age of 16.16±1.90 years were included. The girls were divided into seven groups by age: 8-12; 12.01-13; 13.01-14; 14.01-15; 15.01-16; 16.01-17; and 17.01-18. The hormonal characteristics of our cohort are presented in Table 1 for all participants. The mean serum AMH level for the whole cohort was 5.20±4.19 ng/mL. The mean serum FSH and LH levels for the whole cohort were 5.30±1.94 IU/mL and 6.81±4.82 IU/mL, respectively. The percentiles of serum AMH levels are presented at Table 2. There was a significant difference between the mean values of AMH in age groups when comparing the ≤12 group with the 17-≤18 group ($p=0.011$). There was no significant difference between the other age groups.

A fourth order polynomial function was the best fitting curve for AMH percentiles in our cohort. R^2 values of the curves were listed below.

10^{th} percentile: $y=0.0114x^4-0.6827x^3+15.166x^2-147.91x+534.8$ ($R^2=0.9566$)

25^{th} percentile: $y=0.0106x^4-0.6483x^3+14.784x^2-148.25x+552.6$ ($R^2=0.9408$)

50^{th} percentile: $y=0.0119x^4-0.7197x^3+16.186x^2-159.91x+587.7$ ($R^2=0.9357$)

75^{th} percentile: $y=0.0568x^4-3.4249x^3+76.835x^2-759.08x+2789.5$ ($R^2=0.9383$)

90^{th} percentile: $y=0.067x^4-3.9546x^3+86.668x^2-834.81x+2987.1$ ($R^2=0.9139$)

The nomogram of serum AMH levels is shown in Figure 1. There was a statistically significant but weak correlation between AMH and age and AMH and FSH level (r=0.148, p<0.001 and r=-0.092, p=0.010) (Table 3).

Discussion

This study suggested that AMH may be a reliable ovarian reserve marker for children and adolescents. We also established reference values for serum AMH levels for a population of Turkish girls.

Table 1. Hormonal characteristics of the cohort

Age, years (n)	FSH (IU/mL) (mean ± SD)	LH (IU/mL) (mean ± SD)	AMH (ng/mL) (mean ± SD)	AMH (ng/mL) (median)
8-12 (36)	4.50±2.40	4.56±4.74	3.22±3.74	2.42
12.01-13 (27)	5.60±1.95	6.00±4.42	3.58±4.34	2.61
13.01-14 (69)	5.35±1.78	6.28±5.32	4.15±3.11	3.20
14.01-15 (117)	5.45±1.94	7.37±5.21	5.57±4.62	4.07
15.01-16 (126)	5.26±1.98	6.78±4.79	4.87±3.81	3.69
16.01-17 (169)	5.31±2.04	6.75±4.38	5.47±3.99	4.35
17.01-18 (241)	5.32±1.78	7.19±4.77	5.78±4.43	4.43
Total (785)	5.30±1.94	6.81±4.82	5.20±4.19	4.04

FSH: Follicle-stimulating hormone, LH: Luteinizing hormone, AMH: Anti-Mullerian hormone, SD: Standard deviation

Table 2. Percentile values of serum AMH in Turkish girls

Age, years (n)	5th percentile	10th percentile	25th percentile	50th percentile	75th percentile	90th percentile	95th percentile
8-12 (36)	0.36	0.52	1.47	2.42	4.19	5.44	8.89
12.01-13 (27)	0.37	0.66	1.27	2.61	3.78	7.03	17.16
13.01-14 (69)	1.01	1.44	2.13	3.20	5.74	8.84	10.98
14.01-15 (117)	0.98	1.49	2.31	4.07	7.28	11.68	17.15
15.01-16 (126)	0.86	1.59	2.44	3.69	6.39	9.33	11.43
16.01-17 (169)	1.14	1.52	2.79	4.35	7.04	10.37	14.18
17.01-18 (241)	0.93	1.58	2.79	4.43	7.45	11.64	16.92

AMH: Anti-Mullerian hormone

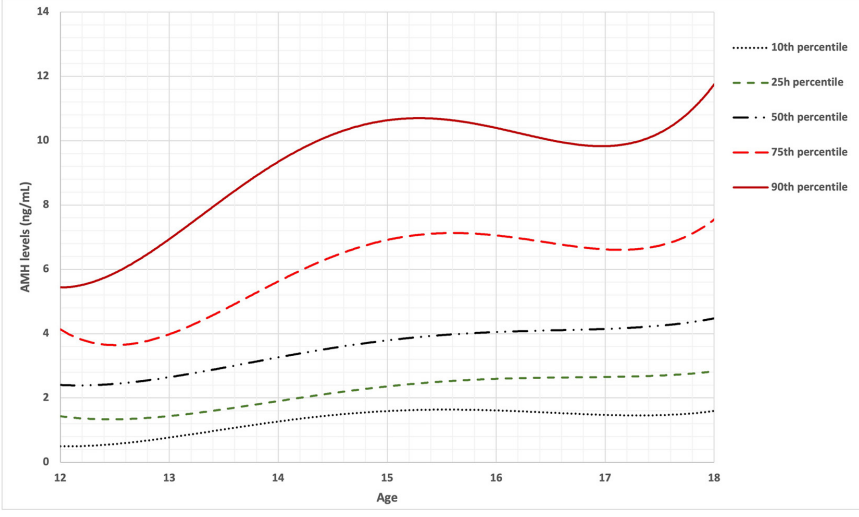

Figure 1. AMH nomogram in Turkish girls based on 4th degree polynomial function. Reference lines of serum AMH for the 10th, 25th, 50th, 75th, 90th percentiles vs. age were demonstrated
AMH: Anti-Mullerian hormone

Table 3. Correlation of AMH with age and FSH

	AMH	
	r	p
Age	0.148	<0.001
FSH	-0.092	0.010

FSH: Follicle-stimulating hormone, AMH: Anti-Mullerian hormone

AMH levels in adolescence have an increasing pattern and peak before pubertal onset. Even though the mean age of menarche in Turkish girls was reported as between 12-13 years (14), we didn't observe a peak around the age groups of ≤12 and 12-≤13. Unlike other studies, we have found a significant but weak correlation of AMH with age and FSH (6,8). Thus, we believe that our nomogram will be a guide for interpreting random AMH measurements during consultation of girls in pediatrics and gynecology clinics.

In the postnatal period, FSH surge increases AMH levels by stimulating ovarian folliculogenesis and follicular growth (6,15,16). Due to ongoing recruitment and follicle growth initiation in a very large pool, a steady increase is observed in serum AMH during childhood, independent of gonadotropins (17). Hagen et al. (3) showed that AMH levels increase significantly by 17% before pubertal onset due to preceding follicular recruitment (18). After pubertal onset due to the growth of follicles beyond the stage of AMH production (redistribution of follicle pool), AMH levels decrease by 30% (3,19). Central inhibition of gonadotropin secretion in childhood and FSH surge before puberty complicate the diagnosis of ovarian failure. Therefore, AMH may serve better than FSH as an ovarian reserve marker in adolescence. Our results confirm the earlier studies that demonstrated minor fluctuations in AMH during childhood and adolescence. Stable AMH levels are achieved by the increased recruitment versus extensive follicle loss (19). Our results partially show the prepubertal increase in AMH. Yet, the increasing rate of recruitment in follicles up to 14-15 years of age was shown clearly by the increase in AMH levels, followed by a plateau, as has been described previously (8,17,19-21). AMH levels >3 ng/mL show more variability across the menstrual cycle compared to low levels (22), so the variability observed in higher percentiles in our results may be attributed to this observation. These interindividual differences don't affect the clinical management since lower AMH levels are more useful for the diagnosis of premature ovarian failure (9,10). Furthermore, wide variation of AMH levels in healthy adolescents may be due to the different rates of follicle loss between individuals (6). Therefore, the diagnostic power of high AMH levels for pubertal girls is yet to be determined.

The clinical use of AMH levels in the pediatric age group includes during the follow-up of patients with central precocious puberty and patients with cancer under gonadotoxic chemotherapy or galactosemia, the determination of the presence of testicular tissue and the diagnosis of persistent Mullerian Duct Syndrome, primary ovarian failure and hypogonadotropic hypogonadism, Klinefelter Syndrome and granulosa cell tumors (10,23,24). There are various studies investigating AMH levels in adolescent patients with PCOS but reporting conflicting results. Therefore, AMH was assumed to have poor diagnostic potency for adolescents with PCOS according to the current literature (12,13,25-27). It should be kept in mind that multifollicular ovarian morphology seen on ultrasound during mid-late puberty may not necessarily represent PCOS. That is indeed a physiological change of puberty.

Study limitations

In this study, we lacked information about the patient's menstrual cycle history, age of menarche, body mass index (BMI), clinical hyperandrogenism and ovarian volume. Thus, we cannot comment on the prevalence of PCOS in our cohort. There are conflicting results about the impact of BMI on AMH values in the literature. Some authors suggested that BMI does not affect AMH values in women of reproductive age, with or without PCOS (28) while others have reported a positive correlation between AMH and BMI (29). Moreover, another study stated that the cut off value of AMH for diagnosing PCOS decreased gradually with increasing BMI (30,31). The lack of BMI data for our subjects prevented us from commenting on the impact of BMI on AMH values in Turkish girls. We also found a higher mean for serum AMH levels in adolescence compared to the other studies (17). This may be due to the high prevalence of PCOS in Turkish population (26%, as reported in Global Burden of Disease Study in 2016) between ages of 15-49 years (32). In an earlier study, it was shown that a quadratic equation was the best model to describe declining serum AMH levels with age (33). The quadratic model didn't fit our data since the age of our cohort was different. Our data is unique in the way that it includes the largest number of girls aged 18 and younger reported until now.

Conclusion

Predicting the menopausal age by AMH levels during childhood and adolescence is not reliable because of the different rate of follicle loss between individuals. However, our results provide real-life data for understanding and interpreting AMH values during childhood and adolescence in Turkish girls. Our nomogram may be useful for counselling girls about their ovarian reserve and diagnosing other gynecological diseases. A longitudinal study is necessary for improving the predictive value of AMH values in girls aged 18 and younger.

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Informed Consent: *Retrospective study.*

Author Contributions: *Surgical and Medical Practices: İ.B.Ö.E., M.Ö., Z.B., C.Ç.; Concept: M.Ö.; Design: M.Ö., İ.Ç.; Data Collection or Processing: İ.B.Ö.E., M.Ö., Z.B., C.Ç.; Analysis or Interpretation: İ.B.Ö.E., M.Ö., İ.Ç.; Literature Search: İ.B.Ö.E., M.Ö.; Writing: İ.B.Ö.E., M.Ö., Z.B., C.Ç., İ.Ç.*

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Single-center experience of laparoscopic hysterectomy: analysis of one thousand five hundred and fifteen patients

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Abstract

Objective: Laparoscopic hysterectomy has become an increasingly used surgery in recent years. The aim of this study was to evaluate the clinical features and perioperative outcomes of patients who underwent laparoscopic hysterectomy for benign or malignant indications in a single center during a period of eight years.

Material and Methods: Data of patients who underwent laparoscopic hysterectomy in the gynecological oncology department of a university hospital over a period of eight years was analyzed retrospectively. Two groups were formed based on being operated for benign or malignant indications. Demographic characteristics and perioperative data of these groups were evaluated.

Results: A total of 1,515 patients underwent laparoscopic hysterectomy. The mean age of the patients was 52.0±9.8 years and mean body mass index (BMI) was 31.3±8.5 kg/m^2. Of these, 1,219 had benign and 296 had malignant histopathology results. In the whole cohort, intraoperative complications were seen in 1.6% and postoperative complications in 3.5%. The patients in the malignant group were older, had a higher BMI and a higher comorbidity rate. The duration of operation and length of hospital stay were significantly longer in this group ($p=0.0001$ for all parameters). However, intraoperative and postoperative complication rates, rate of blood transfusion and amount of transfusion were similar between the two groups ($p>0.05$).

Conclusion: Laparoscopic hysterectomy can be performed with low complication rates in benign and malignant indications, regardless of the patient's contributing factors. However, since experience is important, financial resources and personnel training processes should be supported. (J Turk Ger Gynecol Assoc. 2024; 25: 144-51)

Keywords: Hysterectomy indications, intraoperative complications, laparoscopic hysterectomy, postoperative complications

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Introduction

Hysterectomy is one of the most common gynecological surgical procedures and can be performed by abdominal, vaginal, laparoscopic or robot-assisted approaches (1). Although abdominal and vaginal hysterectomy have been the most preferred methods for many years, vaginal hysterectomy may not be applicable in cases of endometriosis or malignancy. Laparoscopic surgery, on the other hand, has become an increasingly chosen option in recent years due to better visualization of the abdominal cavity when compared to abdominal surgery, improved perioperative outcomes, less postoperative pain, faster recovery period and better cosmetic outcomes (2-4). However, it has also been reported that, particularly related to the experience of the surgical team, complication rates are higher in laparoscopic hysterectomy when compared to other routes (2). In contrast, several studies have indicated that laparoscopic hysterectomy is

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safe and feasible (1,5,6). It has also been reported that while laparoscopic surgery may prolong the operation time in challenging cases, such as with obesity, previous surgery or a huge uterus, it is a safe procedure regarding intraoperative and postoperative complications (3,7). In addition to benign surgery, laparoscopic hysterectomy is frequently performed in the treatment of endometrial cancer and laparoscopy has been found to be safe in terms of perioperative outcomes for this indication as well (8,9).

The aim of the present study was to evaluate the clinical features and perioperative outcomes of patients who underwent laparoscopic hysterectomy for benign versus malignant indications in a single center over a period of eight years.

Material and Methods

The study was approved by the Başkent University Ethics Committee (approval number: E-94603339-604.01.02-155636, date: 31.08.2022).

Retrospective analyses of data of patients who underwent laparoscopic hysterectomy between 2012 and 2020 in the gynecological oncology department of a university hospital were performed. All patients who had undergone laparoscopic hysterectomy by the same surgical team during this time interval were included in the study. Data of patients were obtained from medical records and the hospital database (Nucleus, Monad Software and Consultancy).

Age, body mass index (BMI), comorbidity, American Society of Anesthesiologists scores, surgical procedure, indication of the intervention, complications, need for re-operation and pathology results were evaluated. In addition, patients were classified into two groups, based on the indication for surgery being benign or malignant. Demographic characteristics, as well as intraoperative and postoperative complications, difference between preoperative and postoperative hemoglobin values and blood transfusion data of these groups were compared.

All patients were operated using the same surgical technique. A pneumoperitoneum was created by inserting a Veress needle through the umbilicus and visualization was achieved through a 10 mm trocar. Subsequently, two 5 mm trocars were introduced from the lateral side of the left rectus muscle. One 5 mm trocar was inserted additionally from the lateral side of right rectus muscle in patients who underwent sacrocolpopexy. Two 5 mm trocars from the lateral side of right rectus muscle and one suprapubic 10 mm trocar were also inserted in patients who underwent bilateral pelvic and paraaortic lymph node dissection. A harmonic scalpel (Ethicon) and bipolar cautery were used for coagulation. Round ligaments, utero-ovarian/infindibulopelvic ligaments, and uterine arteries were coagulated and cut bilaterally. The vaginal cuff was cut with a harmonic scalpel. Clermont-Ferrand (Karl Storz GmbH, Tuttlingen, Germany) was used as the uterine manipulator. In all patients, total laparoscopic hysterectomy was performed and the uterus was removed from the abdomen through the vaginal route. In cases where the uterus was very large, the uterus was removed from the vagina by morseling with a scalpel in a bag. The vaginal cuff was closed intracorporeally with a braided suture (Vicryl, Ethicon) with a figure of eight suturing technique.

In patients who underwent sacrocolpopexy, the promontory was reached from the medial of the right ureter, and the mesh was fixed to the vaginal cuff and promontory with non-absorbable braided sutures (Ti-Cron, Medtronic) and peritonized. In patients who underwent lymph node dissection, lymph nodes were taken out of the abdomen in an endobag. Ureters, bladder and bowels were checked after each operation. At the 10 mm trocar entry site, the fascia was closed with a vicryl suture. The duration of the operation was determined as the time from the skin incision to the closure of the incisions, while hospitalization time was determined as the time from surgery to discharge day. Complications observed within 30 days after surgery were considered to be postoperative complications.

Statistical analysis

SPSS, version 25.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Categorical measurements are expressed as numbers and percentages, and continuous measurements were summarized as mean and standard deviation (or median for non-normally distributed data). In the comparison of continuous measurements in the two groups formed according to the presence of malignancy, the distributions were controlled, and the Student's t-test was used for the variables with parametric distribution and the Mann-Whitney U test was used for the variables with non-parametric distribution. The categorical variables between the groups were analyzed using the chi-square test or Fisher's exact test. A $p<0.05$ was considered statistically significant in all tests.

Results

During the study period, a total of 1,515 patients underwent laparoscopic hysterectomy. The mean age was 52.0±9.8 years and mean BMI was 31.3±8.5 kg/m^2. More than half (55.1%) had comorbidities, such as hypertension, diabetes, asthma, cardiac disease, thyroid disease, chronic liver disease, or two or more of these together. Indications for hysterectomy were distributed over a wide range, including dysfunctional uterine bleeding, myoma uteri, pelvic pain, uterine prolapse, adnexal mass, cervical dysplasia, endometrial hyperplasia, and endometrial cancer. The size of uteruses varied between the

8th-20th gestational week. The surgical procedures performed were laparoscopic hysterectomy along with salpingectomy or salpingo-oopherectomy, and in addition, sacrocolpopexy or lymph node dissection and omentectomy, depending on the surgical indication of the patient. The clinical features of the patients, indications for surgery, and the surgeries performed are shown in Table 1. Operation duration for different surgical indications are displayed in Figure 1.

Pathology results included myoma uteri, hyperplasia, adenomyosis, endometriosis, cervical dysplasia, ovarian cyst and malignancies, which were mostly endometrial cancer (Table 2).

Only 1.6% had intraoperative complications, including vascular damage, bowel injury, bladder or ureter injury. None of these complications occurred during the access to the abdominal cavity. The surgery was completed laparotomically in 17 (1.1%) patients. It was observed that all the bowel, bladder and ureteral injuries and vascular laceration in one patient were sutured and repaired laparoscopically. Out of 17 patients, in whom the procedure was converted to open surgery, three patients had major vascular injury (vena cava in two patients and left iliac artery in one patient), one patient had a fixed presacral mass, three patients had ovarian cancer identified by frozen section, and 11 patients, who needed staging surgery, had dense adhesions or could not tolerate the Trendelenburg position due to comorbidities and sufficient visualization could not be provided laparoscopically. Postoperative complications, including trocar site infection, ileus, abscess, vaginal cuff hematoma, cuff dehiscence, fever, urinary retention, D-J stent application, vesicovaginal fistula, deep vein thrombosis, and pulmonary embolism developed in 3.5% of the patients. Mortality was not observed in any of the patients within 30 days postoperatively. A total of 15 patients underwent vaginal re-suturing under sedation, abscess drainage or re-laparoscopy. As for the four patients who underwent re-laparoscopy, re-operation was performed due to cuff hematoma in two patients, ileus in one patient, and need to remove the mesh in the last patient who had discitis after sacrocolpopexy (Table 3).

When the patients were classified into two groups as those with benign and malignant pathology, 1,219 patients had benign and 296 patients had malignant pathology results. The patients in the malignant group were significantly older, had higher BMI, and a higher comorbidity rate. Furthermore, the duration of the operation, postoperative and preoperative hemoglobin differences, and length of hospital stay were significantly greater in this group (p=0.0001 for all parameters). However, intraoperative and postoperative complication rates, rate of blood transfusion and amount of transfusion were similar between the two groups. The clinical features and perioperative outcomes of the two groups are shown in Table 4.

