

Analysis of total laparoscopic hysterectomies for benign disease: The experience of a tertiary center: a retrospective cross-sectional study

Benign hastalıklar için yapılan total laparoskopik histerektomilerin analizi: Üçüncü basamak bir merkezin deneyimi: Retrospektif kesitsel çalışma

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ABSTRACT

Aim: To present the analysis of pre-, intra- and postoperative outcomes of total laparoscopic hysterectomy (TLH) cases performed in our hospital, a tertiary referral center, over a 5-year period.

Materials and Methods: This retrospective cross-sectional study was conducted in the Gynecology Department of an Education and Research Hospital between January 01, 2017 and December 31, 2021. Clinical data and laboratory results were obtained from medical and hospital records. After analyzing these patients, the patients were divided into two groups based on their body mass index and the pre-, intra- and postoperative results were also compared.

Results: The mean age of 516 patients included in the study was 47.4 ± 8.94 years. The most common indication for hysterectomy was endometrial premalignant lesions (23.9%). The overall intra-operative complication rate was 0.78%. The most common intra-operative complication in the overall population was bladder damage. Postoperative complications: 7 wound infections (1 vs. 6); 5 vaginal bleedings (0 vs. 5); 3 abscesses in the vaginal cuff (1 vs. 2); 2 septic shock (0 vs. 2); 2 disorders of the general condition (0 vs. 2); 1 urinary tract infection (1 vs. 0); 1 umbilical hernia (1 vs. 0) and positional nerve damage (0 vs. 1), respectively for the groups.

Conclusion: TLH is now a minimally invasive surgical procedure which can be performed safely and with very few complications as surgical experience and technical equipment improves. A higher complication rate has been reported in earlier cases, but both in the present study and in more recent studies, a very low complication rate was found with the procedure. Therefore, as recommended by the ACOG, this procedure can be used as the preferred method for patients in whom hysterectomy is planned primarily for benign conditions.

Keywords: Hysterectomy; laparoscopy; surgeon experience; uterine fibroid

ÖZ

Amaç: Üçüncü basamak bir sevk merkezi olan hastanemizde 5 yıl boyunca gerçekleştirilen total laparoskopik histerektomi (TLH) olgularının ameliyat öncesi, sonrası ve sonuçlarının analizini sunmaktır.

Gereç ve Yöntemler: Bu retrospektif kesitsel çalışma, 01 Ocak 2017 ile 31 Aralık 2021 tarihleri arasında bir Eğitim ve Araştırma Hastanesi Kadın Hastalıkları Kliniğinde gerçekleştirilmiştir. Klinik veriler ve laboratuvar sonuçları tıbbi kayıtlardan ve hastane kayıtlarından elde edilmiştir. Bu hastalar analiz edildikten sonra, hastalar vücut kitle indekslerine göre iki gruba ayrılmış ve ameliyat öncesi, sonrası ve sonuçları da karşılaştırılmıştır.

Bulgular: Çalışmaya dahil edilen 516 hastanın yaş ortalaması 47,4 ± 8,94 idi. Histerektomi için en yaygın endikasyon endometriyal premalign lezyonlardı (%23,9). Genel intra-operatif komplikasyon oranı %0,78 idi. Genel popülasyonda en sık görülen intra-operatif komplikasyon mesane hasarıydı. Ameliyat sonrası komplikasyonlar: Gruplar için sırasıyla 7 yara enfeksiyonu (1'e karşı 6); 5 vajinal kanama (0'a karşı 5); vajinal kafta 3 apse (1'e karşı 2); 2 septik şok (0'a karşı 2); 2 genel durum bozukluğu (0'a karşı 2); 1 idrar yolu enfeksiyonu (1'e karşı 0); 1 umbilikal herni (1'e karşı 0) ve pozisyonel sinir hasarı (0'a karşı 1).

