Comparison of Unilateral and Bilateral Sacrospinous Ligament Fixation Using Minimally Invasive Anchorage

Vergleich von einseitiger und beidseitiger Fixation am Ligamentum sacrospinale mit minimalinvasiver Verankerung

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ABSTRACT

Objective The aim of this study was to determine the effectiveness of a newly developed anchoring system for unilateral sacrospinous ligament fixation (USSLF) and bilateral sacrospinous ligament fixation (BSSLF) procedures.

Material and Methods Ninety-three patients with pelvic prolapse who were treated surgically with the Anchorsure System® between 2013 and 2018 were included in the study. USSLF was performed in 52 patients (group 1), and BSSLF was performed in 41 patients (group 2). Pelvic organ prolapse was assessed preoperatively and 6 months postoperatively.

Results There were no significant differences between groups 1 and 2 with regard to age, parity, and demographic characteristics. Anatomical improvement rates were similar, irrespective of the type of SSLF used. No bleeding requiring blood transfusion or organ injuries occurred in any patient. Three patients in the group that received BSSLF developed small asymptomatic cystoceles (grade 1 to 2); there was no recurrence of rectoceles or enteroceles. Mild cystocele was found in 1 patient from the USSLF group. There was no significant difference between the groups with respect to the recurrence of cystocele. Recurrence of vaginal vault prolapse was found in 2 patients from the USSLF group (3.84%). There was no significant difference between the groups with regard to recurrence. Febrile morbidity, clinical outcomes, blood loss, duration of operation, intraoperative complications, and length of hospital stay were similar for the two groups.

Conclusions Unilateral and bilateral SSLF techniques produce similar clinical outcomes. USSLF and BSSLF performed using the new anchoring system are safe and effective methods to treat pelvic organ prolapse.

ZUSAMMENFASSUNG

Zielsetzung Ziel dieser Studie war es, die Effektivität eines neuentwickelten Ankersystems für die einseitige und beidseitige Fixation am Lig. sacrospinale zur Behandlung von Prolaps der Beckenorgane zu vergleichen.

Material und Methoden 93 Patientinnen mit Gebärmuttersenkung, die sich zwischen 2013 und 2019 einem chirurgischen Eingriff mit dem Anchorsure System® unterzogen, wurden in die Studie eingeschlossen. Eine einseitige Fixation wurde bei 52 Patientinnen (Gruppe 1) und eine beidseitige Fixation bei 41 Patientinnen (Gruppe 2) durchgeführt. Das Ausmaß der Beckenbodensenkung wurde jeweils vor der Operation sowie 6 Monate nach dem Eingriff evaluiert.

Ergebnisse Gruppe 1 und Gruppe 2 unterschieden sich nicht signifikant voneinander hinsichtlich des Alters, der Parität und der demografischen Merkmale. Ungeachtet des gewählten Eingriffs war das Ausmaß an anatomischer Verbesserung für beide Gruppen vergleichbar. Keine der Patientinnen erlitt

schwere Blutungen mit nachfolgender Bluttransfusion oder Organschädigungen. Drei Patientinnen aus der Gruppe mit bilateraler Fixation entwickelten kleine, asymptomatische Zystozelen (Grad 1–2); keine der Patientinnen entwickelte eine Rektozele oder ein Enterozele-Rezidiv. Eine Patientin aus der Gruppe mit einseitiger Fixation entwickelte eine schwach ausgeprägte Zystozele. Es gab keinen statistisch signifikanten Unterschied zwischen den beiden Gruppen hinsichtlich der Entwicklung von Zystozelen. Zwei Patientinnen aus der Gruppe mit einseitiger Fixation erlitten einen erneuten Scheidenprolaps (3.84 %). Die zwei Gruppen unterschieden sich nicht signifikant im Hinblick auf das Wiederauftreten von Prolaps. Fieberentwicklung, klinisches Outcome, Blutverlust, Operationsdauer, intraoperative Komplikationen und Krankenhausaufenthalt waren in beiden Gruppen vergleichbar.

Schlussfolgerungen Das klinische Outcome nach einseitiger und bilateraler Fixation war vergleichbar. Die einseitige bzw. bilaterale Fixation am Lig. sacrospinale mit dem neuentwickelten Verankerungssystem stellt ene sichere und effektive Methode zur Behandlung der Beckenbodensenkung dar.