Table 1. Clinical features, operation indications and types of surgical procedures

Age (year) (mean ± SD)	52.0±9.8
BMI (kg/m^2) (mean ± SD)	31.3±8.5
Comorbidity, n (%)	835 (55.1)
Operation indication, n (%)	
- Dysfunctional uterine bleeding	331 (21.8)
- Pelvic pain	63 (4.2)
- Prolapsus	142 (9.4)
- Myoma uteri	286 (18.9)
- Adnexal mass	202 (13.3)
- Hyperplasia	157 (10.4)
- Endometrial cancer	239 (15.8)
- Cervical dysplasia	44 (2.9)
- Breast cancer - risk reduction surgery	40 (2.6)
- Early-stage cervical cancer (stage IA1)	6 (0.4)
- Smooth muscle tumors of uncertain malignant potential (STUMP)	2 (0.1)
- Gestational trophoblastic neoplasia	3 (0.2)
Type of surgical procedure - n (%)	
L/S hysterectomy + salpingectomy/salpingo-oophorectomy	1169 (77.2)
L/S hysterectomy + salpingectomy/salpingo-oophorectomy + sacrocolpopexy	152 (10.0)
L/S hysterectomy + salpingectomy/salpingo-oophorectomy + lymph node dissection	194 (12.8)
BMI: Body mass index, SD: Standard deviation	

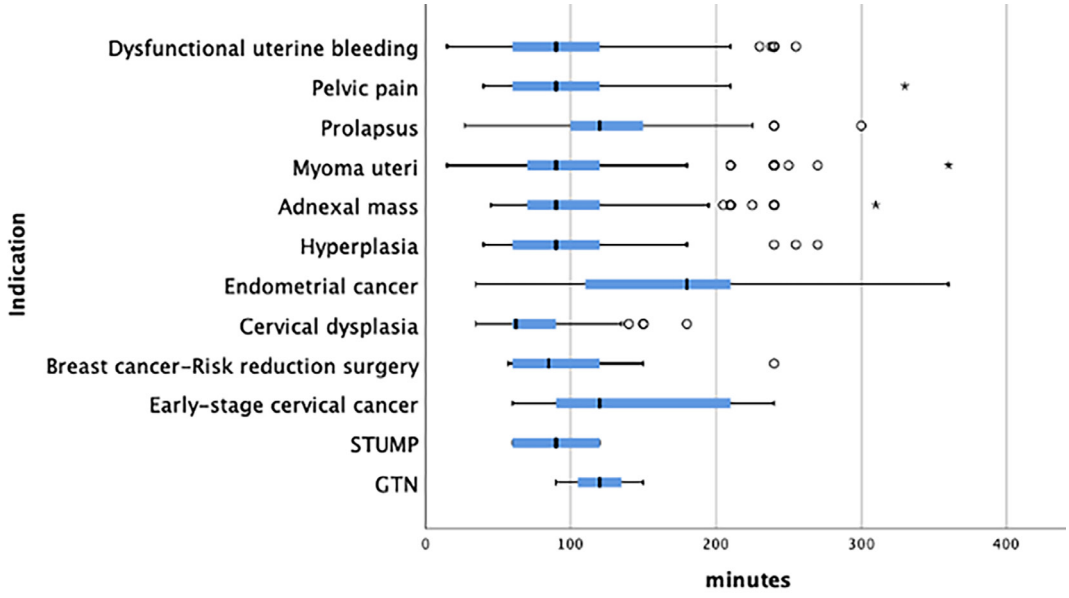

Figure 1. Operation duration for different surgical indications

STUMP: Smooth muscle tumors of uncertain malignant potential, GTN: Gestational trophoblastic neoplasia

Table 2. Pathology results

- Myoma uteri	742 (49.0)
- Adenomyosis	151 (10.0)
- Endometriosis	11 (0.7)
- Hyperplasia	47 (3.1)
- Endometrial cancer	276 (18.2)
- Cervical dysplasia	15 (1.0)
- Cervical cancer	9 (0.6)
- Sarcoma	4 (0.3)
- Lymphoma	2 (0.1)
- Breast cancer metastasis	3 (0.2)
- Trophoblastic invasion	1 (0.1)
- Ovarian cancer	4 (0.3)
- Benign ovarian cyst	78 (5.1)
- Hydrosalpinx	3 (0.2)
- Other (endometritis, cervicitis, irregular proliferative endometrium, polyp)	169 (11.2)

Table 3. Complications and need of re-operation

Intraoperative complications, n (%)	
None	1491 (98.4)
Large vessel injury	4 (0.3)
Intestinal injury	9 (0.6)
Bladder injury	8 (0.5)
Ureter injury	3 (0.2)
Conversion to laparotomy	17 (1.1)
Postoperative complications, n (%)	
None	1461 (96.5)
Pulmonary thromboembolism	2 (0.1)
Surgical site infection	4 (0.3)
Ileus	3 (0.2)
Pelvic abscess	9 (0.6)
Fever	15 (1.0)
Vaginal cuff hematoma	12 (0.8)
Vaginal cuff dehiscence	3 (0.2)
Urinary retention	1 (0.1)
Vesicovaginal fistula	1 (0.1)
D-J stent application	2 (0.1)
Deep vein thrombosis	1 (0.1)
Re-operation, n (%)	
Vaginal re-suturation under sedation	6 (0.5)
Abscess drainage under sedation	4 (0.3)
Re-laparoscopy	4 (0.3)
Repair of incisional hernia	1 (0.1)

Discussion

In the present study, the clinical data, surgical indications, and intraoperative and postoperative complication rates of patients who underwent laparoscopic hysterectomy in a single center were evaluated in a large series, and it was shown that laparoscopic hysterectomy may be safely performed in both benign and malignant pathologies with low complication rates. Total laparoscopic hysterectomy was first described in 1989 and this operation has been performed for more than 20

years for various indications. In the literature, fibroids are the most common benign indication for this procedure. In the study of Bettaiah and Reddy (6), indication of surgery was myomas in 54.4% and treatment-resistant menometrorrhagia in 17.8% of patients. In the surgical indications of Puntambekar et al. (1), fibroids (33.8%) and adenomyosis (22.3%) were the most common. In the present study, 21.8% of the indications were treatment-resistant dysfunctional bleeding and 18.9% were fibroids. However, the rate of myomas in our pathology results was 49.0%, which demonstrates the frequent association of fibroids with different clinical findings in the foreground. In addition, 16.5% of our patients were operated due to malignancy, which may contribute to the difference between our indication rates and previous reports. Apart from the other indications, the treatment of cervical dysplasia is generally limited to loop electrosurgical excision procedure (LEEP) but in the presence of accompanying pathologies or absence of adequate tissue for re-LEEP in case of positive surgical margins, hysterectomy is performed.

The postoperative recovery period is shorter, hospital stay is shorter and patient satisfaction is therefore higher in laparoscopic hysterectomy when compared to abdominal surgery (1). Although it has been shown to be a safe method in many studies, a certain learning curve should be completed (2). The learning curve is defined as the shortening of the operation time without increasing complications, and it has been reported that a plateau can be reached after experience gained in 75 patients for this process, but implementing the learning curve does not warrant a complication-free surgery (10). In several studies it has been reported that 25, 30, 50, 75, or 100 cases are required to complete this process (2). In addition, it has been reported in many studies that the experience of the surgeon plays an important role in reducing complications, and the complication rate of high volume surgeons is lower (1,2,4,11).

Laparoscopic surgery has still not completely replaced open surgery due to fear of possibility of encountering intraoperative complications or conversion to open surgery. However, while a large uterus with multiple fibroids, previous abdominal surgery and obesity were relative complications for minimally invasive approach, today laparoscopy is the method of choice in such challenging cases with obesity or previous surgery in most centers (3). In these cases, abdominal access is particularly important, and the use of alternative sites such as Palmer's point or Lee Huang's point for abdominal entry can reduce complications (3,7). In the present study, the maximum dimension of the large uteruses was up to 20th gestational week-magnitude. In our center, if the uterus is located too near the umbilicus, the Lee-Huang point is used. Likewise, if the patient has previous surgeries, the Palmer point, at which the adhesions are less likely, is preferred.

Puntambekar et al. (1) reported intraopeartive complication rates as 2% (1.5% bladder injury and 0.5% ureter injury) in their series of 1200 cases. Bettaiah and Reddy (6) reported a major complication rate of 0.9% and the conversion rate to laparotomy of 0.93% in their data, which included 858 patients. In another study involving 209 patients, the rate of intraoperative and postoperative complications was reported as 12.9% and the rate of major complications was 3.8%, no vascular damage occurred and the surgery was converted to laparotomy in 3.3% of the patients (5).

Table 4. Clinical features and peri-operative outcomes of patients with benign and malignant pathological results

	Benign (n=1219), (mean/ median)	**Malignant (n=296), (mean/median)**	**p**
Age (years)	50.6±9.2	58.0±10.2	**0.0001**
BMI (kg/m^2)	30.1±6.4	34.9±12.1	**0.0001**
Comorbidity presence, n (%)	627 (51.4)	208 (70.3)	**0.0001**
ASA 3, n (%)	68 (5.6)	106 (35.8)	**0.0001**
ASA 4, n (%)	1 (0.1)	2 (0.7)	
Operation duration (minutes)	90 (15-360)	165 (35-360)	**0.0001**
Hb difference (g/dL)	1 (-5-6)	1 (-2-4.9)	**0.0001**
Intraoperative complications, n (%)	17 (1.4)	7 (2.4)	0.295
Postoperative complications, n (%)	52 (4.3)	11 (3.7)	0.748
Blood transfusion, n (%)	142 (11.6)	40 (13.5)	0.371
Amount of transfusion (units)	2 (1-8)	2 (1-4)	0.962
Time of hospitalization (days)	2 (2-12)	3 (2-15)	**0.0001**

BMI: Body mass index, ASA: American Society of Anesthesiologists, Hb: Hemoglobin

Intraoperative complications often include vascular damage and organ injuries related to the urinary or gastrointestinal systems. Wong et al. (12) reported that lower urinary tract injury was 0.33% in gynecological laparoscopy with benign indications, and bladder injury was three times more common than ureteral injury (0.24% and 0.08% for bladder and ureter injury, respectively). Urinary injury rates of up to 3% during laparoscopic hysterectomy have been reported in different publications (13-15). Particularly in surgeries performed for endometriosis or large fibroids, the risk of urinary injury is reported to be higher (14,16). If the recognition of these types of damage is delayed, further complications such as infection, fistula, and renal failure may occur. Although there are studies advocating that cystoscopy can be performed following hysterectomy in order to reveal damage intraoperatively, its benefit is controversial (17). Our urinary tract complication rate was 0.7%, which is consistent with the literature. All of our cases with ureteral injury were operated for myoma. As for our eight patients with bladder damage, five were operated for myoma, one for ovarian cyst and two for endometrial cancer. All of our urinary tract injuries were recognized intraoperatively, and primary suturation was performed with minimal invasive route, with only one patient requiring D-J stent postoperatively. The rate of intestinal damage during hysterectomy has been reported to be 0.39%, with particularly higher risk in advanced aged patients and for operations performed with the indication of endometriosis. It has been reported that 55% of such injuries occur during Veress or trocar insertion, and 82.3% of them are repaired intraoperatively (18,19). Our rate of 0.6% is consistent with the literature. None of the intestinal injuries occurred during the access to the abdominal cavity in our cases, which is probably due to the careful choice of the abdominal entry point in risky situations. All injuries were recognized immediately and primary repair was performed laparoscopically. The rate of vascular injury, which is another major complication of laparoscopy in benign gynecological surgeries, is 0.09%, mostly involving the epigastric vessels, and 82% of vascular injuries occur during entry into the abdomen. All of our vascular complications, which was at the rate of 0.3%, occurred at vena cava and iliac arteries during lymphadenectomy, while no vascular injury was observed in patients operated with benign indications. In total, our intraoperative complication rate was 1.6% and postoperative complication rate was 3.5%, and only 2 (0.13%) patients underwent laparotomy due to vascular complications. Our overall rate of conversion to laparotomy was 1.1%, which is also similar to the literature.

Postoperative complication rates for laparoscopic hysterectomy have been reported to be around 4-5% (20). In our series, the rate of postoperative complications such as fever, ileus, urinary retention, pelvic fluid collection/abscess, and port site infection was 3.5%, consistent with the literature. In the large series of Puntambekar et al. (1), postoperative complication rates were reported as 7.58%, postoperative hemorrhage was not reported in any patient, 0.67% mild pelvic collection was drained under ultrasound guidance, and other complications including fever, infection, paralytic ileus, and urinary retention were managed conservatively with medical treatment, with no requirement of re-exploration in any of the patients (1). Hematoma or abscess formation on the vaginal cuff, or cuff dehiscence, are reportedly relatively common complications, with cuff dehiscence reported to occur in 1.27% of laparoscopic surgery (21). In the present study, 0.3% of the patients had vaginal cuff dehiscence, 0.8% had hematoma on the cuff, 0.5% of the patients underwent resuturation under sedation, 0.3% had abscess drainage, and 2 (0.1%) patients underwent re-laparoscopy due to cuff hematoma. In a recent review, it was demonstrated that different suture techniques or suture materials do not affect cuff dehiscence (22). However, meticulous vaginal cleaning before the operation, using a harmonic scalpel instead of monopolar energy when cutting the cuff, and obeying the prohibition on postoperative intercourse may be effective in preventing cuff dehiscence (23).

While 1.2% of the patients were re-operated due to complications, others were managed medically. In this context, although 15 (1%) of the patients had fever requiring hospitalization in the postoperative period, no pathology was identified to explain the cause. However, small hematomas or collections, as well as pulmonary atelectasia, may be the cause of this symptom. Nevertheless, the complaints of the patients resolved with follow-up and medical treatment.

In the present study, when patients with benign and malignant pathology were compared, intraoperative and postoperative complication rates, blood transfusion rates and transfusion amounts were found to be similar in both groups. In our center, hysterectomy and surgical staging are performed laparoscopically in almost all patients diagnosed with endometrioid endometrial cancer. In the LAP2 study, in which a randomized comparison of laparoscopy and laparotomy in endometrial surgery was performed for the first time, and in many subsequent studies, perioperative outcomes were evaluated and it was reported that laparoscopy is a safe procedure in endometrial cancer surgery (8,24,25). Low rates of complication and necessity of complication-related laparotomy in our study support the literature. Until recent years, early-stage cervical cancers were also operated laparoscopically in appropriate cases in our center. However, following publication of the results of the Laparoscopic Approach to Cervical Cancer study, patients with cervical cancer are now treated with traditional open surgery (26). Nevertheless, perioperative complication rates were also found to be low in this group of

patients. When the complications that caused the conversion to open surgery were considered, they were found to be vascular complications related to lymphadenectomy procedure, and not the hysterectomy operation itself.

Study limitations

The important limitation of the study is its retrospective design. Also, the focus of this study was laparoscopic hysterectomy and additional surgical procedures other than laparoscopic hysterectomy may cause bias regarding the complication rates. However, the present study aimed to evaluate and compare laparoscopic hysterectomy for both benign and malignant indications, and the complications related to additional procedures were clarified. Assessment of the surgery performed with the same standard technique in a single center with the same surgical team, and the large population included in the study are the strengths of the study.

Conclusion

Laparoscopic hysterectomy may be widely applied in experienced centers with low complication rates for both benign and malignant indications, regardless of the patient's comorbidities, BMI and operation indication. Laparoscopic surgery is becoming increasingly popular due to its technical advantages and patient satisfaction. Therefore, we suggest that the financial resources and personnel training processes of the centers should be supported in this perspective.

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Perinatal outcomes of antenatally diagnosed omphalocele and gastroschisis: a survey from a university hospital

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Abstract

Objective: To evaluate the clinical features and perinatal outcomes of antenatally diagnosed fetuses with omphalocele and gastroschisis.

Material and Methods: This was a retrospective, single-center, cohort study of prenatally diagnosed fetuses with omphalocele and gastroschisis followed-up and delivered at a university hospital. Demographic, pregnancy, birth and perinatal outcomes were compared between gastroschisis and omphalocele.

Results: A total of 75 fetuses with omphalocele and 21 cases with gastroschisis were evaluated. The mean maternal age of women carrying a fetus with omphalocele was significantly higher than the women with gastroschisis ($p=0.001$). Associated structural anomalies were found in 53.3% and 4.7% of fetuses with omphalocele and gastroschisis, respectively ($p<0.001$). The rate of chromosomal anomaly was 8.3% in pregnancies with omphalocele. In liveborn pregnancies, the mean gestational age at delivery and birth weight did not differ between the study groups. Time to postoperative oral intake, duration of parenteral nutrition and length of hospital stay were significantly longer in babies with gastroschisis than omphalocele ($p<0.01$). Rates of termination, intrauterine, neonatal and infant death of fetuses with omphalocele were 25.3%, 6.7%, 10.7% and 2.7% respectively. Time to postoperative oral intake, duration of parenteral nutrition and duration of hospitalization were significantly longer in babies with complex compared to simple gastroschisis ($p<0.01$). Survival rates were 95.2%, 82.9% and 20% in fetuses with gastroschisis, isolated and non-isolated omphalocele, respectively.

Conclusion: Associated structural and chromosomal anomalies were significantly more common in fetuses with omphalocele compared to those with gastroschisis. Prognosis of fetuses with omphalocele depended on the associated structural and chromosomal anomalies, whereas bowel compromise was the main determining factor in gastroschisis. (J Turk Ger Gynecol Assoc. 2024; 25: 152-8)

Keywords: Gastroschisis, omphalocele, prenatal diagnosis, perinatal outcome

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Introduction

Omphalocele and gastroschisis are the two most common congenital malformations affecting the anterior abdominal wall, occurring in 1 out of every 4,000 live births (1). Omphalocele results from a defect in the midline of the abdominal wall, with compromised containment of the intestines and segments of other abdominal visceral organs. These components are enveloped by amnion, Wharton's jelly, and peritoneum (2). In contrast, gastroschisis is due to a flaw primarily situated to the right of the umbilical ring. This results in the protrusion of the intestinal tract and occasionally the urogenital tract into the amniotic cavity, without the presence of a surrounding membrane (2).

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The main prognostic factor for omphalocele is associated structural or chromosomal anomalies, with a reported incidence ranging between 27% and 63% (3). However, for gastroschisis prognosis is primarily determined by the underlying bowel viability and degree of bowel injury (4). Gastroschisis has a favorable outcome with an overall survival rate of 90-95% (5). Both omphalocele and gastroschisis are commonly diagnosed prenatally (4). Prenatal diagnosis is important for these defects as it helps to define associated abnormalities, allows for close follow-up of unfavorable prognostic signs, enables multidisciplinary management, and allows for adequate preparation for the postnatal period.

Postnatal management of neonates with these conditions is primarily conducted by pediatric surgeons and neonatologists, with other specialists sometimes attending to the management, depending on the presence of additional anomalies. The main goal of postnatal period management is to perform surgery with an appropriate technique at the optimal time, provide adequate nutrition using hepatoprotective novel lipid formulations, and start full enteral feeding as soon as possible (1). However, even with comprehensive care, neonates can still be affected by short- and long-term sequelae, such as necrotizing enterocolitis, extensive intestinal loss, and short bowel syndrome, which may require multiple surgeries (1,6).

This study aimed to examine the clinical features and perinatal outcomes of antenatally diagnosed abdominal anterior wall defects in a single tertiary center. In addition, the factors that may affect the prognosis of fetuses with omphalocele and gastroschisis was investigated.

Material and Methods

This retrospective, cohort study included pregnancies diagnosed prenatally with fetal omphalocele and gastroschisis, and the subjects were followed up and delivered at a perinatology clinic of a university hospital between January 2015 and December 2022. Permission for the study was obtained from the İstanbul University-Cerrahpaşa Clinical Research Ethics Committee (approval number: E-83045809-604.01.01-692089, date: 22.05.2023). Informed consent was obtained from all mothers of fetuses included in this study. Omphalocele was defined as protrusion of the intestines, liver, and/or additional organs into the intact umbilical cord and encased by amniotic membrane, Wharton jelly and peritoneum. Gastroschisis was defined as evisceration of the bowel through a paraumbilical abdominal wall defect with an intact umbilical cord and intestines floating freely in the amniotic fluid and lacking a covering membrane.

Ultrasound assessments were performed using a Voluson E10 (GE Medical Systems, Zipf, Austria) device with 3.5- or 5-MHz curvilinear transducers. The gestational age was established using the first day of the last menstruation and subsequently validated through the measurement of crown-rump length during the first trimester ultrasound. A detailed ultrasound examination and anatomic survey of the fetuses were performed, and additional structural anomalies were recorded. Karyotype analyses of the fetuses whose parents accepted the prenatal invasive tests were recorded.

Pregnancies diagnosed as gastroschisis or omphalocele were followed by a multidisciplinary team including perinatologists, pediatric surgeons, and neonatologists. Management plans were formulated by this multidisciplinary team, including follow-up ultrasound scans, decision concerning mode of delivery and conversation with parents regarding possibilities for pregnancy termination. Fetal biometry, Doppler measurements, and amniotic fluid assessment using the vertical measurement of the single deepest pocket were evaluated during the scans. Small for gestational age (SGA) was determined as birth weight falling below the 10th percentile for both gender and gestation (7). For fetuses with gastroschisis, maximal intra-abdominal bowel dilation (IABD) and extra-abdominal bowel dilation (EABD) were evaluated during the scans. Cut-off values of 10 mm for IABD and 18 mm for EABD were used to define complex gastroschisis (8,9). All infants were delivered at our clinic, and neonatal care was provided at the neonatology department within our hospital. Surgical procedures were carried out at the pediatric surgery department within the same hospital. Parameters, such as hospitalization duration, neonatal outcomes, surgical approach (primary repair or mesh utilization), length of postoperative parenteral nutrition, and time until postoperative oral intake were assessed for newborns who underwent surgery.

Variables, including gestational age at diagnosis, parity, maternal age, karyotype results, coexisting fetal abnormalities, birth weight, gestational week at delivery, delivery method, postnatal confirmed diagnosis, and pregnancy outcomes were subjected to analysis. The cases were categorized into six groups based on pregnancy outcome: termination of pregnancy (TOP), abortus, intrauterine fetal death, neonatal death (NND), death in infancy, and survivors. The decision for TOP was made by the official "Termination of Pregnancy Council" within our institution in accordance with national regulations. In order to confirm the diagnosis, fetal autopsy was conducted for all terminated cases. Abortion was defined as intrauterine death before 24 weeks of gestation, NND was defined as death within the initial 28 days of life, and infant death referred to death within the first year. Data regarding surviving infants were gathered through telephone interviews with the parents.

Statistical analysis

Statistical analysis was conducted using the SPSS, version 20.0 for Windows (SPSS Inc., Chicago, IL., USA). Categorical variables are presented as frequencies and percentages, while continuous variables are expressed as mean and standard deviation. Parametric data were assessed using an independent two-sample t-test and One-Way ANOVA. Non-parametric data were compared using the chi-square test, Kruskal-Wallis and subsequent Mann-Whitney U test.

Results

The study population consisted of pregnancies with prenatally diagnosed omphalocele (n=75) and gastroschisis (n=21) in the fetus. Table 1 displays the clinical characteristics and outcomes of the study group. The mean maternal age and incidence of primiparous women for pregnancies with omphalocele and gastroschisis were 30.3±5.9 years and 52% and 25.7±4.5 years and 71.4%, respectively. The mean maternal age of women carrying a fetus with omphalocele was significantly older than the women with fetuses with gastroschisis (p=0.001). The mean gestational age at the initial observation and/or diagnosis was 16.9±3.7 weeks (range; 12-24 weeks) and 16.7±2.6 weeks (range; 13-21) for omphalocele and gastroschisis respectively (p>0.05). Incidence of SGA was significantly higher in pregnancies with gastroschisis than omphalocele (40% vs. 14.9%, p=0.001). Associated structural anomalies were found in 53.3% (40/75) of fetuses with omphalocele and 4.7% (1/21) of fetuses with gastroschisis (p<0.001). Prenatal karyotype analysis was performed in 60 (80%) and 8 (38.1%) pregnancies with omphalocele and gastroschisis, respectively. The rate of chromosomal anomaly was 8.3% in pregnancies with omphalocele whereas fetuses with gastroschisis all had normal karyotype. The mean gestational age at delivery and birth weight of liveborn pregnancies were 36.7±2.8 weeks and 2658±700 grams for omphalocele and 37.4±1.3 weeks and 2464±472 grams for gastroschisis (p>0.05). For newborn babies, time to postoperative oral intake, length of parenteral nutrition and length of hospital stay were significantly longer in babies with gastroschisis than omphalocele (p<0.01).