Sonuç: TLH, cerrahi deneyim ve teknik donanım geliştikçe artık güvenli bir şekilde ve çok az komplikasyonla uygulanabilen minimal invaziv bir cerrahi prosedürdür. Daha önceki vakalarda daha yüksek bir komplikasyon oranı bildirilmiştir, ancak hem bu çalışmada hem de daha yeni çalışmalarda prosedürle ilgili çok düşük bir komplikasyon oranı bulunmuştur. Bu nedenle, ACOG tarafından önerildiği gibi, bu prosedür öncelikle iyi huylu durumlar için histerektomi planlanan hastalarda tercih edilen yöntem olarak kullanılabilir.

Anahtar Kelimeler: Histerektomi; laparoskopi; cerrah deneyimi; uterin myom

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INTRODUCTION

Nowadays, hysterectomy is one of the most common gynecological operations. The number of hysterectomies in the USA is estimated to be about 60,000 per year (1), while in India about 2,310,263 women undergo hysterectomy annually (2). More than 70% of hysterectomies are performed for benign causes such as menorrhagia (abnormal uterine bleeding), uterine fibroids, pelvic pain and uterine prolapse (3). Although these procedures have traditionally been performed abdominally (TAH) and vaginally (VH), minimally invasive techniques are increasingly being used due to innovations of medical technology (4). These techniques include laparoscopically assisted vaginal hysterectomy (LAVH), total laparoscopic hysterectomy (TLH) and robot-assisted laparoscopic hysterectomy.

Reich et al. reported on the first TLH in 1989 (5). Since then, there have been a number of improvements in the field of laparoscopic-assisted hysterectomies. In TLH, all surgical incisions, dissections and suturations (including closure of the vaginal cuff) are performed entirely through the trocars. Compared to other conventional methods, it is characterized by shorter operating times, less blood loss and shorter hospital stays (6). Despite all these advantages, however, TAH is still the most commonly used form of hysterectomy. This is mainly due to the lack of experience of the doctors and support staff, the lack of technical equipment and a higher incidence of complications such as injuries to the ureter, bladder and bowel near the genital organs as well as injuries to the great vessels when inserting the trocars (7).

In this context, we aimed to present the analysis of pre-, intra- and postoperative outcomes of TLH cases performed in our hospital, a tertiary referral center, over a 5-year period.

MATERIALS AND METHODS

This retrospective cross-sectional study was conducted in the Gynecology Department of an Education and Research Hospital between January 01, 2017 and December 31, 2021. The study was approved by the local ethics committee for scientific research (May 26, 2022; No. 2022/06) and was conducted in accordance with the principles of the Declaration of Helsinki. In view of the retrospective nature of the study, the ethics committee waived the required patient consent. All patient data was anonymized or treated confidentially.

Inclusion and exclusion criteria

Patients who had undergone TLH surgery with benign and premalignant disease were included in the study.

Patients were excluded from the study if any of the following applied to them: missing data, TLH surgery for malignancy, uterine fibroids ≥ 10 cm and pregnancy-related circumstances.

Data

The study included 516 patients who had undergone TLH. Data from 516 patients were obtained from patient files and hospital records. Data included age, body mass index (BMI), gravidity, parity, previous cesarean section, previous surgery or gynecologic procedures, TLH indications, laboratory test results [pre- and postoperative white blood cell (WBC) count and hemoglobin (HB)], Delta hemoglobin (Δ HB) (the Δ HB is calculated using the formula [preoperative HB] - [postoperative HB]), hospitalization days, blood transfusions and chronic diseases, etc.

Evaluation of the patients

Patients with benign or premalignant disease for whom hysterectomy is indicated are treated according to the corresponding TLH protocol.

Abdominal and transvaginal ultrasound examinations of the patients were performed prior to surgery using a General Electric Voluson 730[®] (1.5-4.5 MHz probe, Waukesha, WI, USA). Laboratory analyzes [complete blood count (CBC)] of patients who had undergone TLH are analyzed using the Advia[®] 120 hematology system (Siemens Healthcare Diagnostics Inc., Deerfield, Illinois).