Introduction

Sacrospinous ligament fixation (SSLF) is an effective technique that fixes the vaginal vault to the sacrospinous ligament and restores vaginal wall support [1]. Its effectiveness is not a subject of debate and it has a success rate of more than 90 % [2]. However, the indications for this approach have been expanded to include the prophylactic prevention of vaginal vault prolapse during hysterectomy in high-risk patients. SSLF is a safe and feasible method that can be used together with other vaginal procedures, particularly in patients with pelvic organ prolapse (POP). The procedure requires sufficient experience and has a learning curve [1, 3].

Articles discussing the prophylactic use of SSLF during vaginal hysterectomy procedures in patients with surgically weak uterosacral cardinal ligaments only began to be published 10 years after publication of a surgical procedure for vaginal cuff prolapse by Richter in 1968 [1, 4, 5].

SSLF has an effectiveness of 96–98 %, irrespective of whether the uterus is preserved or not [6]. As a transvaginal procedure, SSLF is associated with fewer complications, less preoperative pain, greater cost-effectiveness, shorter hospital stays, less blood loss, and better preservation of sexual intercourse function compared with transabdominal approaches. Furthermore, it provides simultaneous repair of existing gynecological pathologies such as cystocele, enterocele, and rectocele [6]. The most frequent complications of this procedure are bleeding and buttock pain. Life-threatening bleeding after SSLF from sacral or pudendal arteries was reported in 3 patients out of a total of 1229 (0.2 %); the blood transfusion rate for this procedure was reported to be 2 % [7]. The average objective cure rate has been reported to be 75 % for unilateral SSLF (USSLF), whereas the success rate for bilateral SSLF (BSSLF) ranges between 8 and 94 % [7].

Currently, sacrospinous ligament fixation (SSLF) is the most common transvaginal procedure described in the literature. Data on morbidity and outcomes are available because unilateral SSLF is a common procedure. A literature review provided data from more than 1000 patients [8 – 10].

The classic SSLF procedure requires good visualization of the surgical site. Deschamps suture passer is the tool most commonly used to pass a suture through the sacrospinous ligament with the aim of fixating the vaginal apex to the sacrospinous ligament. Good visualization of the surgical site is necessary when using this tool. One or two long retractors with wide surfaces can be used to provide the necessary visualization. Use of this retractor also requires experienced assistants and adequate lighting. A number of devices have been developed to facilitate the safe placement of sutures in deep tissues and to eliminate some of the above-mentioned problems. One approach consists of using hook-like instruments. In our study, we carried out surgery using a pelvic floor repair tissue-fixing anchor, the Anchorsure System® (Neomedic Ltd). This system does not require the help of a surgical assistant experienced in retraction or good lighting and can be performed by a single surgeon.

USSLF and BSSLF procedures were performed in patients with stage 3 and 4 prolapse using the anchoring system. We aimed to analyze the clinical and surgical findings and any intraoperative complications which occurred with these two procedures using this new anchoring system. Secondary outcomes (measures of morbidity) were also compared between the two groups.

Materials and Methods

The study was conducted in the Gynecology and Obstetrics Department of the Health Sciences University of Istanbul Gaziosmanpaşa Training and Research Hospital between January 2013 and February 2018. Our study was planned as a prospective randomized controlled study. Randomization was performed on the day before surgery, using patient protocol numbers in a computer program. The study was approved by the training plans coordination board (EPK) and the ethics board of the hospital. A letter of ethical approval (no. 45) was obtained from the Istanbul Gaziosmanpaşa Training and Research Ethics Committee. All patients included in the study were informed preoperatively about potential complications and the procedural technique; their consent was obtained and they all signed a consent form.

Ninety-three menopausal women (diagnosed as having amenorrhea with follicle-stimulating hormone [FSH] levels > 40 pg/mL) who were sexually active (any sexual activity in the three months prior to surgery) and had POP-Q stage 3 or 4 uterine prolapse were included in the study. Women who had mental, psychological or neurological disease or who had previously had a hysterectomy, and women who were unwilling to participate in the study were excluded. Vaginal hysterectomy (VH) was carried out in all menopausal women.