Table 1. The clinical characteristics and the outcomes of the study group

	Omphalocele	**Gastroschisis**	**p**
n	75	21	
Maternal age (years)	30.3±5.9	25.7±4.5	0.001
Nulliparity, n (%)	39 (52)	15 (71.4)	0.115
Gestation age at diagnosis (weeks)	16.9±3.7	16.7±2.6	0.873
Associated structural anomalies, n (%)	40 (53.3)	1 (4.7)	0.001
Chromosomal abnormality	**(Subgroup size was 60) n=5 (8.3)**	**(Subgroup size was 8) n=0 (0)**	
Trisomy 21	1 (1.7)	-	
Trisomy 18	4 (6.6)	-	
Small for gestational age	7/47 (14.9)	8/20 (40)	0.001
Gestational age at delivery	**(n=47) 36.7±2.8**	**(n=20) 37.4±1.3**	**0.032**
<37 weeks	14 (29.8)	5 (25)	
<34 weeks	5 (10.6)	-	
Birth weight (g)	**(n=47) 2658±700**	**(n=20) 2464±472**	**0.519**
Caesarean section	47/47 (100)	20/20 (100)	
Neonatal			
Parenteral nutrition duration (days)	7.7±4.7	19.5±11.1	0.001
Time to postoperative oral intake (days)	6.7±3.3	11.8±9.2	0.002
Duration of hospitalization (days)	19.5±15.7	31.2±24.3	0.023
Termination of pregnancy	19 (25.3)	-	
Abortion	4 (5.3)	1 (4.7)	0.917
Intrauterine death	5 (6.7)	-	
Neonatal death	8 (10.7)	-	
Infant death	2 (2.7)	-	
Live birth	37 (49.3)	20 (95.2)	0.001
Data are presented as mean ± standard deviation or n (%)			

Of the 75 pregnancies with omphalocele, 19 women (25.3%) terminated their pregnancy and all the terminated fetuses had additional structural anomalies. Rates of abortion were similar in both pregnancies with omphalocele and gastroschisis (5.3% vs. 4.7%, p>0.05). There were no intrauterine, neonatal and infant deaths in the gastroschisis group. The rates of intrauterine, neonatal and infant death of fetuses with omphalocele were 6.7%, 10.7% and 2.7%, respectively. The overall survival rates of fetuses with omphalocele and gastroschisis were 49.3% and 95.2%, respectively (p<0.001).

The distribution of associated structural anomalies of 40 (53.3%) of the fetuses with omphalocele are illustrated in Table 2. The most frequent structural anomaly was cardiac anomalies (52.5%) followed by central nervous system anomalies (20%). All of the fetuses with chromosomal abnormality had an additional structural anomaly [cardiac anomalies (n=3), holoprosencephaly (n=1), hydrops fetalis (n=1)] other than omphalocele. There was one fetus with Beckwith-Wiedemann syndrome (BWS) that was diagnosed after delivery with genetic testing in fetuses with omphalocele. Perinatal and obstetric outcomes of fetuses with isolated and non-isolated omphalocele are shown in Table 3. The incidence of SGA was significantly lower and mean gestational age at delivery, birth weight and survival rate were significantly higher in pregnancies with isolated omphalocele compared to non-isolated omphalocele (p<0.01). The mean gestational age at delivery was 37.4±1.7 weeks and survival rate was 82.9% in pregnancies with isolated omphalocele. There were three abortions (at 16, 22 and 23 weeks of gestation), two intrauterine deaths (at 25 and 26 weeks of gestation) and one infant death (at 4.5 months of age) in pregnancies with isolated omphalocele. Of the 40 pregnancies with non-isolated omphalocele, 19 women (47.5%) terminated their pregnancy and the survival rate was 20%. There were one abortus (at 23

Table 2. The distribution of associated structural anomalies of fetuses with omphalocele

(n=40)	
Cardiac	21 (52.5) (3 Trisomy 18 and 1 Trisomy 21)
Central nervous system	8 (20) (1 Trisomy 18)
Vertebral and/or scoliosis	3 (7.5)
Skeletal	3 (7.5)
Esophageal atresia	2 (5)
Diaphragmatic hernia	2 (5)
Anal atresia	1 (2.5)
Venous system	1 (2.5)
Beckwith-Wiedemann syndrome	1 (2.5)
Data are presented as n (%)	

Table 3. Perinatal and obstetric outcomes of fetuses with isolated and non-isolated omphalocele

	Isolated	**Non-isolated**	**p**
n	**35**	**40**	
Chromosomal abnormality	-	5 (12.5)	
Liver in the sac	32 (91.4)	38 (95)	0.539
Small for gestational age	2 (5.7)	5 (12.5)	0.001
Gestational age at delivery	**(Subgroup size was 30) 37.4±1.7**	**(Subgroup size was 17) 35.6±3.9**	**0.033**
<37 weeks	7 (23.3)	6 (35.3)	
<34 weeks	-	5 (29.4)	
Birth weight (g)	2822±472	2367±930	0.031
Termination of pregnancy	-	19 (47.5)	
Abortion	3 (8.6)	1 (2.5)	0.917
Intrauterine death	2 (5.7)	3 (7.5)	0.307
Neonatal death	-	8 (20)	
Infant death	1 (2.8)	1 (2.5)	0.924
Alive	29 (82.9)	8 (20)	0.001
Data are presented as mean ± standard deviation or n (%)			

weeks of gestation), three intrauterine deaths (at 27, 28 and 30 weeks of gestation), eight NNDs and one infant death (at 7 months of age) in pregnancies with non-isolated omphalocele. All of the newborns with omphalocele had primary closure of the abdominal wall defect.

Of the 21 pregnancies with gastroschisis, eight (38.1%) and 13 were determined to be complex and simple, respectively, based on prenatal ultrasound criteria. Neonatal outcomes of complex and simple gastroschisis cases are shown in Table 4. Time to postoperative oral intake, duration of parenteral nutrition and duration of hospitalization were significantly longer in babies with complex gastroschisis compared to simple gastroschisis ($p<0.01$). All of the newborns with gastroschisis underwent primary closure of the abdominal wall defect within the first day of life. There was no NND in pregnancies affected by gastroschisis.

Discussion

In the present study, the mothers of fetuses with gastroschisis were significantly younger than mothers of fetuses with omphalocele, which is consistent with previous studies (4,10,11). For abdominal wall defects, the rates of successful prenatal diagnosis have risen over the past two decades, reaching levels as high as 100% (3). The average gestational age at the first observation and/or diagnosis of omphalocele and gastroschisis were 16 weeks in our study group, which is similar to those reported in other series (11,12). The mean gestational age at delivery and birth weight of liveborn pregnancies were not significantly different in our omphalocele and gastroschisis cases. However, in the majority of previously reported series, pregnancies with gastroschisis were born earlier and with lower birthweight than those with omphalocele (11-13). The rates of preterm delivery of about 30% and 25% for omphalocele and gastroschisis in our study population are similar to previously reported rates (10,12). All babies with omphalocele and gastroschisis were delivered by Caesarean section (CS). This high rate of CS can primarily be attributed to the strategic choice of delivering in a tertiary care center, where access to suitable neonatal and pediatric surgical services facilitated by CS compared to vaginal delivery. Despite various studies showing no superiority of CS over vaginal delivery for neonates with gastroschisis and omphalocele, similar high rates of CS have been reported in other studies (10,12,14). Pregnancies with omphalocele are reported to be associated with higher incidences of intrauterine, neonatal, and infant deaths than gastroschisis (1,11,12). This was also the case in the present study consistent with previous studies, our findings confirmed that survival rates of offspring in pregnancies affected by gastroschisis is significantly higher than omphalocele (1,11,12). Incidences of chromosomal anomaly and co-existing structural anomalies were significantly higher in pregnancies with omphalocele than gastroschisis in the present study. This finding is in accordance with previous studies (2,3,11,12). The rate of chromosomal anomaly was 8.3% in pregnancies with omphalocele whereas fetuses with gastroschisis all had normal karyotype in our study population. The reported incidences of chromosomal anomalies for omphalocele range widely from 8% to 57% (1,15). Trisomy 13, 18, and 21 were frequently found to coexist with omphalocele, with Trisomy 18 being the most prevalent (15,16). Trisomy 18 was also the most frequent associated chromosomal anomaly in cases of omphalocele in the present series. All of the fetuses with omphalocele and chromosomal abnormality had additional cardiac or central nervous system anomalies. The incidence of associated structural anomalies in fetuses with omphalocele was 53.3% overall. The previously reported incidences of associated structural anomalies in fetuses with omphalocele varies between 25% to 97% (1,15). The most frequent structural anomaly was cardiac (52.5%) followed by central nervous system anomalies (20%) in our series. The distribution of associated structural anomalies in fetuses with omphalocele is similar to those reported by other series (3,11,17). There was one case of BWS, which was diagnosed after delivery with genetic testing in our omphalocele group. BWS is an autosomal dominant condition occurring in 5%-25% of fetuses diagnosed with an omphalocele. Additional findings include macrosomia, macroglossia, organomegaly, placentomegaly, and polyhydramnios (15). Since many features of BWS manifest late in gestation or postnatally, prenatal diagnosis is challenging and only a few cases can be diagnosed prenatally

Table 4. Neonatal outcomes of complex and simple gastroschisis cases

	Simple Gastroschisis	Complex Gastroschisis	p
n	13	8	
Mean parenteral nutrition duration (days)	11.5±2.9	31.4±6.9	0.001
Mean time to postoperative oral intake (days)	5.3±2.9	21.5±6.2	0.001
Mean duration of hospitalization (day)	18.6±7.6	50.1±29.1	0.002
Data are presented as mean ± standard deviation			

(18). The prenatal diagnosis of BWS can be achieved through amniocentesis by employing a multi-tiered approach involving chromosomal microarray, methylation testing, and sequence analysis (15).

Survival and overall prognosis of fetuses with omphalocele depends on the associated genetic, structural and chromosomal anomalies (1,15). The incidence of TOP in non-isolated omphalocele cases was 47.5% in our study. Survival rates of 82.9% and 20% were observed in fetuses with isolated and non-isolated omphalocele respectively, in the present study. Similar survival rates for isolated and non-isolated omphalocele cases have been reported by other studies (11,19,20). As survival of isolated omphalocele fetuses are favorable, thorough evaluation, including detailed anatomic assessment, fetal echocardiogram, prenatal diagnostic testing, and genetic counseling is recommended for pregnancies with omphalocele (15). Prenatal diagnosis will allow parental counseling, the possibility of TOP, assistance with pregnancy management and delivery under optimal conditions.

In cases of gastroschisis, survival rates greater than 90% have been reported (5,11,21). Our data corroborates this finding, and the survival rate of our gastroschisis group was 95.2%. There was one abortus at 21 weeks gestation in fetuses with gastroschisis. Chromosomal abnormalities or additional structural anomalies are rarely seen in fetuses with gastroschisis and prenatal invasive testing is not routinely recommended (2,22). Fetuses with gastroschisis all had normal karyotype and one fetus (4.7%) had muscular ventricular septal defect in the present study. The most severe prenatal complication associated with gastroschisis is the infrequent but unpredictable occurrence of fetal death (23). This could result from an in utero midgut volvulus or, more likely, from the sudden impairment of umbilical blood flow due to the eviscerated bowel (2). Fortunately, we did not observe in utero fetal death in our study population.

The postnatal prognosis of newborns with gastroschisis primarily hinges on the prenatal extent of intestinal damage and the subsequent functional state after birth (5). Bowel compromise is likely due to prolonged exposure of the intestines to amniotic fluid, as well as the compression of bowel and vasculature near the abdominal wall defect (6). Mainly due to impaired bowel function, time to postoperative oral intake, length of parenteral nutrition and length of hospital stay were significantly longer in babies with gastroschisis than omphalocele in our study population. Our findings align with previous studies, indicating that newborns with gastroschisis typically experience a more complex neonatal course compared to those with omphalocele (12,24).

In the neonatal period gastroschisis is commonly divided into two groups: simple and complex. Neonatal complex gastroschisis is defined as gastroschisis accompanied by intestinal atresia necrosis, perforation, and/or volvulus, whereas in the simple form these anomalies are absent (25). Neonatal complex gastroschisis thus includes infants which present with severe bowel injury at birth, and have the highest risk of unfavorable outcome, including sepsis, NND, short bowel syndrome, prolonged reliance on mechanical ventilation, extended hospital stays, and an extended duration of parenteral nutrition (6). There exists a multitude of contradictory perspectives regarding the role of ultrasound assessment in predicting complex forms of gastroschisis and which features are useful prognostic indicators. We have defined >10 mm for IABD and >18 mm for EABD for complex gastroschisis (8,9). Among patients diagnosed with gastroschisis in our cohort, 38% had prenatal ultrasound findings of complex gastroschisis. We had no neonatal mortality and short bowel syndrome in our study group. However, time to postoperative oral intake, duration of parenteral nutrition and duration of hospitalization were significantly longer in babies with prenatal ultrasound findings of complex rather than simple gastroschisis. These findings are consistent with findings from other studies (12,21,25).

Study limitations

The limitations of this study included its retrospective design and the relatively small number of cases. Despite these limitations, we conducted a comparison of various parameters in our patients and compared our findings with the existing literature. Our study reflects the perinatal and neonatal outcomes of pregnancies with omphalocele and gastroschisis managed in a tertiary care center. Further studies should investigate the long-term outcomes of offspring to gain a better understanding of their health and development.

Conclusion

Associated structural and chromosomal anomalies were significantly more common in fetuses with omphalocele compared to those with gastroschisis. Survival rates were 95.2%, 82.9% and 20% in fetuses with gastroschisis, isolated and non-isolated omphalocele, respectively. Prognosis of fetuses with omphalocele depends on the associated structural and chromosomal anomalies, whereas bowel compromise is the main determining factor in gastroschisis.

Ethics Committee Approval: *Permission for the study was obtained from the İstanbul University-Cerrahpaşa Clinical Research Ethics Committee (approval number: E-83045809-604.01.01-692089, date: 22.05.2023).*

Informed Consent: *Informed consent was obtained from all mothers of fetuses included in this study.*

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Molecular mechanisms of PI3K isoform dependence in embryonic growth

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Abstract

Objective: The phosphoinositide 3-kinase (PI3K) pathway is an important signaling mechanism for cell proliferation and metabolism. Mutations that activate PIK3CA may make cells p110α dependent, but when phosphatase tensin homolog (PTEN) is lost, the p110β isoform of PI3Ks becomes more important. However, the exact mechanism underlying the prevalence of p110s remains unclear. In this study, our aim was to elucidate the processes behind PI3K isoform dependency in a cellular model of embryonic development.

Material and Methods: In order to understand PI3K isoform prevalence, mouse embryonic fibroblasts (MEFs) were used and p110β, PTEN and Rac1 activity was modulated using retroviral plasmids. Expression levels and cellular growth were assessed by performing immunoblots and crystal violet assays.

Results: The levels of PTEN had only a partial effect on the prevalence of PI3K isoforms in MEFs. The dependency on p110α diminished when PTEN was depleted. Of note, when PTEN expression was repressed, there was no full transition in dependency from one PI3K isoform to the other. Interestingly, the viability of PTEN-depleted MEFs became less dependent on p110α and more dependent on p110β when p110β was overexpressed. Nevertheless, the overexpression of p110β in conjunction with PTEN knock-downs did not result in a complete shift of isoforms in PI3Ks. Finally, we investigated Rac1 activation with a mutant allele and determined a more potent increase in p110β prominence in MEFs.

Conclusion: These findings suggest that multiple cellular parameters, including PTEN status, PI3K isoform levels, and Rac1 activity, combine to influence PI3K isoform prevalence, rather than a single determinant. (J Turk Ger Gynecol Assoc. 2024; 25: 159-66)

Keywords: PI3K isoform prominence, PTEN, Rac1, mouse embryonic fibroblasts

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Introduction

The phosphoinositide 3-kinase (PI3K) pathway is a fundamental signaling cascade that regulates many physiological activities, such as cellular metabolism, growth, proliferation and survival. Dysregulation of PI3K signaling is implied in various diseases, ranging from cancer to metabolic disorders, making it a prime target for therapeutic intervention (1). However, the complexity of PI3K signaling arises from the existence of multiple isoforms with distinct functions and regulatory mechanisms. Understanding isoform selectivity within the PI3K pathway is paramount for unraveling its physiological roles and developing targeted therapies.

Class I PI3Ks are further categorized into class IA and class IB based on their regulatory subunits and activation mechanisms. Class IA PI3Ks are comprised of a catalytic subunit (p110α, p110β, and p110δ- encoded by *PIK3CA*, *PIK3CB* and *PIK3CD* respectively) and a regulatory subunit (p85α, p85β, p55γ, p55α, or p50α), whereas class IB PI3K consists of p110γ and regulatory subunits (p101 or p84) (2). Each isoform exhibits tissue-specific expression patterns and functional specificity, contributing to the complexity of PI3K signaling. PI3Ks possess lipid kinase activity and catalyze the phosphorylation of phosphatidylinositol lipids at the 3'-hydroxyl group (3). Phosphorylated phosphatidylinositols function as secondary messengers in cells, triggering the activation of the serine

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threonine kinase Akt, as well as other downstream proteins. Phosphatase tensin homolog (PTEN) is the primary inhibitor of PI3K (4).

The differential roles of class IA and class IB PI3K isoforms have been elucidated through a combination of genetic knockout studies, biochemical assays, and structural analyses. For instance, p110α is predominantly associated with growth factor signaling and oncogenic transformation, whereas p110β plays a crucial role in insulin signaling, cardiovascular function, and inflammatory responses (5). Similarly, p110δ and p110γ are primarily involved in immune cell function, making them attractive targets for immunomodulatory therapies (2). Moreover, genetic alterations in tumorigenesis can dictate the isoform prevalence in cancer. Cells bearing *PIK3CA* mutations can become dependent on p110α activity, whereas loss of PTEN function usually promote a p110β dependent state (6).

Isoform-specific inhibitors have emerged as promising candidates for precision medicine, enabling targeted therapies with reduced off-target effects (1). However, attaining isoform selectivity is difficult due to the structural similarity of PI3K isoforms and the interdependence of signaling networks. Computational modeling and structure-based drug design have aided in the creation of selective inhibitors of certain PI3K isoforms (7).

Moreover, the regulation of PI3K signaling is intricately linked to crosstalk with other signaling pathways, such as Ras-MAPK, mTOR and Rac1 pathways (8). Crosstalk between PI3K isoforms and these pathways adds another layer of complexity to the regulation of cellular responses. The interplay between PI3K and Rac1, for example, orchestrates intricate cellular processes, ranging from cytoskeletal dynamics to cell migration and proliferation. Rac1, a small G protein, serves as an isoform-selective effector of PI3K, regulating p110β dependent cellular signaling (9).

The crosstalk between PI3K and Rac1 is bidirectional and elaborate (10). PI3K activation induces the recruitment and activation of Rac1 via its downstream effectors, such as guanine nucleotide exchange factors, leading to actin polymerization and cytoskeletal rearrangements essential for cell migration and invasion. Conversely, Rac1 activation particularly enhances p110β activity through feedback loops, amplifying downstream signaling pathways involved in cellular growth and survival (11,12). Moreover, recent studies have unveiled intricate regulatory mechanisms governing the PI3K-Rac1 interplay. For instance, scaffolding proteins like G protein-coupled receptor kinase-interacting proteins and PAK-interacting exchange factor (PIX) facilitate the spatial and temporal coordination between PI3K and Rac1, ensuring precise cellular responses to extracellular stimuli (13).

Carcinogenesis is linked to dysregulation of the interaction between PI3K isoforms and their effectors, wherein abnormal signaling may facilitate tumor cell invasion, metastasis, and resistance to treatment. Conversely, targeting this axis presents a promising therapeutic strategy for cancer treatment, as evidenced by the development of small molecule inhibitors targeting PI3K and Rac1 signaling pathways. Consequently, study of the determinants of PI3K isoform selectivity can inform us about how cellular conditions can dictate cell growth and proliferation.

Unraveling the complexities of this interplay holds significant promise for PI3K-dependent development of embryonic tissues as well as the development of novel therapeutic interventions targeting cancer and other diseases driven by dysregulated signaling pathways.

In this study, we investigated the molecular mechanisms underlying isoform selectivity in PI3K signaling, with a particular focus on the status of PTEN tumor suppressor, class IA PI3K isoforms and the small G protein, Rac1. We attempted to establish molecular genetic cellular models with distinct PIK3CA or PIK3CB dependences for cell proliferation and tried to switch this dependence by modulating PTEN function. Our results suggest that a single step modification of PTEN cannot account for a complete isoform switch. Our findings further implicate that activation of secondary factors e.g. Rac1 small G-protein activation can dictate the requirement of individual PI3K isoforms for the growth embryonic cells. Our findings, consequently, offer a more comprehensive understanding of the role that isoform selectivity plays in PI3K signaling and the implications of this phenomenon for translational medicine.

Material and Methods

Compounds and reagents

Crystal violet powder was supplemented from Sigma Aldrich. BYL719, KIN193, GDC001 and MK2206 were all purchased from SelleckChem.

