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ORIGINAL CONTRIBUTIONS

Clinical Outcomes of Laparoscopic Greater Curvature Plication and Laparoscopic Sleeve Gastrectomy: a Case-Matched Control Study

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Abstract

Background Laparoscopic greater curvature plication (LGCP) is a new bariatric procedure that is similar to laparoscopic sleeve gastrectomy (LSG) in that it uses a restrictive mechanism. Comparative studies between LGCP and LSG were still limited. The aim of this study was to compare the clinical outcomes of the two procedures based on the same clinical conditions.

Methods From January 2012 to December 2015, 260 patients with morbid obesity underwent LGCP and LSG in a single center. Data on patient demography, operation time, complications, hospital stay, body mass index loss, percentage of excess weight loss (%EWL), and improvement in comorbidities were collected. A propensity-matched analysis, incorporating pre-operative variables, was used to compare the short-term outcomes between LGCP and LSG.

Results Propensity matching produced 48 patients in each group. Patients who underwent LGCP were predominately female (75.5%, 41.1% of the LSG patients were female, $p = 0.028$). Baseline BMI and excess weight were significantly lower in the LGCP group ($p < 0.001$). The LSG group showed a greater decrease in excess body weight than the LGCP group (LSG, 47.36 ± 12.95% in 3 months, 57.97 ± 19.28% in 6 months, 66.28 ± 25.42% in 12 months; LGCP, 39.67 ± 12.58% in 3 months, 47.40 ± 19.30% in 6 months, 48.02 ± 20.17% in 12 months, $p = 0.008$, 0.032, 0.010). Perioperative complications and resolution of obesity-related comorbidities were not significantly different between the two groups.

Conclusion LGCP and LSG are both feasible and safe procedures for surgical weight reduction. In short-term follow-ups, LSG demonstrates a better excess body weight reduction while having perioperative complications similar to LGCP.

Keywords Sleeve gastrectomy · Gastric plication

Introduction

In modern society, obesity represents a major health issue that currently affects over 300 million individuals around the world. Not only is it related to increased risks of hypertension, type 2 diabetes, vascular diseases, dyslipidemia, certain cancers, and premature mortality, but it is also associated with psychosocial problems and an impaired quality of life [1–4]. Therefore, to reduce the potential risks associated with obesity-related chronic diseases, patients with morbid obesity require a multidisciplinary therapy. However, medical management of obesity using diet, exercise, behavior modification, and pharmacotherapy showed limited benefits regarding long-term weight loss [1].

Based on the current guidelines, bariatric surgery is currently considered to efficiently produce long-term body weight loss. Over the past few decades, laparoscopic sleeve gastrectomy (LSG) has been recognized as a safe and effective procedure for morbid obesity in terms of not only weight reduction but also markedly improving obesity-related

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comorbidities [5, 6]. It was widely applied in modern society [7]. However, the resection of normal tissue and irreversibility of gastric volume reduction remained major concerns of the patient. In contrast, laparoscopic greater curvature plication (LGCP) has emerged as a new procedure that involves multiple rows of sutures but does not partially resect the stomach. So far, LGCP has had acceptable short-term weight loss results, has exhibited almost no postoperative complications, and has improved patients' comorbidities [8–11]. The two procedures are similar in that they use restrictive mechanisms of weight loss. Several studies have been proposed for the comparison of the long-term outcomes and perioperative complications of the two procedures [12–22]. However, LGCP was thought to be less invasive and was recommended to patients with lower body mass indexes and fewer comorbidities. These studies might have contained bias on the effectiveness of LGCP as compared to LSG. The aim of this study was to compare the clinical outcomes of the two procedures based on similar clinical conditions.

Methods

All the procedures performed in this study were in accordance with the ethical standards of our institutional research committee. Informed consent was waived in this study due to the data collection being performed retrospectively.