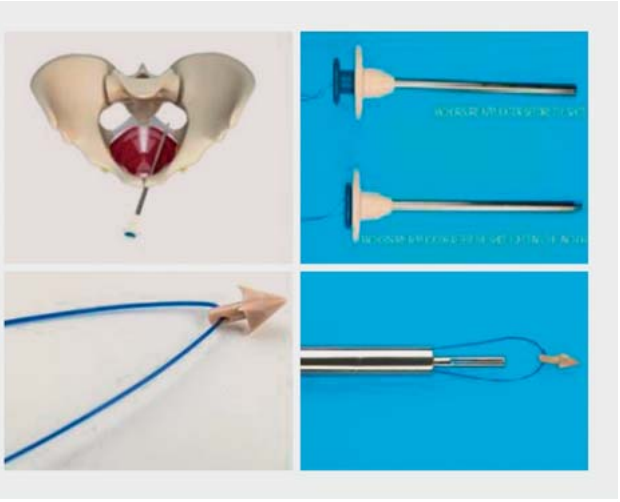
Patients in group 1 underwent VH with unilateral sacrospinous fixation. Patients in group 2 underwent VH with bilateral sacrospinous fixation. Anterior and/or posterior colporrhaphy was also performed when indicated. USSLF was performed in 52 patients

who were randomly selected (group 1), and BSSLF was performed in 41 patients (group 2). All sacrospinous ligament fixation procedures were performed using the anchoring device of the Anchorsure System® (▶ **Fig. 1**). The anchoring applicator is a thin, straight device designed for safe anchor placement at the sacrospinous ligament. It allows the anchor to be advanced to a maximum depth of 12 mm for maximum placement control. It is used for spinous fixation when treating vaginal prolapse after hysterectomy [11, 12].

Patients in group 1 and group 2 were operated on by 3 surgeons (MDs) with at least 10 years' experience in gynecological surgery. Vaginal hysterectomy was performed as described in Te Linde's Operative Gynecology [13]. VH was initiated with a circular incision around the vaginal mucosa. The uterosacral ligaments were clamped and sutured after opening the posterior peritoneum. Uterine vessels were clamped and cut after cutting the cardinal ligaments. The supravaginal septum was cut and the vesicouterine cavity was entered. The utero-ovarian and round ligaments were clamped, cut and the uterus was removed. The infundibulopelvic ligaments were clamped and cut, and the adnexae were removed. The pararectal area was identified within the posterior cuff where the vaginal remnant was located, and the rectum was moved away from the surgical site using the digital rectal maneuver to prevent rectal injury. The vaginal mucosa was dissected blindly and sharply from the rectovaginal septal plane, and the right rectovaginal fascial layers were passed through digitally or using the tip of the scissors at the apical level. The spinous process and the sacrospinous ligament were palpated. The rectovaginal fascial layers were enlarged digitally, and a retractor was placed to make room for the anchoring tool. The anchoring device was advanced to the anchoring point under the guidance of the index finger of the other hand. The applicator insert was advanced transvaginally until the anchor was in direct contact with the sacrospinous ligament. Once the tissue to which the anchor would be applied was reached, the anchor was placed in the sacrospinous ligament-coccygeus muscle complex at about 1.5–2 cm medial to the spinous process using the anchoring device. Prolene sutures at the tip of the anchor were then used to fixate the vaginal vault to the sacrospinous ligament. A suture was passed medially through the vaginal cuff in both groups. The procedure was repeated on the other side for patients who underwent bilateral fixation.

Data including patient age, parity, medical problems, menopausal status, and previous surgeries were obtained from the patients' history at the time of the procedure. Patients were categorized during physical examination using the POP-Q classification of prolapse.

Operations performed in addition to the SSLF procedure, duration of surgery, duration of hospital stay, and early complications including bleeding requiring transfusion, nerve injury, gastrointestinal injury and abscess in the urinary system or ischiorectal abscess or hematoma were recorded. Surgical failure and recurrence rates were evaluated 6 months postoperatively. Febrile morbidity (persisting fever of 38 degrees or more lasting for more than 24 hours and requiring the use of antibiotics) was diagnosed. Patients' re-presentations to hospital in the first week after the procedure were monitored for surgical complications.



▶ **Fig. 1** The Anchorsure applicator system. Prolapse & Anchoring System device (Source: Desarrollo E Investigación Médica Aragonesa SL).

Anatomical outcome

A simplified POP-Q system, a valid and reliable staging method to determine the extent of pelvic organ prolapse in individuals and the period of prolapse, was used to evaluate patients [14]. After patients had evacuated their bladders and were placed in the lithotomy position, they were asked to strain or cough vigorously. Measurements were taken based on 4 criteria points which included the cervix, posterior fornix, and anterior and posterior vaginal walls with the hymen level as the reference point. The level of prolapse was rated for each point as follows: stage 1, the most distal part of the prolapse is more than 1 cm over the hymen; stage 2, the most distal part of the prolapse is located between 1 cm over and 1 cm below the hymen; stage 3, the most distal part of the prolapse is more than 1 cm below the hymen; and stage 4, full eversion of the lower genital tract [14]. POP-Q staging was performed preoperatively in patients and again 6 months postoperatively in both groups. The results were recorded.