Cell culture

The p110α flox/flox; p110β flox/flox mouse embryonic fibroblasts (MEFs) and HEK293 cells were cultured under standard growth conditions. This included high-glucose DMEM, L-glutamine, and 110 mg/L sodium pyruvate, along with 8% FBS (Biowest) and pen-strep solution (100 IU/mL and 100 mg/mL, Gibco). The cells were incubated in a humidified incubator at 37 °C. The cells used in the study were tested for mycoplasma and no contamination was detected. MEFs have been established from homozygotes of p110βflox/flox and p110αflox/flox mice that were embryonic day 13.5. Using the conventional 3T3 method, primary MEFs were immortalized, and polyclonal knock-outs were produced in accordance with previous publications (9). Adenovirus transductions encoding cre-recombinase (AdCre; Iowa Viral Vector Core, Iowa City,

Iowa, USA) was applied to floxed MEFs in order to produce knock-out cells, whereas AdLacZ was used as a control.

Plasmids and retroviral transductions

The pMX GFP Rac G12V plasmid was generously provided by Joan Brugge [Addgene plasmid # 14567, (14)]. Rac1(N17) was cloned from YFP-Rac1(N17) [a gift from Joel Swanson-Addgene plasmid # 11395, (15)] vector in pMX vector with BglII and HindIII compatible ligation. Two different shRNA constructs were used to deplete PTEN expression in MEFs (shPTEN#1: CCGGGCTAGAACTTATCAAACCCTTCTCGA GAAGGGTTTGATAAGTTCTAGCTTTT, shPTEN#2: CCGGCGACTTAGACTTGACCTAT ATCTCGAGATATAGGTCAAG TCTAAGTCGTTTTTG) using pLKO.1-puro plasmid (16). Stable MEF lines were produced via retro/lentiviral transductions. HEK293 cells were transfected with gag-pol/delta 8.3, vsv-g, and retro/lentiviral plasmids using lipofectamine 3000 (invitrogen) to produce retro/lentiviral particles. Retro/lentiviral particle-containing supernatants were collected at 48 and 72 hours post-transfection, combined, subjected to sterile filtration, and used for infections within 2-3 days.

Antibodies and western blotting

The antibodies were purchased from Cell Signaling Technology: anti-HA, anti-p110α, anti-p110β, anti-PTEN, anti-pAkt (Ser473), anti-pAkt (Thr308), anti-pS6 (Ser235/Ser236) and anti-pS6 (Ser240/Ser244). Santa Cruz was the source of the anti-Rac1 antibodies. The source of anti-β-actin antibodies was Sigma. We bought HRP linked secondary antibodies from Advansta. A standard western blotting approach was applied (17). The procedure involved scraping the cells into ice-cold PBS and lysing them in RIPA buffer (EcoTech) supplemented with 1 mM sodium orthovanadate (CST), 1 mM dithiothrietol (Bio-Rad), and protease/phosphatase inhibitor tablets (Roche) at 4 °C. After being electrophoresed with 10-12% SDS-PAGE, 15-20 μg of total protein were blotted onto nitrocellulose membranes (CVS). The membrane stripes were blocked for a duration of thirty minutes using 5% milk without fat in TBS. Subsequently, they were exposed to primary antibodies in a solution containing TBS, 5% milk, and 0.1% Tween-20 overnight at a temperature of 4 °C. Afterwards, the membranes were exposed to secondary antibodies, which were diluted in a buffer of 5% milk-TBS, for a duration of two hours at ambient temperature. Using an Amersham Imager 600, the Pierce ECL Western Blotting Substrate (ThermoFischer Scientific, US) electronic images of the blots were generated.

Cellular proliferation assays

The crystal violet experiments were conducted by inoculating 2×10^4 MEF cells in 6-well plates (SPL). The cells were washed with PBS and then treated with a solution of 10% acetic acid (Sigma) and 10% ethyl alcohol for 4 hours at ambient temperature. The staining procedure involved the use of 0.2% crystal violet (Sigma) and 10% ethyl alcohol for 30 minutes. Afterward, the wells were rinsed with distilled water and left to dry in the air. The crystal violet stain, which was associated with cells, was extracted using 2 mL per well of a 10% acetic acid solution. This extraction process was carried out for 20 minutes on a shaker. Spectrometric analyses were then performed at an optical density of 595 nm. The values were standardized to the baseline measurement (day 0) in each experiment. The figures shown represent the mean value obtained from three independent experiments.

Statistical analysis

The differential comparison between two groups was conducted using a two-tailed Student's t-test. T-tests were conducted with GraphPad Prism. Data are deemed statistically significant when the p-values are less than 0.05. The data is reported as the mean value plus/minus the standard deviation, based on three independent experiments, unless otherwise specified. The quantification of the band intensities (densitometry analysis) of western blot data was evaluated by ImageJ, according to the normalization of the band intensities of each protein to the band intensities of the loading controls.

Results

PI3K isoforms have diverse functions across different cell lines, and their reliance varies among cell types. We used MEFs in our studies as MEFs represent embyronic cellular models with a stable karyotype (18). The proliferation of cells in MEFs mostly relies on the p110α isoform in comparison to other isoforms of PI3K. When the drugs BYL719 (an inhibitor of p110α) and KIN193 (an inhibitor of p110β) were given, it was shown that MEFs were more responsive to the inhibition of p110α, which is consistent with earlier studies (Figure 1A). Cross cancer genomic investigations have shown that 30% of tumor samples harbor PTEN loss (19). Remarkably PTEN-loss induced carcinogenesis allows cancer cells to rely more heavily on the p110β rather than the p110α isoform (20). The current understanding of the molecular processes behind this occurrence remains incompletely understood. Hence, we aimed to examine the influence of PTEN deficiency on isoform reliance in MEFs. PTEN expression was eliminated using two distinct shRNA plasmids. PTEN knock-down efficiency was shown by the use of anti-PTEN immunoblots. Our findings indicate that the shPTEN #2 construct exhibits a greater effectiveness in reducing PTEN levels compared to shPTEN #1 (Figure 1B). Simultaneously, PTEN shRNA treatment resulted in an augmentation in Akt phosphorylation, as PTEN activity

inhibited the PI3K pathway. We investigated whether MEFs still rely on the p110α isoform in the setting of diminished PTEN expression. In order to find out if the loss of PTEN caused a change in isoform dependence, we subjected MEFs expressing shPTEN to treatment with small molecule inhibitors that specifically target distinct PI3K isoforms. The shPTEN-#2-MEF cells, which exhibited a superior suppression of PTEN, demonstrated increased resistance to elevated doses of p110α inhibitor in comparison to wild type (wt) and shPTEN-#1 MEF cells (Figure 1C). These data suggest that the degree to which MEF cells rely on p110α is influenced to some extent by the levels of PTEN. Nevertheless, the sensitivity to p110β inhibition remained unchanged, despite the downregulation of PTEN in these cell lines.

Furthermore, this finding was confirmed by biochemical analysis in a brief inhibitor treatment experiment, where we examined the levels of pAkt and pS6. The administration of BYL719 for 2 hours resulted in a near-complete elimination of pAkt or pS6 in both the control and PTEN repressed MEF lines. In contrast, KIN193 exhibited significantly less effectiveness in reducing Akt and S6 phosphorylations in both the control and PTEN repressed MEFs (Figure 1D). Cells however, retained their high sensitivity to pan-PI3K inhibition by GDC.

Human malignancies often have PI3Ks mutated or amplified. Patients may have *PIK3CA* activating mutations, however this does not apply to the remaining PI3K isoforms (21). The proliferative capacity of p110β, δ, and γ isoforms is enhanced when they are excessively expressed as unaltered proteins. To

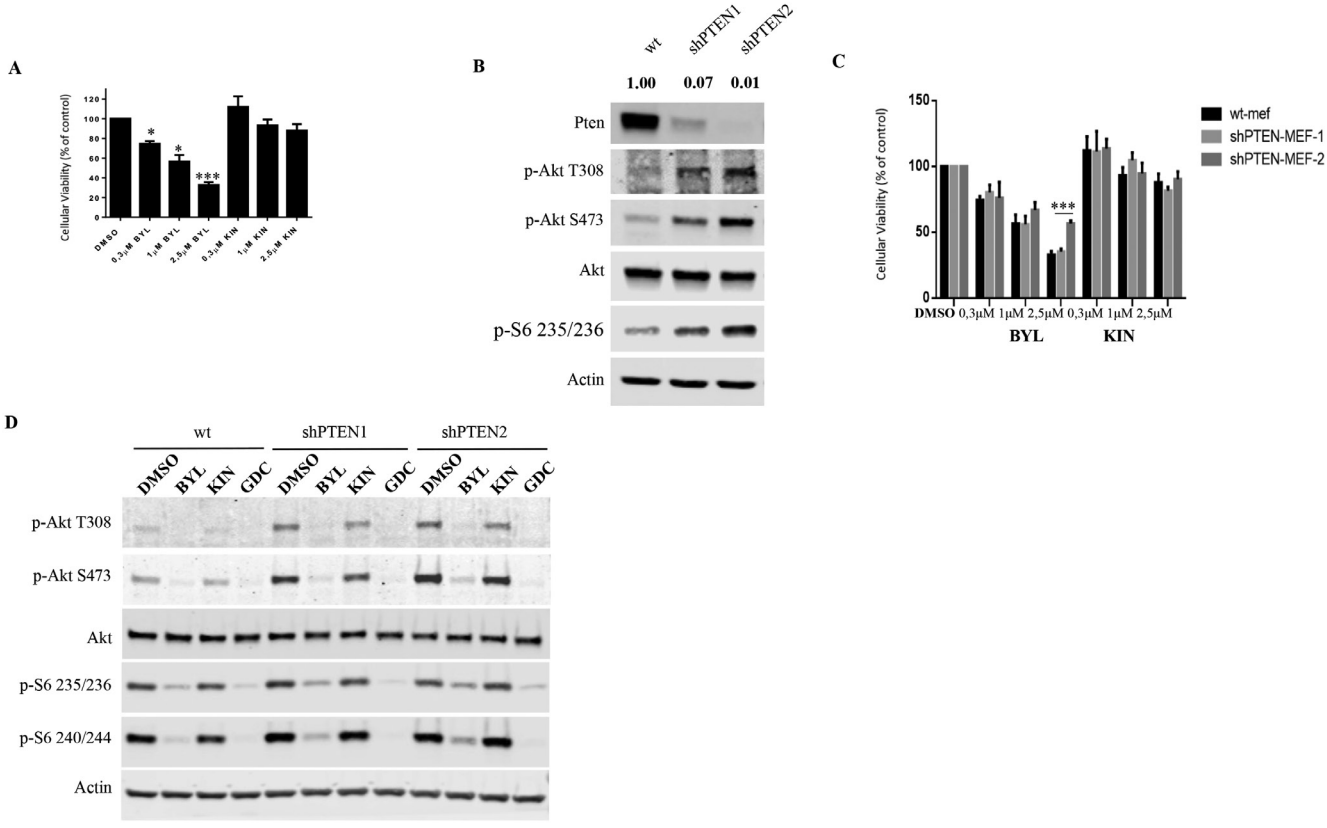

Figure 1. Molecular characterization of PI3K isoform prevalence in MEFs. (A) MEFs were treated with varying doses of p110α and p110β specific inhibitors. Crystal violet assays were performed to determine the cellular proliferative capacity. (B) PTEN was knocked-down in MEFs using shRNA constructs. Knock-down efficiency was determined in an immunoblot using anti-PTEN antibodies. pAkt and pS6 antibodies were used to assess the level of PI3K activation whereas anti-actin were used as loading control. (C) PTEN knocked-down MEFs were treated with varying doses of p110α and p110β specific inhibitors. Crystal violet assays were performed to determine the cellular proliferative capacity. (D) Control or PTEN knocked down MEFs were treated either with DMSO or isoform specific PI3K inhibitors, BYL-719 (BYL) and KIN-193 (KIN), specific for p110α and p110β or GDC, a pan-PI3K inhibitor respectively. The efficacy of PI3K signaling in these cells was assessed using immunoblots for p-Akt (T308 and S473) and p-S6 (S235/236). Actin served as the control for loading. *p<0.05, ***p<0.005, (the error bars in the graphs represent the standard deviation, and the experiments were conducted independently three times)

MEFs: Mouse embryonic fibroblasts, PTEN: Phosphatase tensin homolog

investigate whether increasing the levels of p110β-wt in MEFs leads to a change in the PI3K preponderance in favor of the p110β isoform, ectopic p110β-wt expression was performed (Figure 2A). Proliferation experiments were conducted using 1-2 μM of BYL719 and KIN193. No alterations in susceptibility or resistance to BYL719 and KIN193 treatment were seen when p110β was highly expressed in MEFs (Figure 2B, C). MEFs still exhibited a greater reliance on p110α and a lesser reliance on p110β, irrespective of increased p110β expression. The positive controls utilized in this experiment were GDC0941 at a concentration of 1 μM and MK2206 at a concentration of 2 μM. GDC0941 is an inhibitor that targets all isoforms of PI3K, whereas MK2206 is an inhibitor that specifically targets all isoforms of Akt (Figure 2C). Due to the more thorough inhibition of the PI3K pathway under these settings, we found a reduced rate of cell growth in those cell lines.

Next, our objective was to investigate whether MEFs still rely on PI3K activity in general upon reduced PTEN expression. We utilized genetically modified α,β+/+ MEF cells that had LoxP sites in the first exons of *PIK3CA* and *PIK3CB*. AdCre were used to excise out the floxed sequences concurrently, resulting in the generation of double knock-outs for the p110α and p110β proteins (17). Using the crystal violet test, we demonstrated that cells treated with AdCre exhibited significantly lower viability compared to untreated control cells (Figure 2D). The results indicated that PTEN knock-down did not reverse the growth abnormalities caused by the knockdown of ubiquitously expressed class IA PI3K isoforms. Cells continue to need class IA PI3Ks for proliferation even in the absence of PTEN.

To establish if PI3K isoform predominance was governed by multiple genetic components, we overexpressed p110β under the conditions of PTEN repression. We transfected p110β-wt in shPTEN-MEFs and tested proliferation in the presence of isoform-specific inhibitors. Combining PTEN knock-down with p110β overexpression reduces p110α reliance even further. Despite this, these cells did not exhibit a greater degree of dependence on p110β at intermediate doses of the inhibitor (Figure 2E).

When greater amounts of BYL719 (2 μM) and KIN193 (5 μM) were administered, the overexpression of p110β resulted in a partial resistance to the treatment of p110α inhibitor (BYL). In addition, the overexpression of p110β-wt significantly enhanced the susceptibility of MEFs to the p110β inhibition (KIN) (Figure 2F). Furthermore, we noticed a tendency towards resistance in the GDC0941 treatment group, though it lacked statistical significance. Based on literature, GDC0941 demonstrates only

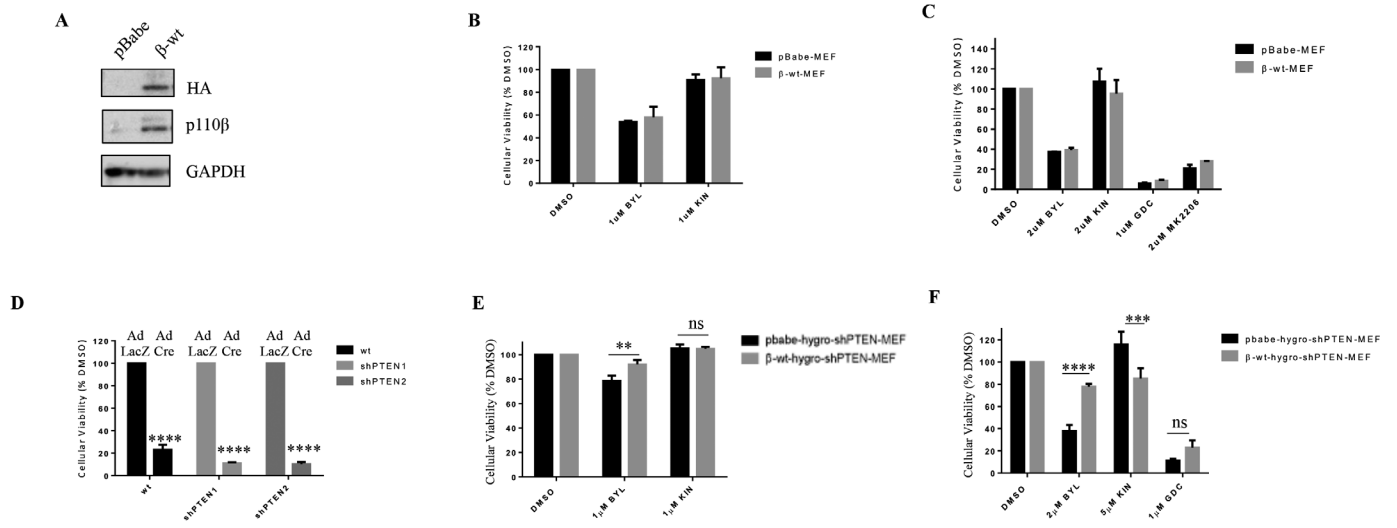

Figure 2. PTEN loss in combination with an increase in PIK3CB expression promote an elevated dependence on p110β. (A) Overexpression of p110β is achieved through retroviral expression of PIK3CB in MEFs. (B) Isoform specific PI3K inhbitors were applied to wt or p110β overexpressing MEFs, crystal violet assays were performed. (C) An increased amount of isoform specific PI3K inhbitors as well as pan-PI3K (GDC) and pan-AKT (MK2206) inhibitors were applied to wt or p110β overexpressing MEFs, crystal violet assays were performed. (D) The shPTEN1 and 2 MEFs were subjected to two rounds of AdCre treatments, and their proliferation rates were assessed using the crystal violet test. As a negative control for PI3K knockdown, LacZ expressing adenoviruses were used. The spectrometric quantification of crystal violet staining data was performed for MEFs treated with adenovirus. (E) The cellular viability of p110β overexpressing shPTEN-MEFs treated with BYL719 and KIN193 was measured. (F) Crystal violet assays for shPTEN MEFs treated with higher concentrations of BYL719 and KIN193 were quantified. **p<0.01, ***p<0.005, ****p<0.0001, ns: Not significant, p>0.05) (the error bars in the graphs represent the standard deviation, and the experiments were conducted independently three times)

MEFs: Mouse embryonic fibroblasts, PTEN: Phosphatase tensin homolog

moderate effectiveness against the p110β and γ isoforms in comparison to the p110α and δ isoforms (22). As GDC0941 is less effective at stopping p110β in p110β overexpressing MEFs, the cells in turn might have changed their dependence on p110β, enabling them to proliferate in the face of p110α blockade.

These findings indicate that increasing the expression of p110β only, is not enough to make MEFs more reliant on p110β. Similarly, the inhibition of PTEN alone did not demonstrate sufficient effectiveness in making cells more reliant on p110β. However, when p110β is overexpressed and PTEN is abolished, cells become less dependent to p110α and more vulnerable to p110β inhibition. Nevertheless, even when these modifications are combined, they do not appear to cause a full transition from reliance on p110α to p110β. Many parallel signaling pathways might cooperate with PI3K for sustaining cell proliferation. Rac1 is a small G protein that has the ability to control a wide range of processes, such as the activation of transcription factors, cell growth, cell transformation, programmed cell death, and the organism's innate immunity (10).

In order to understand the interaction of PI3K with Rac1, we decided to utilize a constitutively active version of Rac1 (Rac1 V12) as well an inactive mutant (Rac1 N17). These mutations either inhibit or promote GTPase activity of the enzyme regulating its function. We established stable MEF lines which express these Rac1 mutants and have not detected noticeable changes in the expression level of either p110α or p110β isoforms, or in phosphorylation of core PI3K pathway components, Akt and S6 (Figure 3A). As Rac1 is implicated in p110β dependent regulation of cell growth, we wished to understand the PI3K isoform dependency in the presence of these mutant Rac1 versions. Although, KIN treatments lead to similar growth profiles for control (13% inhibition) or Rac1 N17 (9.5% inhibition) expressing MEFs in comparison to DMSO, we saw an increased dependency on p110β upon expression of an activated Rac1 allele (53% growth inhibition) (Figure 3B). Interestingly, p110α prominence in these MEFs did not change upon expression of either Rac1 version. These results suggest that MEFs expressing an activated Rac1 allele become more reliant upon p110β for their growth in comparison to wild type MEFs.