The inclusion criteria were as follows: body mass index (BMI) ≥ 35 kg/m^2 with or without comorbidities and BMI ≥ 27.5 kg/m^2 with inadequately controlled lifestyle alternations and medical treatment [23]. By sharing decision-making, the patients were well informed with all the advantages and drawbacks for both the procedures, and making selection of procedures based on a patient's preference. We retrospectively reviewed 260 consecutive patients undergoing LGCP or LSG from January 2012 to December 2015 in a single center. Clinical data including gender, age, baseline BMI, weight excess, comorbidities, and biochemical examinations were collected. A propensity-matched analysis, incorporating pre-operative variables, was used to compare the outcomes between LGCP and LSG. Matching criteria were sex, age $\pm$ 10 years, BMI $\pm$ 6 kg/m^2, pre-operative comorbidities, and pre-operative biochemical exams (including HbA1c, fasting serum glucose and insulin levels, C-peptide, serum lipid profile, and creatinine levels).

All the patients were regularly followed up at 1, 3, 6, and 12 months after surgery. Primary endpoints included perioperative morbidity and mortality rates. Secondary endpoints were operative time, hospital stay, BMI, percentage of excess body mass index loss (%EBMIL), percentage of excess weight loss (%EWL), percentage of total weight loss (%TWL), and improvement of comorbidities.

Surgical Technique

For both the LGCP and LSG groups, the patients were placed in modified reverse + Trendelenburg position with both arms abducted. The surgeon stood in between the legs; the camera operator stood on the right side of the patient. Elastic and intermittent pneumatic compressing stockings were applied. A transumbilical two-site-modified single-incision laparoscopic surgery technique was used [24]. Three skin incisions were made at two sites of the abdomen, including two skin incisions along the natural fold of the umbilicus for a 10-mm port for the videoscope and a 12-mm port for a working channel, and one skin incision at the left lateral abdominal wall for a 5-mm port for another working channel. A 30°, 10-mm laparoscope was inserted into the 10-mm port to ensure an adequate operative arrangement. A 2-0 Prolene T suture (Ethicon, Johnson & Johnson, Somerville, NJ) with a straight needle was used for liver retraction and good exposure of the operative field. Both procedures started with the dissection of the greater gastric curvature 4 cm from the pylorus up to the angle of His. In both techniques, pouch calibration was achieved by passing a 32-Fr endoscope toward the pylorus.

In the LGCP group, plication was performed by applying two rows of extra-mucosal sutures. The first row was composed of interrupted stiches of 2-0 silk sutures. The second row was applied with interrupted stiches of 2-0 Ticron sutures (Fig. 1). In the LSG group, stapling started 4–5 cm from the pylorus with a green cartridge and was followed by a series of blue cartridges. Prolonged waiting times were used for the pre-compression and interstroke periods which allow 2 min in each firing interval to secure stapling [25–27]. This was followed by continuous running suture with 3-0 v-LOC.

In both groups, a drain was placed parallel to the gastric pouch. After surgery, an upper gastrointestinal endoscopy was routinely performed to assess the final stomach capacity. A

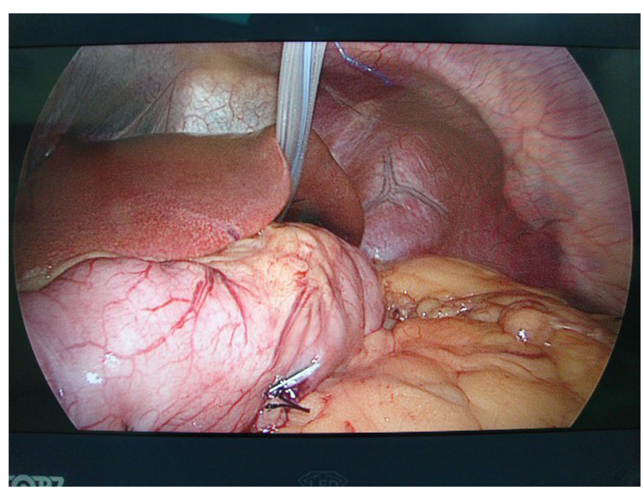

Fig. 1 Intraoperative picture of completed gastric plication procedure

16-Fr nasogastric tube was inserted after removal of the endoscope.

Postoperatively, the nasogastric tube was removed on the next day. The oral fluids were given after flatus passage. Patients were kept on a liquid diet for at least 2 weeks after surgery, and then, soft foods were given. Solid foods were gradually introduced 30 days after surgery.