Statistical analysis

When evaluating the study data, in addition to descriptive statistical methods such as mean values and standard deviation, Student's t-test was used to compare normally distributed parameters and evaluate quantitative data, and Mann-Whitney U-test was used to evaluate parameters which were not normally distributed. The level of significance was accepted as $p < 0.05$.

Results

Demography

Between 2013 and 2018, VH and SSLF was performed in 93 patients with uterine prolapse in our clinic. USSLF was performed in 52 patients (group 1) and BSSLF was performed in 41 patients. The mean age in group 1 and group 2 was 62.76 ± 6.6 years and

▶ **Table 1** Characteristics of the groups.

Parameters	Group 1 Unilateral sacrospinous (n = 52)	Group 2 Bilateral sacrospinous (n = 41)	p value
Age (years)	62.76 ± 6,6	61,25 ± 8,7	0.3437
BMI (kg/m^2)	27.84 ± 4.61	29.1 ± 3.01	0.1337
Parity	3.88 ± 1.2	4.06 ± 1.04	0.4486
History of surgery			
▪ caesarean section	8 (15.3)	7 (17.07)	0.8185
▪ tubal ligation	11 (21.1)	8 (19.5)	0.85
▪ Colporrhaphy anterior	8	5	0.6595
▪ Colporrhaphy posterior	6	4	0.7843
▪ No prior surgery	26	24	0.416
▪ modified Gilliam-Dolares	1	–	0.3750
Topical or systemic estrogen use	8 (15.3)	6 (14.6)	0.92
Smoking status	4 (7.69)	3 (7.31)	0.94
DM	5 (9.61)	4 (9.75)	0.9988
Hypertension	10 (19.2)	8 (19.51)	0.974
Duration of menopause (years)	14.02 ± 3.03	15.09 ± 2.87	0.0870
COPD	5 (9.6)	3 (7.31)	0.6972
Preoperative POP-Q stage (range)	3.4 ± 0.4	3.5 ± 0.3	0.1862

DM: diabetes mellitus, COPD: chronic obstructive pulmonary disease

▶ **Table 2** Additional procedures performed concurrently with vaginal hysterectomy and sacrospinous ligament fixation.

Procedure	Group 1 Unilateral sacrospinous ligament fixation (n = 52)	Group 2 Bilateral sacrospinous ligament fixation (n = 41)	p value
	n (%)	n (%)	
Anterior colporrhaphy	42 (80.7)	35 (85.3)	0.5622
Posterior colporrhaphy	30 (57.6)	28 (68.2)	0.2977
Enterocele repair	7 (13.4)	6 (14.6)	0.865
TOT	11 (21.1)	10 (24.3)	0.7152

TOT: transvaginal tape-obturator

61.25 ± 8.7 years, respectively. Mean parity of group 1 and group 2 was 3.88 ± 1.2 and 4.06 ± 1.04, respectively. All patients were post-menopausal. All ninety-three patients had systemic diseases that did not constitute contra-indications for surgical procedures (mostly obstructive pulmonary diseases, diabetes mellitus, and hypertension). No statistical differences were found between the two groups with respect to age, body mass index (BMI), duration of menopause, topical or systemic estrogen use, chronic obstructive pulmonary disease, diabetes mellitus, hypertension, smoking, previous surgical history, parity, and POP-Q stage. The groups were similar in terms of their demographic characteristics and findings on physical examination (▶ **Table 1**).

Additional procedures performed simultaneously with the SSLF procedure are shown in ▶ **Table 2** for both groups. VH was performed in all patients in both groups. Procedures that most commonly accompanied the VH + SSLF procedure included anterior colporrhaphy, posterior colporrhaphy, enterocele repairs, and transvaginal tape-obturator (TOT) procedures. No significant differences were found between the two groups with respect to the frequency of additional procedures.

Adverse events

Mean hospital stay of patients from the USSLF group and the BSSLF group was 2.3 ± 0.9 days and 2.4 ± 0.8 days, respectively. The mean time used to fixate the sacrospinous ligament to the vaginal cuff was 76.6 ± 10.7 minutes for group 1 and 80.5 ± 11.8 minutes for group 2. No statistically significant differences were noted between the two groups with respect to mean duration of

▶ **Table 3** Comparison of variables and intraoperative, immediately postoperative, and late complications between the two groups.