Discussion

The PI3K signaling pathway triggers a sequence of highly controlled biochemical reactions at the plasma membrane that are necessary for the initiation and progression of the cell cycle. There are several variants of PI3Ks in eukaryotic cells. The specific roles of these diverse PI3K isoforms in cell cycle progression, as well as their activation patterns at different phases of the cell cycle, are not fully understood. Furthermore, changes in the PI3K pathway are identified in a diverse array of cancer types. PTEN is a frequently mutated gene that is usually shown to be deleted in multiple kinds of cancers, such as breast, prostate, and ovarian cancer. When PTEN is not present, p110β becomes the predominant isoform among class IA PI3Ks. Nevertheless, the precise mechanism behind the change in isoform dependency in a PTEN-null setting remains unclear. Pan-PI3K inhibitors were employed to treat cancers that are promoted by deregulated PI3K, however, these inhibitors exhibit significant adverse effects (23). Instead, inhibitors specifically targeting the p110β isoform, such as AZD8186 and KIN193, were explored for the treatment of PTEN-null cancer types. Treatments with standalone p110β inhibition led to temporary suppression of the PI3K pathway, followed by the development of resistance to inhibition in the cells. Research has indicated that tumor cells may develop a dependence on other types of PI3K when treated with inhibitors. Along these lines, it was shown that when PTEN-null cells were treated

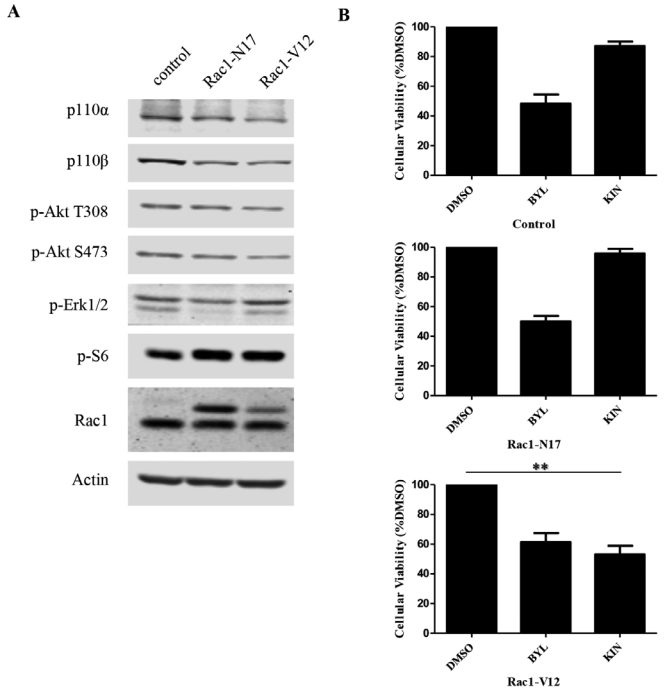

Figure 3. Activated Rac1 expression tilts the balance towards increased p110β prominence. (A) MEFs expressing mutant Rac1 versions were analyzed for expression of the depicted proteins and phosphor-proteins in immunoblots. Actin served as the control for loading. (B) Either empty vector control or mutant Rac1 expressing MEFs were treated with moderate doses of p110α and p110β specific inhibitors (BYL, KIN). Crystal violet assays were performed to determine the cellular proliferative capacity. **p<0.01, (the error bars in the graphs represent the standard deviation, and the experiments were conducted independently three times)

MEFs: Mouse embryonic fibroblasts

with p110β monotherapy, the p110α isoform was activated by IGFR or other RTKs (24). Discovering the molecular connection between PTEN loss and p110β dependency might improve existing treatment options and result in more efficient suppression of potential feedback pathways. The present study used immortalized MEFs, primarily due to their untransformed characteristics, low mutational burden and the presence of wild type PTEN expression. Our research aimed to determine whether cells require p110α and p110β for their cellular proliferation, regardless of the presence or lack of wild type PTEN. Firstly, our findings indicated that even when PTEN was efficiently suppressed, MEFs still required the activity of the ubiquitous class IA isoforms for cell growth. Under shPTEN treatment settings, we examined the dependence on the *PIK3CA* and *PIK3CB* genes. While PTEN suppression did not result in a shift in single isoform dependency in MEFs, there was a reduction in the effectiveness of p110α.

We overexpressed p110β in MEFs to see if it causes a more potent p110β dependency. However, we observed no shift in isoform reliance from p110α to p110β. Next, we coupled p110β overexpression and PTEN suppression. The combination resulted in a partial tolerance to p110α inhibitors and increased susceptibility to p110β inhibitors. GDC0941, a pan-PI3K inhibitor, became less effective against p110β overexpressed MEFs in the absence of PTEN. MEFs with insufficient PTEN relied more on the p110β isoform due to GDC0941's low selectivity for it. Overexpressing p110β alone did not increase cellular dependence on p110β. Different isoforms of PI3Ks engage with distinct small GTPases to regulate their activity. For example, the Ras protein binds specifically with the p110α isoform, while Rac1 preferentially activates the p110β isoform (11). In order to understand whether Rac1 collaborated with p110β and improved its potency in MEF growth, we exogenously expressed an activated Rac1 allele (V12) in MEFs and monitored their proliferative potential in response to isoform specific PI3K inhibition. Our data indicated that MEFs expressing an activated Rac1 allele became significantly more dependent on p110β. These findings corroborate prior reports describing a signaling node composed of GPCRs, trimeric G-proteins, Rac1 and p110β possibly establishing a feedback loop, enhancing the potency of the p110β generated signal (9,11,12).

Study limitations

We did not create a triple compound molecular genetic model that included all the genetic modifications. To achieve a more complete isoform transition, an activated Rac1 expression may need to be added to the existing combination in the MEFs in future studies.

Conclusion

We observed that alterations in PTEN and p110β expression in untransformed MEFs had a limited impact on the prevalence of PI3K isoforms. The inhibition of PTEN led to an overall activation of the PI3K pathway, but caused a decreased dependence on p110α. Our data suggest that no single genomic alteration would likely determine PI3K isoform dependency. A combination of several genetics elements, such as PTEN status, p110 expression levels and Rac1 activation status, have a profound effect on the relative effectiveness of the individual PI3K isoforms. These factors together shape the context-dependent reliance on p110α and p110β, making it particularly critical in certain pathological conditions, such as cancers with PTEN loss, where it supports key signaling pathways driving tumor growth and survival.

Ethics Committee Approval: *Not applicable.*

Informed Consent: *Not applicable.*

Author Contributions: *Concept: O.Ç.; Design: S.A., O.Ç.; Data Collection or Processing: S.A., O.Ç.; Analysis or Interpretation: S.A., O.Ç.; Literature Search: O.Ç.; Writing: O.Ç.*

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Association of candidate gene (*INSR & THADA*) polymorphism with polycystic ovary syndrome: meta-analysis and statistical power analysis

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Abstract

Polycystic ovary syndrome (PCOS) is a common endocrine and metabolic disorder that impacts women before reaching menopause. In addition to notable features (irregular ovulation, elevated androgen levels, and the existence of numerous ovarian cysts), individuals with PCOS frequently encounter diverse metabolic, cardiovascular, and psychological conditions. The onset of PCOS is influenced by a combination of factors, and various genetic variations are believed to play a significant role in its progression. The objective of the current study was to explore the link between genetic variations in the candidate genes *thyroid-adenoma-associated (THADA)* gene and insulin receptor (*INSR*) and susceptibility to developing PCOS. We conducted an extensive search across various databases, including Google Scholar, PubMed, Science Direct, Scopus, and EMBASE, to compile relevant case-control studies and literature reviews for subsequent statistical analysis. In the present study, the Preferred Reporting Items for Systematic Reviews and Meta-Analyses checklist was followed, a guideline for Systematic Reviews and Meta-Analysis. While a previous meta-analysis explored the correlation between *INSR* rs1799817 and *THADA* rs13429458 and their association with susceptibility to PCOS, our current study did not integrate any findings from these prior investigations. Our research encompassed articles published between 2017 and 2023, and we employed MetaGenyo software to assess the collected data. Statistical power analysis was performed using G*Power 3.1 software. Odds ratios and their corresponding 95% confidence intervals were calculated for each genetic model. Fifteen studies that met the criteria were analyzed. Out of these, ten studies, involving 1,189 cases and 1,005 controls, examined the *INSR* rs1799817 gene polymorphism, while five studies, including 783 cases and 553 controls, investigated the *THADA* rs13429458 gene polymorphism. The meta-analysis results indicated that there was no statistically significant association between the *INSR* rs1799817 gene polymorphism and the risk of PCOS ($p>0.05$). In contrast, the *THADA* rs13429458 gene polymorphism showed a significant association with PCOS risk under the over-dominant model ($p<0.05$). The present meta-analysis demonstrated a notable association between the *THADA* rs13429458 gene polymorphism and the likelihood of developing PCOS. Further rigorous studies with expanded sample sizes and diverse ethnic representation will be important to comprehensively evaluate and validate these findings. (J Turk Ger Gynecol Assoc. 2024; 25: 167-78)

Keywords: PCOS, *INSR*, *THADA*, gene polymorphism, meta-analysis, hyperandrogenism

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Introduction

Polycystic ovary syndrome (PCOS) is a common hormonal disorder that primarily impacts individuals with ovaries and tends to manifest frequently during their reproductive years. PCOS is a multifaceted condition characterized by a variety of symptoms. Irregular menstrual cycles, or in some cases, the absence of menstruation, are common indicators (1). PCOS was initially identified in women by Stein and Leventhal in 1935. It stands as a primary cause of hyperandrogenism and oligoovulation during the reproductive years, often being a significant factor contributing to infertility (2). PCOS is a prevalent issue, impacting approximately 8-21% of women in

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their reproductive years on a global scale. While it can develop post-menarche, most cases are identified between the ages of 20 and 30 years. Worldwide, about 1.55 million women of reproductive age are affected by PCOS, resulting in 0.43 million people living for years with the associated disability (3). While the exact etiology of PCOS remains unclear, a number of different factors are thought to be involved. Primarily, hormonal imbalances, in particular elevated luteinizing hormone (LH) levels and normal or suppressed follicle-stimulating hormone (FSH) levels, resulting in an altered LH/FSH ratio are characteristic. In addition, the clinical signs of hyperandrogenism are linked with hyperinsulinemia and insulin resistance. While the specific predisposing factors for the progress of PCOS are uncertain, there are observations indicating a genetic basis in some cases, and obesity has been identified as a contributing factor due to its association with hyperinsulinemia, potentially increasing the risk of developing PCOS (4). Variations in genetic single nucleotide polymorphisms (SNPs) and single nucleotide variants play a role in affecting steroidogenesis, the activity of ovarian theca cells, and the secretion of hormones from the hypothalamus and pituitary gland (5). Epigenetic elements, like exposure within the womb and increased androgen levels in a mother's environment, may lead to lasting, inheritable traits that contribute to PCOS. This condition, marked by hyperandrogenism, irregular steroid production, insulin resistance, and central obesity, stems from a dysfunctional connection between the hypothalamus, pituitary gland, and ovaries (6). Excessive androgen secretion by ovarian theca cells, triggered by the growth of fatty tissue, leads to the development of numerous small follicles and an imbalance in sex hormones, potentially causing endometrial carcinoma (7). Chronic oxidative stress and inflammatory markers harm both oocyte quality and endothelial function, indicating infertility. Timely screening and diagnosis are vital for preventing PCOS and managing metabolic irregularities. Prioritizing physical and mental well-being, embracing a healthy lifestyle, and fostering a favorable environment all play pivotal roles in mitigating the challenges posed by PCOS (8). According to the latest research, compelling evidence suggests that genetic factors significantly contribute to the development of PCOS. Although various studies have examined gene variants across different biological pathways, the influence of PCOS inheritance patterns on its pathophysiology remains uncertain (9). Candidate genes associated with steroid hormone biosynthesis and metabolism, the action of gonadotropins and gonadal hormones, as well as those related to obesity and energy regulation, insulin secretion, and action, have been investigated and implicated in the development of PCOS (10). Global findings indicate that allelic variants or SNPs within genes related to ovarian steroidogenesis, folliculogenesis, and insulin-regulated glycemic control could potentially disrupt homeostatic signaling mechanisms, ultimately contributing to the development of PCOS (11). It is important to highlight that the pathophysiological pathways in different phenotypes of PCOS may vary, impacted by both genetic and environmental factors. Multiple interlinking aspects could impact the expression of PCOS, making it highly unlikely that a singular cause can be identified for this condition (12). Therefore, it is essential to examine numerous candidate genes linked to PCOS to identify its precise genetic foundation.

Seven Genome-Wide Association Studies have endeavored to establish connections in diverse populations between specific SNPs within candidate genes and PCOS (13-19). The thyroid adenoma-associated protein (THADA), produced by the *THADA* gene, is located on chromosomal band 2p21. This protein is expressed in various tissues, including the pancreas, thyroid, testes, thymus, adrenal gland, small intestine, and stomach (20). The *THADA* gene has been linked to disruptions in energy metabolism, leading to a decrease in energy production and an elevated risk of obesity. This increased susceptibility to obesity, in turn, enhances the likelihood of developing PCOS (21). Notably, *THADA* gene variations impact beta cell function and insulin secretion, potentially influencing insulin resistance in PCOS and diabetes. Endocrinologists are interested in these findings for their relevance to understanding and addressing these conditions (22). Insulin resistance is a key dysfunction associated with PCOS, mainly linked to the *insulin receptor (INSR)* gene located on chromosome 19. Several investigations have additionally indicated that women diagnosed with PCOS face an elevated risk of experiencing gestational diabetes, miscarriages, preeclampsia, and preterm labour (23). The insulin gene is believed to play a crucial role in both insulin secretion and action, as well as in signaling pathways. Any variations in the *INSR* gene can potentially alter INSR function, increasing the susceptibility to developing PCOS (24).

Examining polymorphisms in candidate genes related to the likelihood of developing PCOS offers an insight into the complex interaction between genetic susceptibility and disease development. This method enables researchers and healthcare professionals to explore the underlying genetic predisposition and potential molecular mechanisms influencing PCOS pathogenesis, leading to a more nuanced understanding of the condition. Hence, we undertook a meta-analysis using suitable case-control studies to examine the relationship between the gene polymorphisms of *INSR* and *THADA* and the likelihood of developing PCOS.

Subjects, materials, and methods

During the research, we adhered to the Preferred Reporting Item Guideline for Systematic Reviews and Meta-Analysis

checklist. This meta-analysis was conducted following the guidelines depicted in Figure 1a, b. Additionally, the International Prospective Register of Systematic Reviews (PROSPERO) validated the study's reliability by confirming the registration of its prospective review protocol (PROSPERO ID 502139).

Data source

A comprehensive literature search was carried out across various databases, including Embase, NCBI, Google Scholar, Elsevier, Science Direct, and PubMed from 2017 to 2023, using the following keywords "*PCOS*", "*INSR*", "*THADA*", "gene polymorphism", "case vs. control", "*SNPs*", and "*INSR* gene". The words of Boolean logic, such as OR/AND, were combined with the employed keywords. The database has been updated and eliminating duplicates was performed through screening reviews, recent studies, and past meta-analyses. Furthermore, the reference lists of the identified articles were also screened.

Study selection

Inclusion criteria

This meta-analysis considered the studies that fulfilled the following inclusion criteria: (i) the study had to investigate *INSR* and *THADA* gene polymorphism with PCOS risk; (ii) the research only involved human subjects; (iii) the distribution of allele and genotype information in a case-control study was presented; and (iv) the full text was available in the English language.

Exclusion criteria

Studies were excluded that did not meet the following criteria: (i) studies not investigating *INSR* and *THADA* gene polymorphism and PCOS; (ii) reviews and previous meta-analysis related to PCOS risk; (iii) case reports, animal studies that overlap with other research; and (iv) studies with duplicate data.

Data extraction

Based on the inclusion criteria, we collected the following information in a consistent format, and any discrepancies were reviewed with co-authors until a conclusion could be reached. To learn more about the allelic frequencies and genotypes of the case and control individuals, the retrieved publications were appropriately read. In some cases, not all the genotypic information was shown in the studies. In these situations, the data was calculated using other information, such as allelic frequencies. Research studies where the necessary data was unable to be extracted from the control and case groups were rejected. First author name, PubMed ID, Hardy-Weinberg Equilibrium (HWE) score, year of publication, language, sample size, ethnicity, study

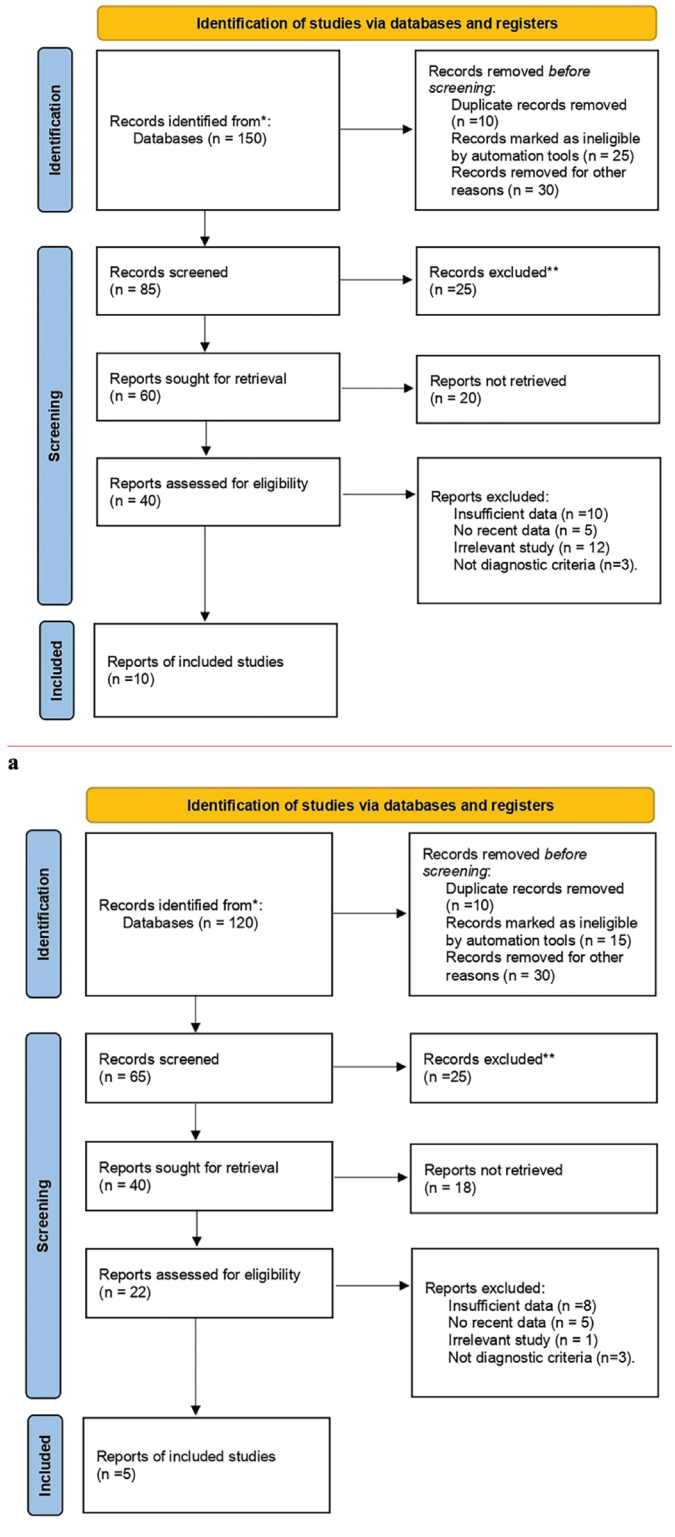


Figure 1. (a) Flow chart for literature screening of *INSR* rs1799817 gene polymorphism. (b) Flow chart for literature screening of *THADA* rs13429458 gene polymorphism
INSR: Insulin receptor, THADA: Thyroid-adenoma-associated gene

design, and other information were taken from each study. To improve screening flexibility, a table for data extraction was created and tested twice. Consistency was maintained throughout the eligibility criteria and data screening process.

Quality of study using risk bias

A thorough risk bias assessment is essential to correctly assessing the methodological quality and possible biases of the studies included in a meta-analysis. In the present study, the risk of bias was assessed using the Cochrane risk of bias tool (ROB2) software. The studies were divided into three categories based on their level of bias: "high risk," "some concern," or "low risk."

Statistical analysis

The relationship between *INSR* and *THADA* gene polymorphisms and PCOS susceptibility was examined using a range of statistical methods. The relationship was estimated by calculating odds ratios (ORs) along with their corresponding 95% confidence intervals (CIs). The statistical significance was determined with a threshold set at $p<0.05$. An index of inconsistency (I^2) was employed to assess how consistent the results were across various research efforts in order to measure their uniformity. The I^2 score indicates the extent of variability or diversity among studies, ranging from 0% to 100%. A low I^2 number indicates consistency in results across investigations, while a high value signifies greater diversity or variability. Due to a heterogeneity value below 50%, the research opted for a fixed effect model. If the random effect model with a probability of exceeding 50% was used. The Q statistics were used to conduct a chi-square test to determine the presence of heterogeneity. The test results indicated a statistically significant diversity between the two studies. Summary ORs were evaluated using a Z-test ($p<0.05$), and heterogeneity among studies was assessed using the Q statistic and I^2. Moreover, a sensitivity analysis was carried out to examine the influence of omitting certain studies, especially those where the controls did not adhere to HWE. Furthermore, Egger's regression method was used to evaluate the potential presence of publication bias. All the statistical analyses were conducted using the MetaGenyo software.

Power analysis

The acquired metadata was analyzed through a power assessment under conditions with a 95% CI (0.05 α error). The power for each study sample size (both case and control groups) was combined and examined separately for each chosen gene. The calculation of power was performed using G*Power 3.1 software.

Protein-protein interactions

The STRING (v11.0) online search tool database can predict functional proteins and protein-protein interactions (PPIs) with a score of ≥0.4 for identified PCOS-linked polymorphisms.

Results

Search results

This study sought to investigate the relationship between the gene polymorphisms *INSR* rs1799817 and *THADA* rs13429458 with PCOS risk. A comprehensive search across databases yielded 10 studies comprising 1,189 cases and 1,005 controls for the *INSR* rs1799817 polymorphism, and five studies with 783 cases and 553 controls for *THADA* rs13429458 polymorphism. Table 1 presents key characteristics extracted from the included case-control studies (25-38). Among the 15 selected studies, 14 were conducted within the Asian population, while the remaining study focused on a European population.

Risk bias

ROB2 was used to conduct a thorough assessment of the methodological quality of the included studies, as illustrated in Figure 2a, b. Each row in the chart corresponds to an individual study, while each column indicates a distinct bias category. The color scheme in the image indicates the reviewer's evaluation of the risk level linked to each bias type in each study - red denotes high risk, yellow signifies moderate risk, and green indicates low risk. The majority of the included studies displayed a minimal risk of bias. These findings suggest that most of the studies were executed and documented in ways that effectively minimized the likelihood of bias or systematic errors.