Details of TLH surgery

A supraumbilical port with a diameter of ten millimeters was used as the primary trocar. Three lateral trocars were used. A bipolar electrocautery or a LigaSure laparoscopic sealer (5 and 10 mm) was used to coagulate the pedicles and scissors were used for cutting. Colpotomy was performed with a monopolar electrocautery hook. Salpingo-oophorectomy was performed if the patient was postmenopausal or had significant ovarian pathology, otherwise the ovaries were preserved. The vaginal cuff was usually closed laparoscopically using the intracorporeal knotting technique. In all cases, the bilateral ureters were traced from the pelvic edge to the bladder entrance, especially in previously operated cases in which the course of the ureter was disturbed due to adhesions. In some cases, the adhesions were removed by sharp and blunt dissections using bipolar electrocautery and scissors.

Study design

A total of 516 patients who met the inclusion criteria mentioned in the Material and Methods section and underwent TLH surgery were included in the study. After analyzing these patients, the patients were divided into two groups: those with a BMI < 30 kg/m² (Group I) and those with a BMI ≥ 30 kg/m² (Group II), and the pre-, intra- and postoperative outcomes were also compared.

Statistical analyses

Data analysis was performed using IBM's Social Sciences Statistical Package Version 29.0 (SPSS ver 29.0). The Kolmogorov-Smirnov and Shapiro-Wilk tests were used to check whether the numerical data correspond to a normal distribution. Numerical data are expressed as mean \pm standard deviation. Categorical variables were expressed as numbers (percentages) and analyzed using the chi-square test, with odds ratios (OR) expressed with 95% confidence intervals. In this study, which consisted of two independent groups, the t-test for independent samples was also used for parametric variables. Statistical significance was accepted as a p value <0.05 .

RESULTS

After reviewing patient files and hospital records, 564 patients were found who had undergone TLH surgery. After applying exclusion criteria, 516 patients were included in the study. The 516 patients were divided into two groups: those with a BMI <30 kg/m² (Group I) (n=260) and those with a BMI ≥ 30 kg/m² (Group II) (n=256). The flow chart of the participants is shown in Figure 1.

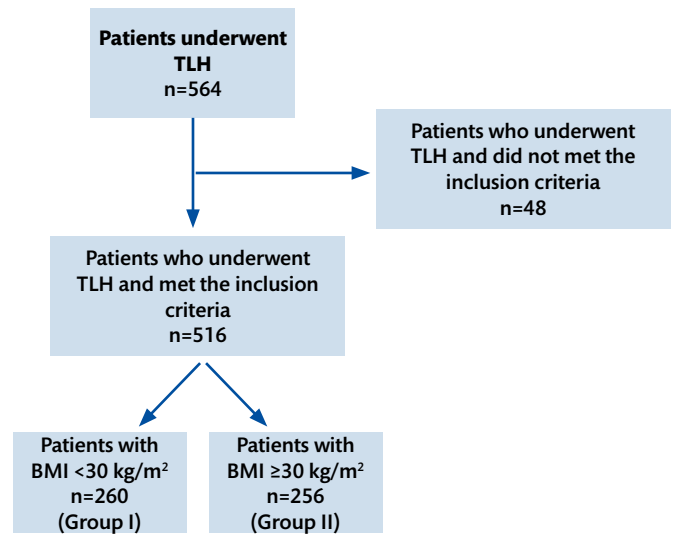


Figure 1. The flow chart of the participants

The demographic, pre- and post-operative laboratory analysis of the patients is shown in Table 1. The mean age of the 516 patients was 47.4 ± 8.94 years. Group II had a statistically significantly higher average age than Group I. Although there was no statistically significant difference in the pre-operative WBC and HB values between the groups, the post-operative HB was statistically

Table 1. The demographic, pre- and post-operative laboratory analysis of the patients