Clinical outcomes and complications	Group 1 Unilateral sacrospinous ligament fixation with vaginal hysterectomy n: 52	Group 2 Bilateral sacrospinous ligament fixation with vaginal hysterectomy n: 41	p value
Operating time (min)	76.6 ± 10.7	80.5 ± 11.8	0.098
Hospital stay (days)	2.3 ± 0.9	2.4 ± 0.8	0.0604
Estimated blood loss (ml)	133 ± 40.9	140.4 ± 50.8	0.43
Complications			
Bladder injury, n (%)	0	0	ns
Rectal injury, n (%)	0	0	ns
Febrile morbidity	0	0	ns
Ischiorectal abscess	0	0	ns
Required blood transfusion	0	0	ns
Nerve injury	0	0	ns
Hematoma	0	0	ns
Significant recurrence	2 (3.84)	0	ns
Pop-Q stage postoperatively	0.7 ± 0.4	0.6 ± 0.3	0.1862
Cystocele recurrence	1 (1.9)	3 (7.3)	0.2056

ns: not significant

surgery, mean hospital stay, or mean blood loss. No bladder, rectal or nerve injury or serious bleeding requiring blood transfusion occurred intraoperatively. No patient developed any early complications such as ischiorectal abscess, hematoma or febrile morbidity. Stage 1 cystocele developed in 3 patients in the BSSLF group; there was no recurrence of rectoceles or enteroceles. In the USSLF group, a stage 2 cystocele was found in 1 patient. There were no statistically significant differences between the two groups with respect to cystocele occurrence. Based on the results of anatomical healing in patients, neither procedure was superior to the other. POP-Q staging was performed 6 months postoperatively, and no statistically significant differences were found between the two groups. The success rate for the group that underwent USSLF procedures using the anchoring system was 96.1% (50/52 women). The success rate for the BSSLF group, however, was 100% (41/41 women). No statistically significant difference was found between the two groups. Recurrence (vaginal cuff prolapse) was found in 2 patients in the USSLF group at follow-up 6 months postoperatively. There was no recurrence in the BSSLF group. The two cases with recurrence presented with stage 2 and stage 3 vaginal cuff prolapse, respectively. Laparoscopic sacrocolpopexy was performed in these patients (▶ **Table 3**).

Discussion

In our prospective randomized study, we investigated whether unilateral sacrospinous ligament fixation using the Anchorsure system or bilateral sacrospinous fixation procedures were superior. The system we used is based on placement of an anchor [15] and solves the problem of catching the suture without retraction. We used this device in all 93 patients.

We carried out a MEDLINE search and reviewed 22 articles on sacrospinous ligament fixation. SSLF was carried out in 1229 patients, and data for 1062 of these patients was obtained. The objective cure rate in these studies ranged between 8 and 94%. Recurrent pelvic relaxation developed in 109 patients out of 1062 (18%). Of these patients, 7 out of 81 with cystocele, 20 out of 32 with vaginal vault eversion, and 4 patients out of 24 with rectocele required re-operation. Based on the data obtained, it was concluded that SSLF is effective for the treatment of vaginal vault prolapse [7]. Lantzsch et al. reported on 123 patients who underwent SSLF; after a mean follow-up of 4.8 years, the rate of recurrent vault prolapse was 3.25%, and the cystocele rate was 8% [16]. Based on a retrospective analysis of 486 patients who had undergone pelvic reconstructive surgery, Porges and Smilen found that adding SSLF to VH in patients with stage 3 prolapse reduced the risk of recurrence from 15.8 to 6.7% [17]. In the series by Cruikshank and Cox consisting of 135 patients who underwent VH, SSLF was added to the procedure in 48 patients (35%). Vault prolapse was found in only one patient at the end of a mean follow-up period of 2 years [18].

Some studies do not recommend carrying out SSLF during VH. Colombo et al. [19] performed a retrospective case control study comparing 62 patients who underwent SSLF concurrently with VH and a control group of 62 patients who underwent culdoplasty; prolapse was found to have recurred in 17 (27%) patients in any vaginal area after follow-up periods ranging between 4 and 9 years, while recurrence in the control group was 9 in the same period (15%) ($p = 0.14$). Recurrence of vault prolapse was found in 5 patients (8%) and 3 patients (5%) in the SSLF and control groups, respectively ($p = 0.72$). The investigators concluded that prophylactic SSLF should not be recommended to patients with

uterovaginal prolapse. In our study, recurrence (vaginal cuff prolapse) was found in only 2 patients who underwent prophylactic unilateral SSLF in addition to hysterectomy. The success rate in the USSLF group using the anchoring system was 96.1% (50 women out of 52). Two patients in the USSLF group required re-operation. In the bilateral SSLF group, however, the success rate was 100% (41 women out of 41); no vaginal vault prolapse was found in any patient.