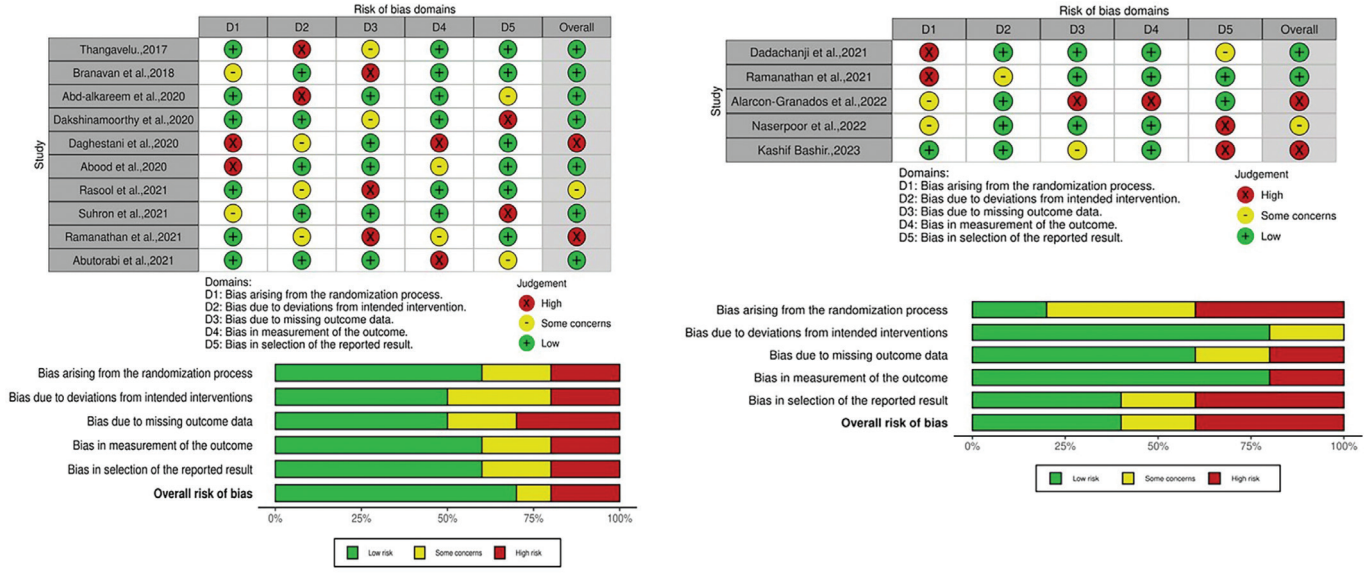
Quantitative data analysis of *INSR* and *THADA* Gene polymorphism with PCOS

Based on the genotypes analyzed in the present meta-analysis, two gene polymorphisms of *INSR* rs1799817 and *THADA* rs13429458 were specifically selected. The investigation into their association with PCOS was conducted using multiple comparison models, taking into consideration the HWE. The *INSR* rs1799817 gene polymorphism showed no significant association in allelic, recessive, dominant, and over-dominant models ($p>0.05$; Table 2). In addition, the subgroup analyses with allelic, recessive, dominant, and over-dominant models were non-significant ($p>0.05$). However, *THADA* rs13429458 gene polymorphism showed significant association with the over-dominant model ($p<0.05$), whereas allelic, recessive, and dominant models showed non-significance ($p>0.05$; Table 3). The results of the subgroup analysis indicated a noteworthy association with the over-dominant model, while other genetic models showed non-significant associations.

Table 1. Characteristics of selected case-control studies for association of *INSR* and *THADA* gene polymorphism with PCOS

SNPs	Study	Country	Ethnicity	Genotype frequency of cases			Genotype frequency of controls			Total cases	Total controls	HWE-p-value
INSR				CC	CT	TT	CC	CT	TT			
rs1799817	Thangavelu et al. (34)	India	Asian	19	76	74	22	67	80	169	169	0.1881
	Branavan et al. (29)	Sri Lanka	Asian	48	1	6	95	1	14	55	110	0
	Abd-alkareem and Omear (25)	Iraq	Asian	8	49	30	2	14	14	87	30	0.5428
	Dakshinamoorthy et al. (26)	India	Asian	81	151	21	30	186	92	253	308	0
	Daghestani (32)	Saudi Arabia	Asian	64	47	15	87	21	10	126	118	0
	Abood et al. (28)	Iraq	Asian	9	20	21	11	36	3	50	50	0.0007
	Rasool et al. (27)	India	Asian	156	74	19	67	21	12	249	100	0.0001
	Suhron and Zainiyah (30)	Indonesia	Asian	12	30	8	10	28	12	50	50	0.3891
	Ramanathan et al. (31)	India	Asian	9	3	8	2	2	6	20	10	0.0976
	Seyed Abutorabi et al. (33)	Iran	Asian	73	56	1	57	2	1	130	60	0.0002
THADA				AA	AC	CC	AA	AC	CC			
rs13429458	Dadachanji et al. (36)	India	Asian	236	101	11	98	49	3	348	150	0.2655
	Ramanathan et al. (31)	Asian	Asian	16	0	4	9	0	1	20	10	0.0016
	Alarcón-Granados et al. (35)	Colombia	European	37	12	0	41	6	2	49	49	0.0202
	Naserpoor et al. (37)	Iran	Asian	32	26	8	26	12	6	66	44	0.0382
	Bashir et al. (38)	Pakistan	Asian	136	54	110	106	100	94	300	300	0

THADA: Thyroid-adenoma-associated gene, INSR: Insulin receptor, PCOS: Polycystic ovary syndrome, SNP: Single nucleotide polymorphisms, HWE: Hardy-Weinberg Equilibrium



a b

Figure 2. (a) Risk of bias summary for *INSR* rs1799817 gene polymorphism. (b) Risk of bias summary for *THADA* rs13429458 gene polymorphism
INSR: Insulin receptor, THADA: Thyroid-adenoma-associated gene

Publication bias and sensitivity analysis

Each variable was carefully examined to detect any potential publication bias caused by constraints in sample size and reporting bias. A forest plot showed heterogeneity (Figures 3-6). A sensitivity analysis was conducted, revealing that excluding individual studies did not significantly alter the

Table 2. Summary estimates for odd ratios and 95% confidence interval in different ethnicity for INSR rs1799817 polymorphism

Model	Study	Number of studies	Test of association			Test of heterogeneity			Publication bias
			OR	95% CI	p-value	Model	p-value	I^2	p-value (Egger's test)
Allele contrast (A vs. a)	Overall	10	0.9231	(0.5775; 1.4755)	0.738194101	Random	0	0.8942	0.1151
Recessive model (AA vs. Aa + aa)	Overall	10	0.8931	(0.4390; 1.8168)	0.755038939	Random	0	0.8795	0.5237
Dominant model (AA + Aa vs. aa)	Overall	10	1.2726	(0.7009; 2.3108)	0.428294474	Random	0	0.7914	0.3975
Overdominant (Aa vs. AA + aa)	Overall	10	1.3952	(0.8632; 2.2552)	0.17400012	Random	0	0.7677	0.5221
OR: Odds ratio, CI: Confidence interval, INSR: Insulin receptor									

Table 3. Summary estimates for odd ratios and 95% confidence interval in different ethnicity for THADA rs13429458 polymorphism

Model	Study	Number of studies	Test of association			Test of heterogeneity			Publication bias
			OR	95% CI	p-value	Model	p-value	I^2	p-value (Egger's test)
Allele contrast (A vs. a)	Overall	5	1.0321	(0.8648; 1.2317)	0.725995	Fixed	0.6913	0	0.0083
Recessive model (AA vs. Aa + aa)	Overall	5	1.1900	(0.9417; 1.5037)	0.145096	Fixed	0.1349	0.4301	0.0684
Dominant model (AA + Aa vs. aa)	Overall	5	0.8072	(0.5919; 1.1008)	0.176014	Fixed	0.6975	0	0.5667
Overdominant (Aa vs. AA + aa)	Overall	5	0.7106	(0.5495; 0.9188)	0.009175	Fixed	0.0011	0.8122	0.3585
OR: Odds ratio, CI: Confidence interval, THADA: Thyroid-adenoma-associated gene									

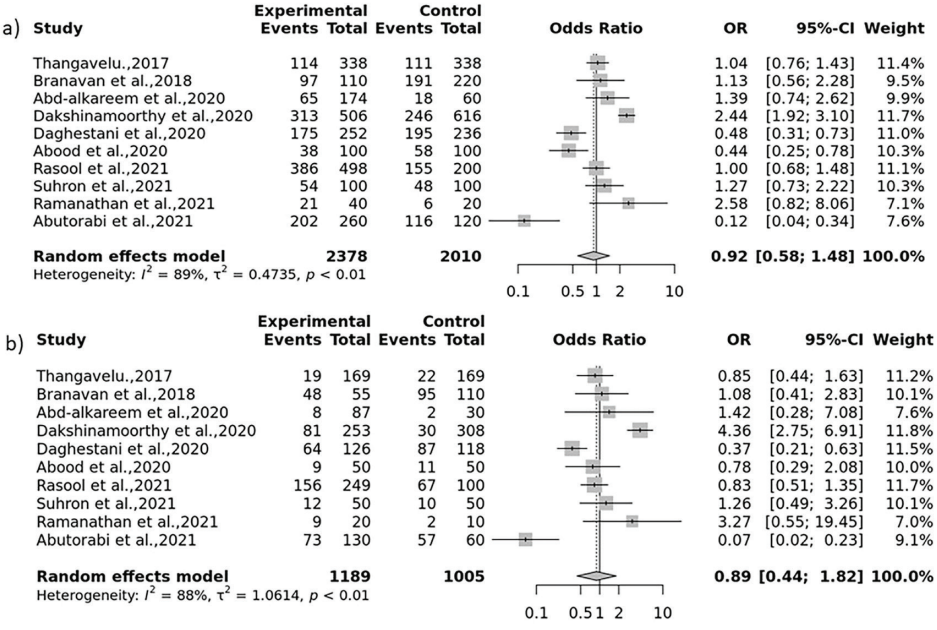


Figure 3. Forest plot for the association of *INSR* rs1799817 gene polymorphism with PCOS risk. (a) Allelic model and (b) Recessive model

INSR: Insulin receptor, PCOS: Polycystic ovary syndrome, OR: Odds ratio, CI: Confidence interval

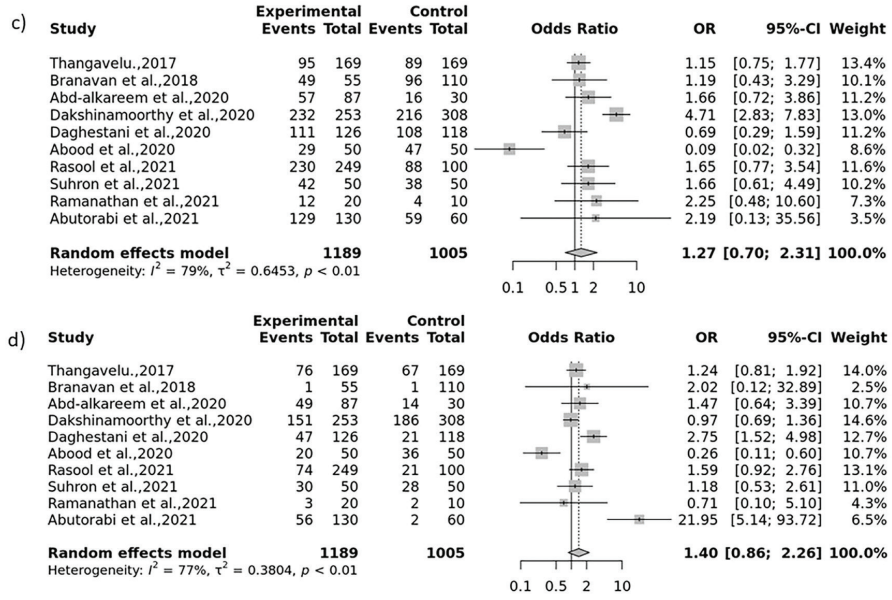


Figure 4. Forest plot for the association of *INSR* rs1799817 gene polymorphism with PCOS risk. (c) Dominant model and (d) Over-dominant model

INSR: Insulin receptor, PCOS: Polycystic ovary syndrome, OR: Odds ratio, CI: Confidence interval

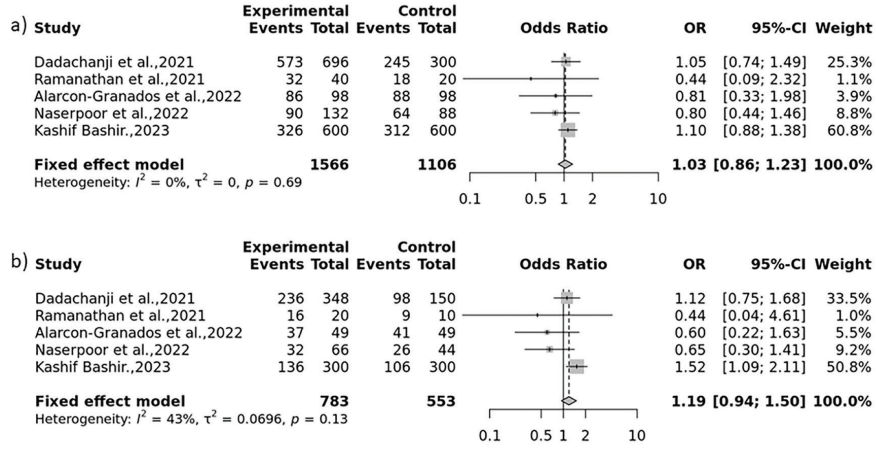


Figure 5. Forest plot for the association of *THADA* rs13429458 gene polymorphism with PCOS risk. (a) Allelic model and (b) Recessive model

THADA: Thyroid-adenoma-associated gene, PCOS: Polycystic ovary syndrome, OR: Odds ratio, CI: Confidence interval

overall outcome. This suggests that our findings are statistically robust and not significantly influenced by the exclusion of any single study (Figure 7). A funnel plot was used to evaluate the possible existence of publication bias. However, no obvious indications of bias were found (Figure 8).

Power analysis, circos plot, and construction of PPI network

We conducted a power analysis to determine the significance level of each study's selected SNPs. According to our findings, the sample size in the selected literature met the significant level requirement, encompassing an α error probability of 1e-007. The findings of the power analysis can be found in Table 4. The Circos plot, arranged from outer to inner, represents annotated genes, chromatin states, transcription factors, and histone modifications, each linked with specific SNPs based on their pairwise linkage disequilibrium (r2) (Figure 9). A PPI network analysis was performed on polymorphic proteins related to *INSR* and *THADA* using the STRING database. The network includes 21 nodes and 123 edges for both *INSR* and *THADA* genes. Although there is no direct interaction, the *INSR* and *THADA* genes are linked through intermediary genes (Figure 10).

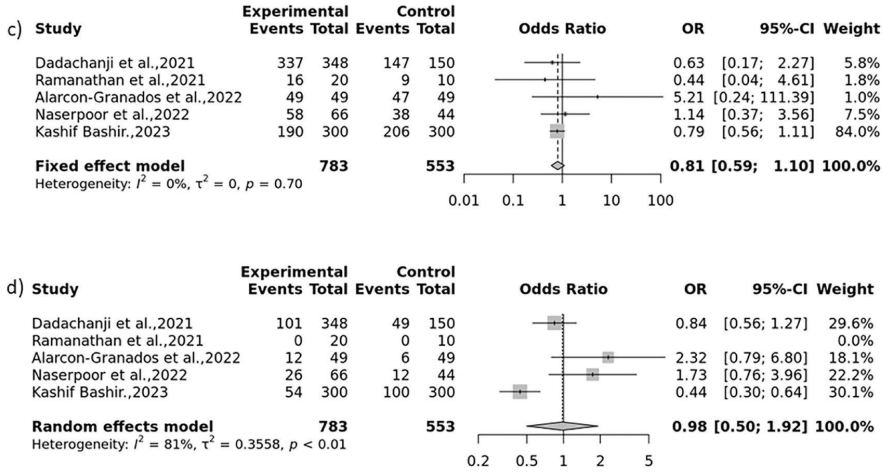


Figure 6. Forest plot for the association of *THADA* rs13429458 gene polymorphism with PCOS risk (c) Dominant model and (d) Over-dominant model

THADA: Thyroid-adenoma-associated gene, PCOS: Polycystic ovary syndrome, OR: Odds ratio, CI: Confidence interval

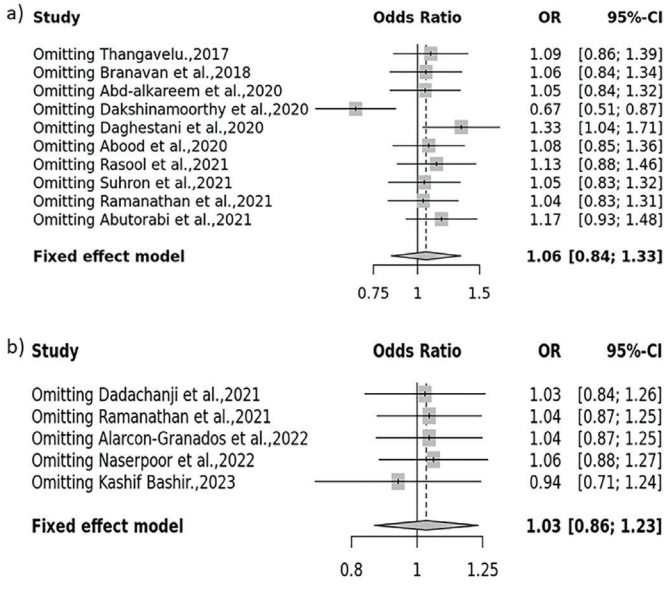


Figure 7. The forest plot representing sensitive analysis between the selected gene polymorphisms and PCOS susceptibility. (a) *INSR* rs1799817 gene polymorphism with the susceptibility of PCOS. (b) *THADA* rs13429458 gene polymorphism with the susceptibility of PCOS

INSR: Insulin receptor, THADA: Thyroid-adenoma-associated gene, PCOS: Polycystic ovary syndrome, OR: Odds ratio, CI: Confidence interval

Discussion

PCOS is a complicated endocrine disorder with multifactorial origins, impacting a significant proportion of reproductive-aged women globally. Its implications extend beyond reproductive ages, persisting throughout a person's lifetime and may often be associated with metabolic disorders such as type 2 diabetes mellitus (DM), obesity, and dyslipidemia. This underscores the substantial impact of PCOS on population health (39). The intricate mechanisms involved in PCOS encompass genetic factors regulating various aspects of steroidogenesis, steroid hormone function, gonadotrophin action, insulin activity, persistent inflammation, and energy metabolism (Figure 11) (40). Candidate genes, *INSR* and *THADA*, have been implicated in PCOS. *INSR* is linked to insulin resistance, a prevalent feature in PCOS contributing to elevated insulin levels and increased risk of metabolic disorders. *THADA*, initially associated with thyroid adenomas, may play a role in PCOS, although the specific mechanisms remain unclear. PCOS is a condition influenced by both genetic and environmental factors, and ongoing research aims to unravel the specific genetic contributions of various genes in its development. According to some study findings, there is no substantial correlation between *PCOS* and *THADA* gene polymorphism (31-36). However, earlier research indicated a notable association between candidate gene polymorphism and PCOS (37,38). A previous meta-analysis investigated the correlation between *INSR* and *THADA* gene polymorphisms and susceptibility to PCOS (41,42). However, the current study did not integrate findings from earlier research. Conducting updated meta-analyses is crucial for maintaining the relevance and accuracy of scientific knowledge. By incorporating the latest research findings, these analyses ensure that conclusions are based on the most current and robust evidence. Our meta-analysis, integrating information from 1,189 cases and 1,005 controls for the *INSR* rs1799817 gene polymorphism, and 783 cases and 553 controls for the *THADA* rs13429458 gene polymorphism, provides valuable insights into their correlation with PCOS. The results indicate that *INSR* rs1799817 gene polymorphism was

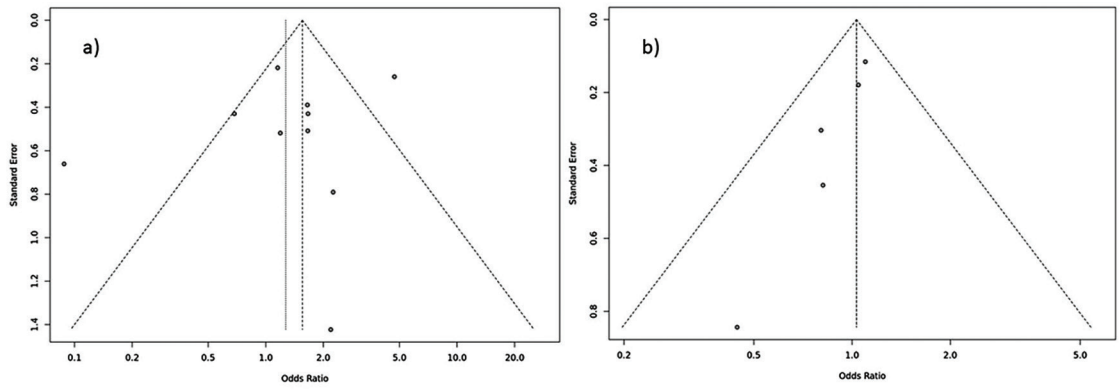


Figure 8. Publication bias was analyzed by funnel plot on the selected gene polymorphisms with the susceptibility of PCOS. (a) *INSR* rs1799817 gene polymorphism with the susceptibility of PCOS. (b) *THADA* rs13429458 gene polymorphism with the susceptibility of PCOS.
INSR: Insulin receptor, THADA: Thyroid-adenoma-associated gene, PCOS: Polycystic ovary syndrome

Table 4. Selecting an appropriate sample size is crucial for reliable findings and accurate statistical evaluation in genetic association studies, particularly when investigating specific polymorphisms. Estimating sample size is critical for determining statistical power