	Total patients n=516	Groups		p
		Group I n=260	Group II n=256	
Age (years) (mean \pm SD)	47.4 \pm 8.94	46.0 \pm 10.56	48.9 \pm 6.63	<0.001^a
Gravidity (mean \pm SD)	2.9 \pm 1.76	2.5 \pm 1.67	3.3 \pm 1.77	<0.001^a
Parity (mean \pm SD)	2.3 \pm 1.34	2.0 \pm 1.32	2.5 \pm 1.32	<0.001^a
Previous CS n (%)	No	442 (85.7)	218 (85.2)	0.746 ^b
	Yes	74 (14.3)	38 (14.8)	
BMI (kg/m²) (mean \pm SD)	29.7 \pm 11.05	24.8 \pm 2.50	34.6 \pm 13.86	<0.001^a
Preoperative WBC (cells/mm³) (mean \pm SD)	7.050 \pm 1.916	7.048 \pm 1.967	7.052 \pm 1.867	0.492 ^a
Preoperative HB (g/dL) (mean \pm SD)	11.3 \pm 3.63	11.3 \pm 3.99	11.3 \pm 3.23	0.404 ^a
Postoperative WBC (cells/mm³) (mean \pm SD)	10.665 \pm 3.417	10.899 \pm 3.467	10.428 \pm 3.357	0.059 ^a
Postoperative HB (g/dL) (mean \pm SD)	9.4 \pm 3.22	9.7 \pm 3.29	9.0 \pm 3.13	0.012^a
Delta HB (g/dL) (mean \pm SD)	1.93 \pm 4.44	1.57 \pm 4.69	2.29 \pm 4.15	0.033^a
Blood transfusion (mean \pm SD)	0.09 \pm 0.44	0.08 \pm 0.413	0.10 \pm 0.474	0.333 ^a
Transfusion n (%)	None	490 (95)	249 (95.8)	0.554 ^b
	Postoperative ES	13 (2.5)	4 (1.5)	
	Preoperative ES	8 (1.6)	4 (1.5)	
	Postoperative Ferric Carboxymaltose	4 (0.8)	2 (0.8)	
	Intraoperative ES	1 (0.1)	1 (0.4)	
	Total Transfusion	26 (5)	11 (4.2)	
Hospital stay (days) (mean \pm SD)	2.23 \pm 1.41	2.09 \pm 1.12	2.37 \pm 1.65	0.013^a

Abbreviations: CS: cesarean section; ES: erythrocyte suspension; HB: hemoglobin; SD: standard deviation; WBC: white blood cell

A p value of <0.05 indicates a significant difference. Statistically significant p-values are in bold.

^aStudent t test ^bChi Square test

significantly lower in Group II than in Group I ($p=0.012$). Δ HB values were statically significant higher in Group II than Group I ($p=0.033$). There was no statistical difference between the groups in terms of the number of transfusions ($p=0.333$). The duration of hospitalization was statistically significantly higher in Group II ($p=0.013$).

The analysis of chronic diseases and the patients' history of previous surgical interventions (gynecologic or non-gynecologic) is shown in Table 2. While there was no difference between the groups in terms of previous gynecologic surgery, there was a statistically significant difference in terms of previous non-gynecologic surgery ($p<0.001$). While appendectomy and cholecystectomy surgeries were more common in Group II, mastectomy surgeries were significantly more common in Group I. Among chronic diseases, cases of hypertension were higher in Group II ($p<0.001$).