It appears that cystocele development is one of the leading long-term complications of sacrospinous ligament fixation. The reason for this is that the vaginal axis is shifted to a posterior and more horizontal position, resulting in greater exposure of the anterior vaginal wall to increased intraabdominal pressure. Figures ranging between 0 and 92% have been reported for cystocele development after SSLF [20]. In a study of 36 patients who underwent SSLF with a mean follow-up of 42 months, Holley et al. [21] reported that cystocele developed in 33 patients (92%), rectocele developed in 6 (17%), and enterocele was found in 2 patients (6%), while recurring vault prolapse was seen in 3 patients (8%). In contrast, in a retrospective case control study of patients who had SSLF with or without anterior colporrhaphy, Smilen et al. [20] suggested that SSLF did not result in increased development of cystocele when performed alone; however, adding anterior colporrhaphy to SSLF increased the risk. In the study by Szess and Karram, the postoperative anterior vaginal wall relaxation rate was reported to be 7.6% (81 out of 1062). In another study, the same authors reported a similar recurrence rate of 7% after anterior colporrhaphy [7, 22]. Sacrospinous ligament fixation can also be used for cystocele correction, as shown in the study by Fünfgeld et al. [23].

In our study, 3 (7.3%) patients developed stage 1 cystocele, and there was no recurrence of rectocele or enterocele. In the USSLF group, however, stage 2 cystocele was seen in 1 patient (1.9%). There were no significant differences between the two groups with respect to the recurrence of cystocele.

The recurrence of prolapse after SSLF can be due to several factors, including inherent tissue weakness in the patient, neuropathy related to wide vaginal dissection, or anatomical distortion caused by the surgical procedure. The reason for the lower rate of recurrent prolapse in our study may be due to the fact that the anchoring system we used required less vaginal dissection compared with SSLF carried out using the classic open technique.

Although rare, SSLF can have serious intraoperative and postoperative complications. The most frequent complication is hemorrhage related to pudendal vascular injuries. Other complications include pudendal and sciatic nerve injuries, bladder injuries, gluteal pain, and suture abscess [7, 24, 25]. Pohl and Frattarelli found that bilateral SSLF resulted in increased blood loss of 25–50 mL and that the operative time was 20–30 minutes longer compared with unilateral SSLF [26]. No serious intraoperative complications developed in our study.

We found only one article in the literature that directly compared unilateral and bilateral SSLF [25]. Jones et al. performed BSSLF in 62 patients and USSLF in 41 patients. Anatomical cure rates for both procedures were similar, irrespective of SSLF type (37/41 women [90.2%], unilateral SSLF, compared with 53/62 [85.5%], bilateral SSLF; $p = 0.56$). Women in the unilateral SSLF group had more blood loss and longer operative times than those in the bilateral SSLF group ($p = 0.02$). No statistically significant differences were found between the two groups in terms of intraoperative complications, transfusion rates, urinary retention, febrile morbidity, re-admissions to hospital, cystitis, postoperative incontinence, and hospital stay [27]. In our study, vaginal vault prolapse recurrence was found in 2 patients in the USSLF group. No recurrence of vaginal vault prolapse was seen in the BSSLF group. There were no significant differences between the two groups. In contrast to Jones' study, in our study there were no statistically significant differences between the two groups with regard to blood loss or duration of surgery. We attribute this to the anchoring system requiring less dissection and consequently reducing the amount of bleeding [27]. In the literature, sacrospinous fixation with Anchorsure under local anesthesia has also been described in older patients and patients with anesthesia risks [28].

Conclusions

Our study is limited by the relatively small number of patients and short follow-up period. But our study is noteworthy as it is only the second article in the literature that directly compares unilateral and bilateral SSLF procedures. Morbidity rates for bilateral SSLF performed using a new suturing device with an anchor appear to be no different from those seen with unilateral SSLF.

Compliance with Ethical Standards and Ethics Approval

The study was approved by the Ethics Committee of Gazi Osman Paşa Taksim Research and Education Hospital. Informed consent was obtained from all recruited subjects.

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None.

Conflict of Interest

The authors declare that they have no conflict of interest.

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