Gene	SNP	No. of studies	Cases	Control	α err prob	Power (1-β err prob)
INSR	rs1799817	10	1189	1005	0.05	0.9500036
THADA	rs13429458	5	783	553	0.05	0.9502123
INSR: Insulin receptor, THADA: Thyroid-adenoma-associated gene, SNP: Single nucleotide polymorphisms						

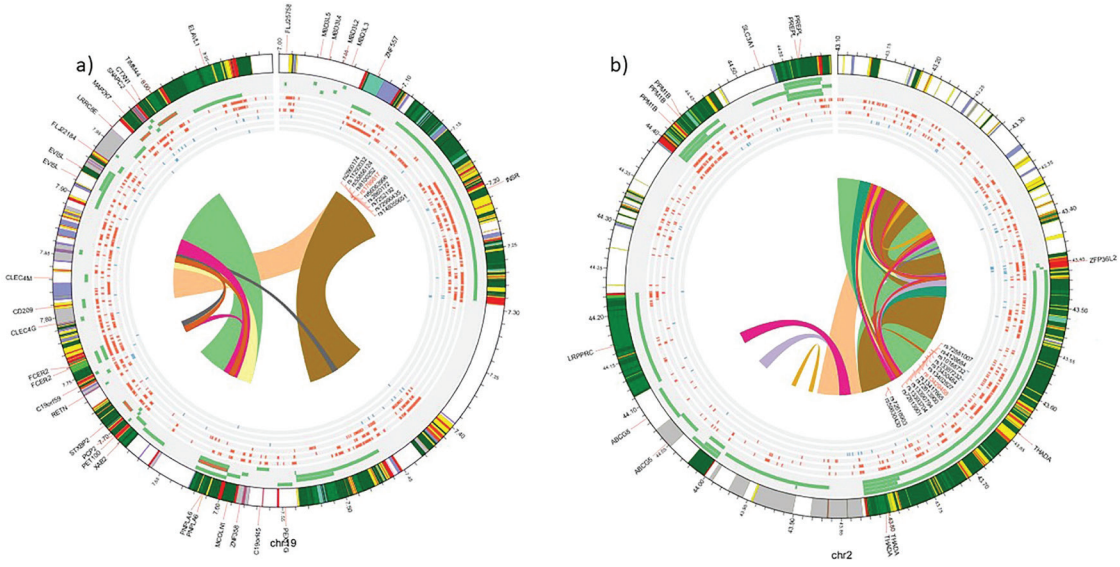


Figure 9. Circos plot that visually represents the chromosomal relationships among the selected SNPs, with a focus on (a) rs1799817 and (b) rs13429458
SNP: Single nucleotide polymorphisms

not associated with PCOS across allelic, recessive, dominant, and over-dominant genetic models, suggesting it may not contribute to PCOS risk. Conversely, *THADA* rs13429458 gene polymorphism significantly correlated with PCOS risk in the over-dominant model but not in other genetic models (allelic, recessive, and dominant), implying its potential contribution to PCOS risk. A sensitivity analysis demonstrates that no single study significantly influences the overall outcomes. Our study concludes that both *INSR* and *THADA* gene polymorphisms adhere to the principles of HWE. To assess publication bias,

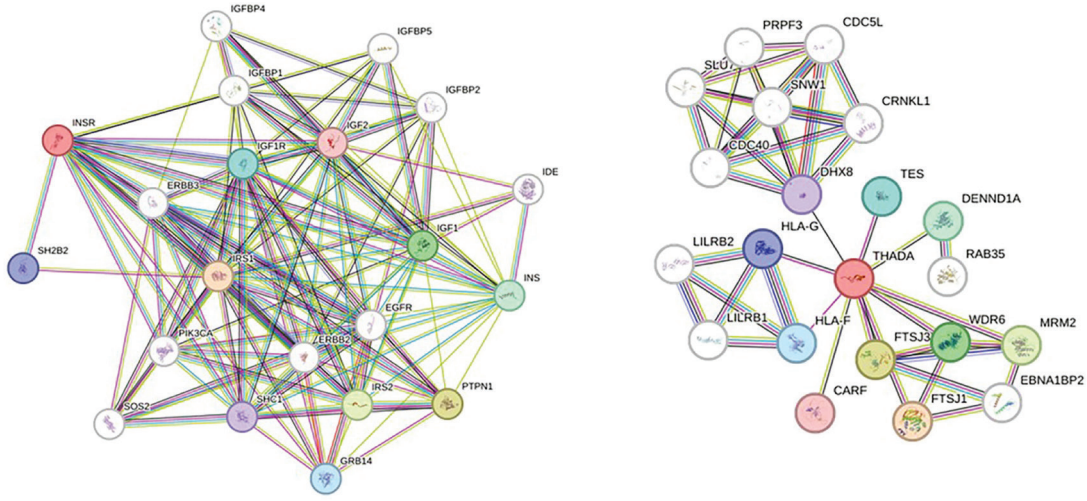


Figure 10. The protein-protein interaction network of differentially expressed genes among the selected genes associated with PCOS
PCOS: Polycystic ovary syndrome

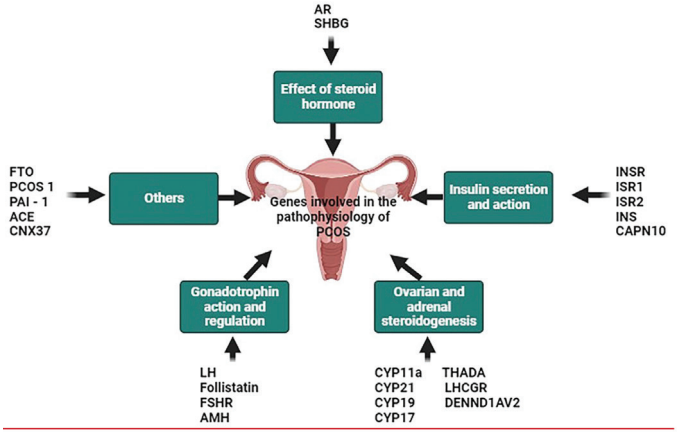


Figure 11. The schematic diagram represents the genes involved in the pathophysiology of PCOS
PCOS: Polycystic ovary syndrome, INSR: Insulin receptor, THADA: Thyroid-adenoma-associated gene, LH: Luteinizing hormone

funnel plots, and Egger's test were employed, revealing no indications of bias. Methodological quality, evaluated using the ROB2 tool, indicated lower risk levels across various aspects of research design in each included study, supporting the reliability of the results. Therefore, our findings are robustly supported by statistical evidence. Rigorous data extraction and analysis procedures were employed, and a power analysis confirmed that the sample size in the selected studies meets the required significance level. According to Albahlol et al. (43) a potential correlation between genetic variations in the *VDR* gene and increased susceptibility to PCOS in Egyptian women was found. Fathy et al. (44) suggested the presence of polymorphism rs6495096 in the *CYP11A1* gene could elevate the susceptibility to PCOS among Iranian women. Alsobaie et al. (45) confirmed that rs8192675 *SLC2A2* is linked to the occurrence of PCOS in women, particularly showing a robust association with those developed type 2 DM in Saudi Arabia. Subbaraj and Sindhu (46) found that *FSHR* (rs6166) was not associated with PCOS. Conversely, they observed a positive correlation between *IL10* (rs1800896) and PCOS, while *IRS-1* (rs1801278) variations were linked to an adverse association (46). Goussalya et al. (47) demonstrated that *IL-6* and *IRS* gene polymorphisms were associated with PCOS. Analyzing gene polymorphisms in PCOS is crucial for understanding its genetic basis, improving diagnostic accuracy, and developing personalized treatments. Genetic variations help identify individuals at risk, allowing for proactive interventions and tailored therapies. Additionally, this research enhances our understanding of PCOS at the molecular level, contributing to advancements in drug development and treatment strategies.

Study limitations

The present study possesses certain limitations. We did not investigate the potential impact of gene-environment interactions and other demographic factors. Subgroup investigation was not carried out due to a lack of sufficient studies, with the majority of the included studies concentrating on Asian populations. It is recommended to conduct further research involving diverse populations to enhance the applicability of the findings.

Conclusion

In summary, the present meta-analysis examined the possible association among polymorphisms in candidate genes, *INSR* and *THADA* and the risk of PCOS by analyzing data from both significant and non-significant studies. The overall findings suggest that there may not be an association between the *INSR* rs1799817 polymorphism and PCOS. Conversely, the *THADA* rs13429458 gene polymorphism seems to be associated with a risk of PCOS. Further research is required with larger sample sizes, environmental datasets, and diverse ethnic populations to validate and support these findings. Regularly updating meta-analyses is essential to incorporate the latest research findings, ensuring that conclusions are based on the most current and robust evidence. This practice enhances the accuracy of results, identifies evolving trends, and guides decision-making with up-to-date insights, contributing to the ongoing improvement of scientific understanding in specific fields.

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Intraoperative laparoscopic ultrasound during laparoscopic myomectomy: a narrative review

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Abstract

Intraoperative laparoscopic ultrasound (IOLUS), a dynamic imaging technique, has emerged as a valuable instrument for guiding surgery in various medical specialties. As IOLUS provides accuracy, improved visualization, and real-time guidance, the integration of IOLUS into many surgical procedures has occurred and IOLUS assists surgeons during advanced procedures. Today, laparoscopic myomectomy has become a prominent surgical procedure in gynecology. Despite its benefits, laparoscopic myomectomy presents certain challenges. The risk of residual fibroids is higher in laparoscopic myomectomy compared to abdominal surgery. The limited depth perception and restricted range of motion can also be obstacles for surgeons, especially when dealing with deeply embedded fibroids. IOLUS has the potential to overcome these limitations. In this study, our aim was to conduct a review of the literature concerning the use of IOLUS during laparoscopic myomectomy. (J Turk Ger Gynecol Assoc. 2024; 25: 179-83)

Keywords: Intraoperative laparoscopic ultrasound, laparoscopic myomectomy, minimally invasive surgery

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Introduction

Laparoscopic myomectomy has gained prominence as a surgical procedure in the field of gynecology. In contrast to traditional open surgery, laparoscopic myomectomy presents several advantages, such as minimized scarring, shorter hospital stays, and a faster postoperative recovery (1). However, despite its benefits, laparoscopic myomectomy does present certain challenges. Laparoscopic myomectomy is considered an advanced laparoscopic surgery, especially in cases with many fibroids. The challenges include the need to find multiple myomas, potential use of many laparoscopic sutures, longer surgery time, and a higher risk of bleeding. These factors collectively categorize laparoscopic myomectomy as a complex laparoscopic surgical procedure (2).

Intraoperative laparoscopic ultrasound (IOLUS) is an imaging technique that involves the use of an endoscopic ultrasound probe, enabling real-time visualization of internal structures. IOLUS provides high-resolution images that assist surgeons in navigating anatomical structures (Figure 1). Throughout its history, IOLUS has undergone refinement, including improvements in image quality, probe design, and integration with laparoscopic instruments. IOLUS has established itself as a valuable tool, enhancing the precision and outcomes of surgeries across various medical specialties. When faced with challenges related to limited depth perception and restricted range of motion, IOLUS can provide a solution, ultimately improving the effectiveness of the procedure. Today, IOLUS is a cornerstone in general surgery, with surgeons using it for the precise localization of tumors, mapping, and assessing tumor invasion in the diagnosis and treatment of various diseases (3). In a study published by Wakelin et al. (4) in 2022, which involved 36 patients and compared computerized tomography (CT), endoscopic ultrasound, and IOLUS in the preoperative staging of esophago-gastric carcinoma, IOLUS was also notably superior to CT in the assessment of distant metastases. The

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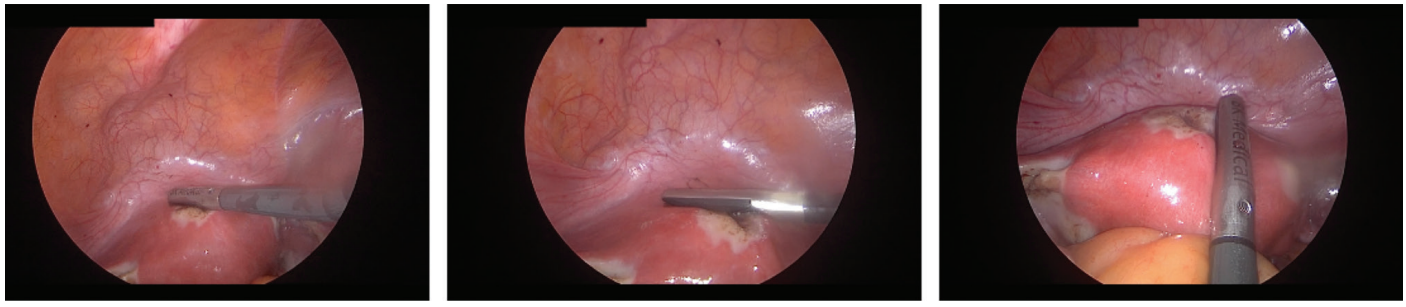

Figure 1. Intraoperative laparoscopic ultrasound during laparoscopic myomectomy surgery

reported sensitivity of IOLUS in detecting liver lesions can reach 100% (3). In urology, IOLUS helps surgeons accurately locate renal tumors and navigate delicate structures, ensuring maximal tumor removal while preserving kidney function (5).

The first use of IOLUS in laparoscopic myomectomy was described in 2004 (6). During laparoscopic myomectomy, once the telescope and instruments are in position, the IOLUS probe is introduced through a separate port. On the sterile handle of the IOLUS, there are buttons designed to control the movement of the probe's head, enabling adjustments in two planes. The maneuverability of the probe allows surgeons to guide it effectively, providing real-time ultrasound images on their monitor. This aids in visualizing internal structures, accurately locating fibroids, and assessing their features. IOLUS's interactive nature ensures that surgeons can adapt their approach based on live feedback from the ultrasound images.

There is a limited number of studies in the literature about IOLUS during laparoscopic myomectomy. The objective of this study was to perform a narrative review of the available literature regarding the application of IOLUS during laparoscopic myomectomy.

Materials and Methods

In this narrative review, an extensive literature search was performed using the PubMed database to identify pertinent studies related to IOLUS in the fields of gynecology, general surgery and urology. The search employed keywords such as "IOLUS," "gynecology," "general surgery," and "myomectomy." It was restricted to articles published exclusively in the English language.

History of IOLUS

The history of IOLUS can be traced back to the evolution of endoscopy and ultrasound technologies (7). During the 1960s, intraoperative ultrasound (IOUS) using A-mode or non-real-time B-mode imaging began. However, it was not widely adopted due to image interpretation challenges. In the late 1970s, special probes for surgery were developed. By the 1980s, IOUS became common in various surgical fields worldwide, especially hepatobiliary and pancreatic surgery. After 1990, as surgical techniques advanced towards minimally invasive approaches, the concept of real-time ultrasound imaging during surgery emerged. This led to the development of IOLUS, where the ultrasound probe could be used to guide surgical procedures and provide immediate feedback to the surgeon. While being an older technique, the initial use of IOLUS in laparoscopic myomectomy was documented in a case report in 2004 (6). In this case, a laparoscopic ultrasound transducer helped with the identification and accurate location of a myoma within an otherwise seemingly normal uterus.

Advantages of IOLUS-assisted laparoscopic myomectomy

The use of IOLUS for laparoscopic myomectomy offers a range of benefits in gynecological surgery. These benefits result from the real-time imaging and precision that IOLUS affords during the procedure.

IOLUS provides a clear and magnified view of the surgical field, allowing surgeons to identify location, size, and depth of fibroids with greater accuracy. This information is crucial for planning the best approach to removal. Especially deeply embedded or hard-to-see fibroids become more accessible with IOLUS guidance (8). In a recent study by Patel et al. (9), laparoscopic ultrasound was performed to examine 42 patients. This approach revealed 54 additional fibroids in 27 patients (64%), averaging two extra fibroids per patient. The median size of fibroids detected was 1.5 centimeters, with the majority being FIGO grade 3 (43%) and grade 2 (33%). This study underscores the value of IOLUS in identifying smaller intramural fibroids, particularly in patients with multiple fibroids. Urman et al. (8) documented 17 cases of symptomatic, deep, intramural myomas that remained unseen during laparoscopy. These myomas were successfully identified and removed using IOLUS during laparoscopic myomectomy without any complication. In another study comparing IOLUS with intraoperative transvaginal ultrasound, it was shown that IOLUS was better at detecting residual fibroids, although both methods are effective (10). In this study, 78 women underwent laparoscopic myomectomy. In this context, IOLUS identified a total of 140 residual fibroids,

whereas transvaginal ultrasound detected 127 (p=0.03). In a comprehensive study, IOLUS was compared with transvaginal ultrasound and magnetic resonance imaging (MRI) (11). The study involved 135 women who had symptomatic myomas and underwent laparoscopic ultrasound-guided radiofrequency volumetric thermal ablation of the myomas. IOLUS effectively detected a total of 818 myomas (mean 6.1 per subject, standard deviation 4.9), which was nearly twice the number of myomas compared to transvaginal ultrasound and 1.5 times more than MRI. As a result, IOLUS demonstrated its superiority by detecting the greatest number of myomas when compared to transvaginal ultrasound or MRI.

A distinctive benefit of IOLUS, unlike other imaging methods, is its capability to facilitate therapeutic interventions within the same session. It is acknowledged that the risk of residual fibroids is higher in laparoscopic myomectomy compared to abdominal surgery (12). However, IOLUS appears to be promising in addressing this issue. In a randomized controlled trial conducted with 156 patients divided into three groups, the recurrence rate was notably lower in the IOUES-guided laparoscopic myomectomy group compared to both the standard laparoscopic and open resection groups ($p<0.01$ in both cases) (13). Additionally, the IOLUS-guided group exhibited a lower residual rate than the standard laparoscopic group ($p<0.05$). However, there were no significant differences in residual rates between the IOLUS-guided group and the open resection group ($p>0.05$). Hence, IOLUS has the potential to identify additional fibroids, improve the outcomes of laparoscopic myomectomy, and decrease the occurrence of residual fibroids and recurrence rates. It should be noted that in certain cases, the presence of a significant number of myomas may lead to a preference for open surgery. This situation is regarded as a "relative contraindication" for laparoscopic myomectomy. However, the ease of intraoperative identification of myoma locations with IOLUS and the reduced risk of residual myomas may mitigate this "relative contraindication".

IOLUS offers additional advantages. Employing IOLUS for precise incision placement and size can result in reduced operation time and enhanced cost-effectiveness in material usage. Some studies in the literature have mentioned an extension in operation duration following IOLUS use; however, this is attributed to the removal of additional residual myomas using IOLUS (9). When conducted by a experienced surgical team familiar with setup and device operation, the use of IOLUS does not result in longer procedure times compared to routine transvaginal ultrasound. Furthermore, IOLUS allows surgeons to navigate complex anatomical structures, ensuring thorough fibroid removal without unnecessary damage to surrounding tissues. The ability to detect potential complications, like excessive bleeding or damage to adjacent organs, during surgery allows for timely intervention. This can minimize postoperative complications and improve patient outcomes. Therefore, by combining the strengths of IOLUS with laparoscopic myomectomy, surgeons can offer patients a more effective and safer treatment option. There is currently a lack of literature on cost-effectiveness analyses related to the use of IOLUS. However, a study conducted by Donoghue et al. (14), focusing on the cost-effectiveness of IOLUS in suspected choledocholithiasis, sheds light on its economic aspects. The study revealed that the cost per use of IOLUS was lower compared to magnetic resonance cholangiopancreatography (£183 vs. £365, respectively), making it a cost-effective option. Moreover, IOLUS offered the added benefit of reducing hospital bed days and saving approximately 240 hours of magnetic resonance cholangiopancreatography imaging time. This study highlighted the potential economic advantages of IOLUS application, which warrants further investigation in gynecology.

Challenges of IOLUS-assisted laparoscopic myomectomy

While the integration of IOLUS with laparoscopic myomectomy offers significant benefits, several challenges and considerations demand attention. Firstly, the proper calibration and setup of IOLUS equipment are paramount for obtaining accurate images. Ensuring the availability of well-maintained equipment is essential for successful implementation. Furthermore, IOLUS necessitates supplementary equipment and personnel. Hospitals and surgical centers must allocate resources for training, maintenance, and support to ensure the seamless integration of this technology. Presently, IOLUS is only accessible in select clinics and has yet to fully realize its potential in the field of gynecology.

Using IOLUS during laparoscopy requires training and experience. Surgeons must become proficient in interpreting ultrasound images. For instance, when employing the probe on the front surface of the uterus, it generates an image similar to that of transvaginal ultrasound. However, when the surgeon performs the ultrasound on the back surface of the uterus, the resulting image becomes a mirrored version of the classical transvaginal ultrasound image. This could potentially lead to confusion during the surgical procedure. Surgeons must establish their orientation based on the endometrium. If a fibroid is seen between the probe and the endometrium on the ultrasound monitor and the probe is positioned on the back surface of the uterus, the fibroid is actually located on the posterior wall of the uterus. Surgeons need to adapt to this situation, making a learning curve essential for the effective use of IOLUS.

Determining which patients will benefit most from IOLUS-assisted laparoscopic myomectomy requires careful consideration. Factors such as fibroid size, location, and individual patient characteristics play a role in the decision-

making process. As an example, while IOLUS may not offer significant benefits in detecting a large sub-serosal fibroid with a clearly defined perioperative location, it presents distinct advantages in the removal of a fibroid embedded in the myometrium, suspected to contribute to bleeding or infertility, situated close to the endometrium, and whose location cannot be determined intraoperatively. While IOLUS can enhance surgical precision, it may slightly extend procedure duration. Therefore, balancing the benefits of enhanced accuracy with the need for efficient surgical workflow is crucial.