A comparison of the indications for TLH, intra- and post-operative complications, concomitant other surgical procedures, etc. is shown in Table 3. The groups showed significant differences in terms

of the indications for TLH ($p<0.001$). Statistically significant TLH indications for Group I are uterine fibroids and gender affirmation surgery. Statistically significant TLH indications for Group II are persistent bleeding and premalignant endometrial lesions. In Group II, ureteral damage was diagnosed in 1 case and bladder damage in 2 cases, while in Group I, bladder damage was diagnosed in only 1 case. Postoperative complications were statistically significantly higher in Group II ($p=0.002$). Postoperative complications: 7 wound infections (1 vs. 6); 5 vaginal bleedings (0 vs. 5); 3 abscesses in the vaginal cuff (1 vs. 2); 2 septic shock (0 vs. 2); 2 disorders of the general condition (0 vs. 2); 1 urinary tract infection (1 vs. 0); 1 umbilical hernia (1 vs. 0) and positional nerve damage (0 vs. 1), respectively. None of our patients developed intra- or postoperative deep vein thrombosis, thrombotic events or pulmonary thromboembolism. The OR for postoperative complications for Group II was 4.84 (95% CI 1.615-14.508).

In three Group II cases, laparoscopy was replaced by laparotomy in the intra-operative phase (cervical premalignant lesion, adnexal mass and endometrial premalignant lesion).

Table 2. The analysis of chronic diseases and the patients' history of previous surgical interventions

		Total patients n=516	Groups		p
			Group I n=260	Group II n=256	
Previous gynecologic surgery n (%)	None	471 (91.3)	239 (91.9)	232 (89.6)	0.366*
	Endometriosis	1 (0.2)	1 (0.4)	0 (0)	
	Myomectomy	6 (1.2)	4 (1.4)	2 (0.8)	
	Other gynecologic surgeries	25 (4.8)	10 (3.9)	15 (5.8)	
	BTL	11 (2.1)	4 (1.6)	7 (2.7)	
	Prolapsus	2 (0.8)	2 (0.8)	0 (0)	
Previous non- gynecologic surgery n (%)	None	385 (74.6)	198 (76.2)	187 (73)	<0.001*
	Appendectomy	32 (6.2)	7 (2.7)	25 (9.8)	
	Cholecystectomy	19 (3.7)	5 (1.4)	14 (5.5)	
	Thyroidectomy	9 (1.7)	3 (1.2)	6 (2.3)	
	Mastectomy	39 (7.6)	27 (10.4)	12 (4.7)	
	Other surgeries	32 (6.2)	20 (7.7)	12 (4.7)	
HT n (%)	No	442 (85.7)	238 (79.7)	204 (79.7)	<0.001*
	Yes	74 (14.3)	22 (8.5)	52 (20.3)	
DM n (%)	No	468 (90.7)	242 (93.1)	226 (88.3)	0.061*
	Yes	48 (9.3)	18 (6.9)	30 (11.7)	
Lung diseases n (%)	No	502 (97.3)	256 (98.5)	246 (96.1)	0.098*
	Yes	14 (2.7)	4 (1.5)	10 (3.9)	
Other chronic diseases n (%)	Cardiovascular	7 (1.4)	5 (1.9)	2 (0.8)	0.102*
	Mammary CA	18 (3.5)	10 (3.8)	8 (3.1)	
	Thyroid diseases	26 (5)	18 (6.9)	8 (3.1)	

Abbreviations: BTL: bilateral tubal ligation; CA: cancer; DM: diabetes mellitus; HT: hypertension

A p value of <0.05 indicates a significant difference. Statistically significant p-values are in bold.

*Chi Square test

Table 3. A comparison of the indications for TLH, intra- and post-operative complications, concomitant other surgical procedures