Future directions of IOLUS in gynecology

As ultrasound technology advances, the quality of IOLUS images is expected to enhance, providing surgeons with better visualization during interventions the integration of machine learning algorithms into IOLUS also holds promise for automated image analysis. Beyond its current application in laparoscopic myomectomy, IOLUS has a potential to broaden its scope to guide a spectrum of minimally invasive gynecological procedures. For example, in a video article published in 2023, the technique of laparoscopic shaving for rectosigmoid endometriosis during deep endometriosis surgery was demonstrated (15). This technique employs a novel approach involving laparoscopic ultrasound guidance to facilitate thorough excision. Another area where IOLUS could potentially be beneficial is in the treatment of isthmocele. One of the most significant challenges in laparoscopic isthmocele repair is identifying the location of the defect. In the literature, methods such as laparoscopic repair with hysteroscopy guidance and the use of a Foley catheter have been described to overcome this difficulty (16,17). However, due to its direct contact with the uterus, IOLUS can be used more easily than all these methods to identify the location of the isthmocele. Similarly, another potential application could involve the intraoperative diagnosis of adenomyosis in complex surgery, such as endometriosis surgery.

Conclusion

IOLUS has the potential to represent a significant advance in the field of gynecological surgery. By providing real-time imaging, enhanced visualization, and precise guidance, IOLUS assists surgeons in locating and removing “hard-to-find” fibroids. While challenges such as setup, the learning curve, and patient selection exist, the potential benefits outweigh these obstacles. As research and technology progress, IOLUS-guided procedures will offer patients safer, more effective, and minimally invasive treatment options. These findings are promising, but the evolution of IOLUS-assisted laparoscopic myomectomy is an ongoing process. Further research, larger-scale studies, and long-term follow-ups are essential to establish its place as a standard practice in gynecology.

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What is your diagnosis?

A 28-year-old woman (gravida 5, para 2, live 2, abortion 1) was referred from elsewhere with multiple foetal anomalies, detected on morphology scan at 21 weeks. This was a natural conception. There was no family history of twins or congenital anomalies. There was no history of intake of teratogenic drugs. She had a dating scan at 9 weeks which was told to be normal.

A follow-up scan at our centre, revealed a foetus with two fused faces-oriented opposite to each other, a single body, and a set of upper and lower limbs. Two-dimensional (2D) grey scale ultrasonography (USG) showed four lateral ventricles, tetraophthalmos (two sets of eyes with lateral ones fused together), two separate noses and mouths, both showing cleft lips (Figure 1).

There was a single heart with transposition of the great arteries (TGA) and ventricular septal defect (VSD), a single spine, thoracic and abdominal cavity, a solitary stomach, and urinary bladder. There were no other foetal anomalies.

Diagnosis was confirmed using three-dimensional (3D) USG where both the faces could be seen, fused together, on the same plane (Figure 2).

The parents were informed about the ultrasound findings and decided to terminate the pregnancy. Termination of pregnancy was done using vaginal misoprostol (PGE1) tablets and resulted in expulsion of the malformed fetus vaginally. There were no problems encountered during the expulsion of fetal head. Autopsy findings confirmed the scan findings (Figure 3a, b). Informed written consent was obtained from the parents for presenting this case.

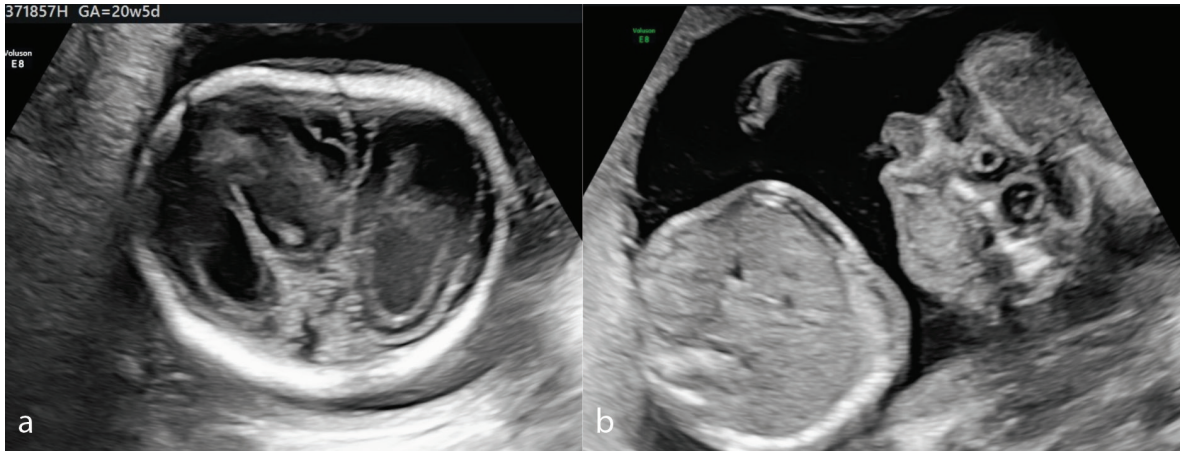

Figure 1. 2D USG shows distorted fetal head with multiple lateral ventricles (a). Tetraophthalmos with fused lateral eyes is seen in the center. There was a single thoracic and abdominal cavity (b)

2D USG: Two-dimensional ultrasonography

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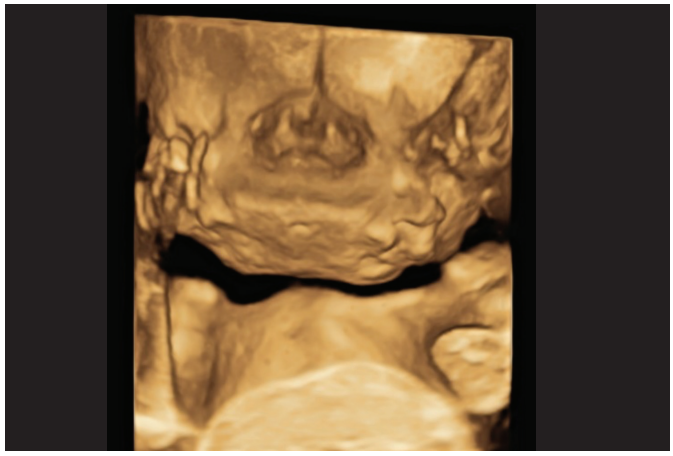

Figure 2. 3D USG shows two faces fused together laterally and oriented in opposite directions

3D USG: Three-dimensional ultrasonography

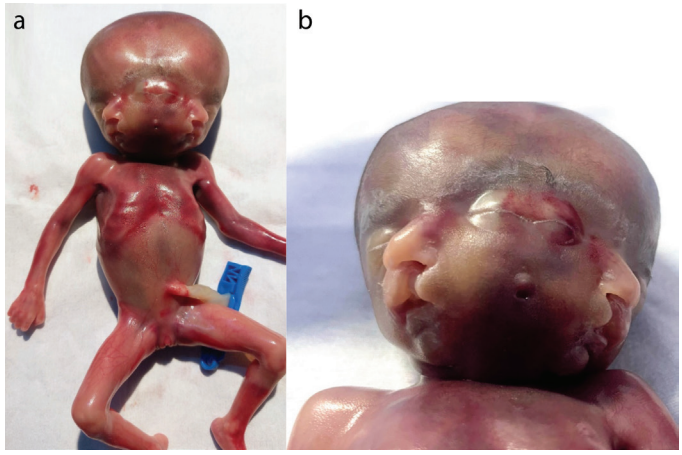

Figure 3. Post-abortal images revealed craniofacial duplication with a single body and a set of upper and lower extremities. No other organs were shared (a). Note the cleft lip on both the faces (b)

Answer

Parapagus diprosopus is a rare type of conjoined twinning which involves craniofacial duplication on a single body. Based on the site of attachment, conjoined twins are classified into: thoracopagus (42%); parapagus dicephalus (12%); cephalopagus (6%) and omphalopagus (6%). Diprosopus means duplication of the face; the trunk and limbs are normal (1).

This is an extremely rare condition, with an incidence of 1in 180,000 to 15,000,000 births (2). To date, only 40 cases have been described in the medical literature (3). Gorlin et al.'s (4) classification of the craniofacial duplication is the most widely accepted. The various types described are: 1) single mouth with duplication of maxillary arch; 2) supernumerary mouths laterally placed with rudimentary segments; 3) single mouth with duplication of mandibular segments; and 4) true facial duplication diprosopus (4).

Complete facial duplication, as in our case, is seen in only 50% of cases. The prevalence is higher in females (3). The aetiology is thought to be a disruption in the Sonic Hedgehog pathway, which controls the craniofacial tissue differentiation in the embryo (5).

The majority of cases (96%) are associated with cranial abnormalities, such as anencephaly, encephalocele, and craniorachischisis, with the most common being anencephaly (6). Other associated anomalies include: cardiac (86%); cleft lip/palate (63%); and congenital diaphragmatic hernia (42%) (3).

In the presented case there was evidence of cleft lip in both the faces (Figure 3a, b) and TGA with VSD. 2D USG raised the suspicion of foetal anomaly but 3D USG confirmed the diagnosis, since both the fused faces could be clearly seen on one plane (Figure 2). Conjoined twins are at risk of other structural anomalies, their presence impacts the prognosis adversely (3,7). In the presented case, the foetus had VSD with TGA.

Prognosis is typically poor with most of these babies dying in the first few months of life due to cardiorespiratory arrest (3). The remainder are either stillborn or abortuses. Hence early prenatal diagnosis is of paramount importance, as it gives enough time for parents to consider termination of pregnancy.

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Interstitial ectopic pregnancy in a patient with absent ipsilateral fallopian tube

To the Editor,

We read the video article titled "Treatment and management of interstitial pregnancy with laparoscopic cornual resection" by Şeker and Elçi (1) with great interest. We also manage our cases of interstitial pregnancy with the same laparoscopic technique. They have rightly mentioned the rarity and severity of interstitial pregnancy. However, its occurrence is even more rare in the absence of an ipsilateral fallopian tube. Only a few such cases are reported (2-5). Here, we report a case of interstitial pregnancy in a totally absent ipsilateral fallopian tube managed with laparoscopic cornual resection.

A 33-year-old female (gravida 4, para 1), with one previous abortion and one ectopic pregnancy, presented with a one and a half month history of amenorrhea. She underwent laparoscopic left salpingectomy 4.5 years previously for left-sided ruptured tubal ectopic pregnancy. Pelvic ultrasound revealed an empty uterine cavity, an eccentrically located gestational sac of six weeks at the left cornual end of the uterus with a surrounding thin rim of myometrium, suggestive of left interstitial ectopic pregnancy.

A diagnostic hysteroscopy was performed and a normal uterine cavity with bilateral ostia was visualized, and no gestational sac was seen. The diagnosis of interstitial ectopic and absent ipsilateral fallopian tube was confirmed on laparoscopy (Figure 1a). The left fallopian tube was absent, and bilateral ovaries and the right fallopian tube were healthy. Intramyometrial vasopressin was instilled to minimize blood loss during the procedure. The interstitial ectopic gestational sac was excised completely with a harmonic scalpel, and the defect was closed with a barbed suture in two layers (Figure 1b-e). No breach of the endometrial cavity was noted. The products of conception were removed in a bag, and the diagnosis of interstitial pregnancy was confirmed on histopathology.

The development of interstitial pregnancy in the isthmic portion after partial salpingectomy is still plausible. However, the underlying mechanism of the development of interstitial pregnancy after total salpingectomy is unclear. The possible mechanisms are:

1. Passage of spermatozoa via the healthy tube followed by Pouch of Douglas, to fertilize the ovum on the side of the absent fallopian tube;
2. Passage of spermatozoa and ovum on the side of the absent tube, fertilization and implantation in the interstitial portion of the absent tube, if patent;
3. Normal fertilization in the healthy tube, followed by migration of the fertilized embryo via ostia and implantation in the interstitial portion of the absent tube.

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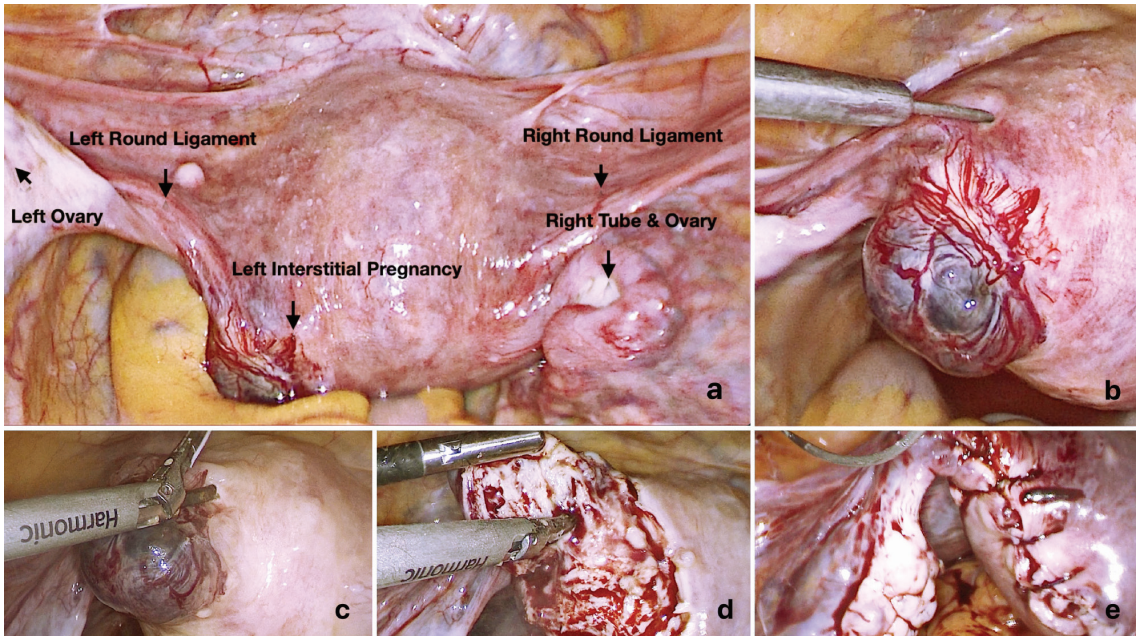

Figure 1. (a) Laparoscopic image showing left interstitial ectopic pregnancy and absent left fallopian tube; (b) intramyometrial injection of vasopressin to minimize blood loss; (c) use of harmonic scalpel to excise the gestational sac; (d) complete excision of ectopic tissue; (e) repair of the defect in two layers

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Author's Response

Dear colleague,

I read your article with great interest. The diagnosis of interstitial ectopic pregnancies is difficult and can be misdiagnosed. We are very pleased that laparoscopic treatment has become established. On the other hand, the absence of an ipsilateral fallopian tube makes the case interesting. The authors also performed a hysteroscopy before the operation. In the differential diagnosis of interstitial pregnancy, the use of hysteroscopy is a useful method in difficult cases. We expect that the use of minimally invasive methods will increase as knowledge on this topic increases.

Yours sincerely,

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When did the confusion between vulvodynia and vaginismus start?

To the Editor,

Vulvodynia, defined as vulvar pain persisting for at least three months without an identifiable cause, potentially accompanied by associated factors, is common yet remains enigmatic (1). "Vulvodynia" and "vaginismus" are frequently confused by both laypeople and healthcare professionals. Vaginismus is characterized by involuntary spasms of the pelvic floor muscles, which can be primary or secondary; secondary vaginismus may result from vulvodynia.

The fifth edition of the Diagnostic and Statistical Manual of Mental Disorders included dyspareunia and vaginismus into the newly created category of "genito-pelvic pain/penetration disorder", which remains a theoretical concept, lacking scientific validation (2). This change may have increased confusion, potentially denying women the appropriate diagnosis and, consequently, the correct management.

It appears that vulvodynia was addressed as early as 1825 BC in ancient Egyptian papyri. Some authors arguably sustain that "satyriasis" (excessive or abnormal sexual desire), described by Soranos (1st century AD) may correspond to vulvodynia. Possible descriptions of vulvodynia can be found in books from Thomas (3), Kellogg (4), and Skene (5), in 1868, 1891, and 1898, respectively. The latter proposed surgical removal of the area of "excessive sensitivity" (5).

The term "vaginismus" was coined by Sims (6), a controversial yet pivotal figure in medical history, in 1862. In his seminal work, he described five cases of women who were either unable to engage in intercourse (four cases) or had only experienced it a few times, incompletely, due to severe pain (Table 1). One woman had an "irritable bowel," which may have corresponded to irritable bowel syndrome. Each woman reported intense pain upon light touching of the vulvar vestibule and hymen. He stated, "the gentlest touch with the finger, a probe, or even a feather, produces the most excruciating agony." Given this description, we believe that these cases represent vulvodynia, rather than vaginismus. Although many women with vulvar pain may develop some degree of secondary vaginismus, introital pain alone does not define vaginismus.

The solution proposed for the problem was surgical: complete excision of the hymen and a V-shaped incision extending from above the hymen to the perineal raphe, followed by the use of dilators. He advised starting using glass or metal dilators within 24 hours after the surgery. While they experienced some soreness, it was not comparable to their previous pain levels. This outcome is unexpected for vaginismus but aligns with what might be anticipated for localized provoked vulvodynia (vestibulodynia).

He concluded that this condition was not uncommon as he and a colleague observed 17 cases over a 24-month period. He reported a surprisingly high success rate (88%), with some women even achieving pregnancy a few months post-procedure. This success rate aligns closely with current outcomes reported for the surgical treatment of localized provoked vulvodynia, despite the differences between his technique and the current ones (7,8).

To our knowledge, the most accurate detailed description of vulvodynia, which included a highly successful treatment approach, was provided by Sims (6). Juliet famously questioned, "What's in a name?". In this instance, an inaccurate term has led to a common condition remaining largely unknown and understudied for over one and a half

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Table 1. Clinical histories and treatment approaches for five women reported by Sims (6)

Year	Age	Clinical history	Gynecological exam	Treatment and outcome
1857	45	Woman of high social position unable to ever have intercourse. Married at age 20. "Painful menstruation" "Irritable bowel" "Sensation of bearing down" "Nervous system in a deplorable condition" Consulted several specialists in the US, Paris, London and other centers without success. Previous treatments included: Surgical removal of a "sanguineous tubercle at the meatus urinarius" 2-3 years after marriage (no benefit) Dilation with "graduated bougies" ("intolerable suffering")	"The slightest touch at the mouth of the vagina produced the most intense agony, throwing her nervous system into great agitation, with general muscular spasm and shivering of the whole frame, as if with the rigors of an intermittent, while she shrieked aloud, her eyes glaring wildly, and tears rolled down her cheeks, all rendering her a pitiable object of terror and suffering." "I succeeded in introducing the index finger into the vagina, up to the second joint, but no further. The resistance to the passage was so great, and the vaginal contraction so firm, as to deaden the sensation of the finger" The exam under anesthesia ("etherization") revealed a normal vagina.	No treatment proposed. Sims believed that surgical division of the muscles and nerves of the vulva could help, but refused to perform the surgery as it would be experimental and the lady was of a high social position.
1858 (?)	Not specified	Woman married two years before, with the "same dread instinct of being touched"	"Utterly impossible to pass a finger into the vagina"	Since the husband threatened to divorce, surgical treatment was proposed. Division only of the edges of the hymeneal membrane on each side of the fourchette - no improvement Division at the same points, but deeper "through the mucous membrane, and through some of the fibres of the sphincter muscle" - tolerated the introduction of 2 fingers, but with significant pain Proposed excision of the hymen, deeper incisions, followed by use of dilators - not allowed by the mother
1859	Not specified	Wife of a clergyman, married for 6 years and unable to have intercourse. Already consulted several surgeons.	"The slightest touch at the reduplication of the hymeneal membrane with a feather or a camel's hair pencil, produced as severe suffering as if she were cut with a knife."	Complete excision of the hymen V-shaped incision starting above the hymen and finishing in the raphe Use of dilators
1859	Not specified	Woman married for 3 years during which "sexual intercourse had been imperfectly accomplished a few times during the first few weeks after marriage". Stopped attempting intercourse ("lived and loved as innocently as two little children") Concerned about having child (family pressure)	Similar to the previous cases	
1859	Not specified	Married for 2.5 years, with a "truly unhappy" husband due to "persistent virginity"	Similar to the previous cases	

centuries since its first description.

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CONGRESS CALENDER

INTERNATIONAL MEETINGS

(for detailed International Meeting please go website: https://www.emedevents.com/obstetrics-and-gynecology)

September 11-13, 2024	XXIX. European Congress of Perinatal Medicine, Vienna, Austria
October 06-09, 2024	34th ISUOG World Congess, Dubai, UAE
October 16-18, 2024	International Gynecologic Cancer Society (IGCS) 2024 Meeting, Dublin, Ireland
October 19-23, 2024	American Society for Reproductive Medicine (ASRM) 80th Annual Meeting, Denver, Colorado, United States
October 19-22, 2024	19th World Congress on Menopause, Melbourne, Australia
October 27-30, 2024	ESGE 33rd Annual Congress, Marseille, France
November 17-20, 2024	The 53rd American Association of Gynecologic Laparoscopists (AAGL) Global Congress on Minimally Invasive Gynecologic Surgery (MIGS), New Orleans, Lousiana, United States
November 21-23, 2024	The 32nd World Congress on Controversies in Obstetrics Gynecology & Infertility (COGI), Lisbon, Portugal
April 23-27, 2025	**XV. Turkish-German Gynecology Congress, Antalya, Turkey**

CONGRESS CALENDER

NATIONAL MEETINGS

(for detailed International Meeting please go website: https://www.kongreuzmani.com/2024)

September 06-08, 2024	3. Uluslararası Pelvik Taban ve Kozmetik Jinekoloji Kongresi, İstanbul, Türkiye
September 18-22, 2024	7. Minimal İnvaziv Jinekolojik Cerrahi Kongresi, İstanbul, Türkiye
October 02-06, 2024	6. Jinekoloji ve Obstetrikte Tartışmalı Konular Kongresi, Antalya, Türkiye
November 14-17, 2024	12. Üreme Sağlığı ve İnfertilite Kongresi, Antalya, Türkiye