		Total patients n=516	Groups		p
			Group I n=260	Group II n=256	
TLH indications n (%)	Uterine fibroids	87 (16.9)	63 (24.2)	24 (9.4)	<0.001*
	Persistent uterine bleeding	111 (21.6)	48 (18.5)	63 (24.6)	
	EPL	123 (23.9)	42 (16.2)	81 (31.8)	
	Adenomyosis	20 (3.9)	10 (3.8)	10 (3.9)	
	BRCA (+) prophylaxis	20 (3.9)	14 (5.4)	6 (2.4)	
	Adnexal mass	46 (8.9)	23 (8.8)	23 (8.8)	
	CPL	39 (7.5)	13 (5)	26 (10.2)	
	PMB	34 (6.5)	14 (5.4)	20 (7.8)	
	Gender affirmation surgery	36 (6.9)	33 (12.7)	3 (1.1)	
BS/BSO/USO surgery with TLH n (%)	BS	133 (25.8)	72 (27.7)	61 (23.8)	0.355*
	BSO	342 (66.3)	171 (65.8)	171 (66.8)	
	USO	41 (7.9)	17 (6.5)	24 (9.4)	
Additional surgical intervention n (%)	No	478 (92.6)	245 (94.2)	233 (91)	0.425*
	Bladder repair	3 (0.6)	1 (0.4)	2 (0.8)	
	Adhesiolysis	21 (4)	9 (3.4)	12 (4.6)	
	Ureteroneocystostomy	1 (0.2)	0(0)	1 (0.4)	
	Perineoplasty / Vaginoplasty	5 (1)	1 (0.4)	4 (1.6)	
	TOT	5 (1)	3 (1.2)	2 (0.8)	
	Cholecystectomy	2 (0.4)	0(0)	2 (0.8)	
	Appendectomy	1 (0.2)	1(0.4)	0 (0)	
Intraoperative Complications n (%)	No	512 (99.2)	259 (99.6)	253 (98.8)	0.308*
	Yes	4 (0.8)	1 (0.4)	3 (1.2)	
Postoperative Complications n (%)	No	494 (95.7)	256 (98.5)	238 (93)	0.002*
	Yes	22 (4.3)	4 (1.5)	18 (7)	

Abbreviations: BRCA:Breast Cancer gene; BS: bilateral salpingectomy; BSO: bilateral salpingo-oophorectomy; CPL: cervical premalignant lesion; EPL: endometrial premalignant lesion; PMB: postmenopausal bleeding; TLH: total laparoscopic hysterectomy; TOT: transobturator tape; USO: unilateral salpingo-oophorectomy. A p value of <0.05 indicates a significant difference. Statistically significant p-values are in bold.

*Chi Square test

The vaginal cuff was closed vaginally in 2 Group II cases. All other TLH cases were closed using the laparoscopic intra-corporeal suture technique.

DISCUSSION

In recent years, laparoscopic surgery has become one of the most frequently used surgical methods in gynecological practice. This surgical method has also become established for hysterectomy, one of the most common surgical procedures in gynecological departments. In the committee statement published by the American College of Obstetricians and Gynecologists (ACOG) in 2017, it was mentioned that VH and LH, which are minimally invasive methods, should be preferred as much as possible in surgical experience and patient selection, and even TLH should be the standard method in

patients for whom VH are not suitable (8). With this in mind, we provide an overview of the analysis of TLHs performed for benign conditions at our tertiary referral hospital where minimally invasive surgery is used effectively.

In the retrospective 5-year period, 564 patients underwent TLH and 516 patients met the inclusion criteria and were included in our study. If we look at the total number of cases in 5 years, we see that 564 TLH cases is a good number. In the Cheung et al. study (9), the number of cases in 5 years was 175, but when we look at the time frame of the study, we realize that it was conducted about 10 years before our study. This low number is probably due to lack of surgical experience and technical inadequacies. In our study, the mean age is 47.4 ± 8.94 years, and also the mean age is 46.0 ± 10.56 years in Group I and 48.9 ± 6.63 years in Group II, respectively. A study by Ashfaq et al. (10) examined the experience

of a single surgeon on the results of TLH procedures and found an average age of 46.42 ± 5.01 years. In a retrospective analysis of 361 TLH cases by Mereu et al. (11), they arrived at a mean age of 49.6 ± 6.5 years.

In our study, the indications for TLH were endometrial premalignant lesions, treatment-resistant uterine bleeding and uterine fibroids. Uterine fibroids were the most common indication in the group with a BMI <30 kg/m², while endometrial premalignant lesions and treatment-resistant uterine bleeding were more common in Group II with a BMI ≥ 30 kg/m². In the literature, uterine fibroids are mentioned as the most common indication, followed by abnormal uterine bleeding and endometriosis (8,10). Another study by Antoun et al (12) concluded that the most common reasons for TLH in the 128 patients were pelvic pain (45%), followed by uterine fibroids (21%) and abnormal uterine bleeding (18%). The results of our study are partly compatible with these indications.

The present study included 74 (14.3%) patients had a history of cesarean section, with no differences between groups ($n=36$ vs. $n=38$); when we examined rates of previous laparotomies, 202 patients had a history of abdominal surgery (including gynecologic, non-gynecologic, and cesarean section). There was no difference between the groups in terms of previous abdominal surgery. Previous abdominal surgery is not a contraindication for laparoscopic surgery, but the risk of bladder and bowel injury is sometimes increased in these patients (13). We have four intra-operative complications: in Group II, ureteral damage was diagnosed in 1 case and bladder damage in 2 cases, while in Group I, bladder damage was diagnosed in only 1 case. However, there were no significant differences between the groups with regard to intra-operative complications. Postoperative complications were higher in Group II with a BMI ≥ 30 kg/m². In the literature, there are conflicting results in studies on BMI-related TLH complications (14-16). Jayashree et al (14) concluded that there were no differences in intra- and postoperative parameters between overweight women compared to women with a normal BMI. Another study by Otake et al (15) found that overweight patients had longer operation times and more postoperative complications than normal-weight patients. In an Egyptian study, the authors concluded that TLH can be successfully performed in obese patients, although a BMI between 30-39.9 kg/m² has higher peri-operative clinical and financial consequences compared to non-obese patients (BMI <30 kg/m²) (16).

There was no statistical difference between the preoperative HB values of the groups, the postoperative HB value of Group II was statistically lower and the Δ HB value, which indicates the intra-operative HB change, was statistically significantly higher than

Group I. Thus, overweight patients have a higher risk of blood loss in TLH cases. In contrast to our study, Andan et al. reported that BMI has no effect on the amount of bleeding in TLH (17). A study by Nawfal et al. also found that BMI did not increase the amount of intra-operative bleeding (18). In agreement with our study, Heinberg et al. (19) reported that the risk of intra-operative blood loss was increased in obese women compared to non-obese women and that this risk tripled with blood loss of more than 500 ml.

In conclusion, due to improved surgical experience and technical equipment, TLH is now a minimally invasive surgical procedure that can be performed safely and with very few complications. In earlier cases, a higher complication rate was reported, but both in this study and in more recent studies, a very low complication rate was found for the procedure. Therefore, as recommended by the ACOG, this procedure can be used as the method of choice for patients who are primarily scheduled for hysterectomy for benign conditions. However, particular attention should be paid to complications and blood loss in overweight women.

The strengths and limitations

The study was conducted in a tertiary referral hospital and standardized protocols and surgical methods were used for all patients. The treatment and care of all patients in the study was coordinated in the gynecology department, which had a sufficient number of concomitant cases.

However, due to the retrospective design of the study, data/information was missing for some patients (duration of laparoscopic surgery, uterine weight, etc.). As the study was planned in a single center, we also lacked experience with different surgical techniques and approaches.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Conceptualization, F.B.F. and Y.A.R.; methodology, F.B.F., Y.A.R. and A.A.; software, A.A., A.K.O. and G.E.; validation, A.A. and G.E.; formal analysis, A.A., A.K.O. and G.E.; investigation, F.B.F., Y.A.R., R.E.P., and V.K.; resources, A.K.O., G.E., and R.E.P.; data curation, G.E. and R.E.P.; writing—original draft preparation, F.B.F.; writing—review and editing, F.B.F. and Y.E-U.; visualization, V.K. and Y.E-U.; supervision, Y.E-U.; project administration, F.B.F., Y.A.R., V.K. and Y.E-U.

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