Statistical Analysis

We used a one-to-one matching analysis between the LGCP and LSG groups on the basis of propensity score to minimize bias due to the nonrandom allocation of treatments among patients [28]. Propensity scores were estimated using a logistic model including the following variables: age, gender, BMI, weight excess, comorbidities, and biochemical data. The propensity score summarizes these features in a single variable that can be included in the analyses comparing perioperative outcomes across two groups. Data were expressed in the form mean value ± standard deviation for continuous normally distributed variables, and frequency with proportions for discrete variables. Continuous variables were compared using the independent-sample *t* test for parametric variables. Categorical variables were compared using the χ^2 test. Comparison of %EWL at 1, 3, 6, and 12 months in both groups of patients was done using *t* tests for multiple comparisons. Statistical analysis was considered to be significant when the probability value was below 0.05. Data analysis was performed using Statistical Package for the Social Sciences software (version 17.0; SPSS, Chicago, IL).

Results

A total of 53 patients underwent LGCP, and 207 patients had LSG for morbid obesity between January 2012 and December 2015. Patients who underwent LGCP were predominately female (75.5%, 41.1% of LSG patients were female, $p = 0.028$). Baseline BMI and excess weight were significantly lower in the LGCP group (34.42 ± 5.02 kg/m^2 and 34.13 ± 15.90 kg in LGCP, while 40.08 ± 6.65 kg/m^2 and 50.92 ± 20.51 kg in LSG, $p < 0.001$). Comorbidities and biochemical data were also different between the two groups. The proportions of hyperglycemia (11.3% in LGCP, 29.5% in LSG, $p = 0.001$) and coronary artery disease (0% in LGCP, 3.9% in LSG, $p = 0.004$) were higher in the LSG group. We matched the two groups based on the clinical characteristics and underlying diseases, and 48 patients of each group were chosen as shown in Table 1. In the LGCP group, 35 (72.9%) patients were female compared to 33 (68.8%) in the LSG group; the difference was not significant ($p = 0.657$). The mean age was 35.42 ± 9.94 years in the LGCP group, while it was 32.42 ± 9.00 years in the LSG group ($p = 0.125$). The mean BMI was 34.79 ± 5.11 kg/m^2 and 35.49 ± 5.55 kg/m^2 in the LGCP and LSG groups, respectively ($p = 0.522$). As shown in Table 1, there were no significant differences in pre-operative comorbidities and pre-operative biochemical exams between the two groups.

All procedures were performed laparoscopically without any conversions to open surgery. The mean operative time was 192.23 ± 42.9 min in the LGCP group, which was longer than the LSG group's mean operative time of 168.10 ± 57.6 min ($p = 0.022$). The mean hospitalization stay was 4.15 ± 0.9 days and 4.40 ± 0.8 days in the LGCP and LSG groups, respectively ($p = 0.158$). There were no perioperative major complications. However, early minor postoperative complications such as nausea, vomiting, and abdominal pain statistically occurred at the same frequency in the two groups as shown in Table 2. There were no mortalities.

Patients were followed up for 12 months. The BMI, %EBMIL, %EWL, and %TWL at 1, 3, 6, and 12 months postoperatively in the two groups are listed in Table 3. There were no significant differences between the two groups in %EBMIL and %EWL at 1 month. However, the LSG group had a greater percentage of excess weight loss at 3 ($p = 0.008$), 6 ($p = 0.032$), and 12 months ($p = 0.010$) postoperatively. Similar results were also noted on %EBMIL and %TWL. As shown in Table 4, there was no significant difference in blood sugar profile between the two groups.

Discussion

In this study, LSG is superior to LGCP in terms of providing greater %EWL at follow-ups of 3, 6, and 12 months. LSG has a shorter operative time than LGCP. No significant difference was found in perioperative complications and the resolution of obesity-related comorbidities.

During the past few years, bariatric surgery has become the more effective option of therapy for morbid obesity as compared to medical management [29–31]. With the improvement of surgical techniques and endostapling devices, LSG had become a popular procedure for morbid obesity. According to previous studies [12–21], 53.8–80% of excess weight loss was obtained at 12 months postoperatively, and a similar result of 66.08% was noted in our study. However, the resection of the stomach with a long staple line was also accompanied by the innate risk of postoperative bleeding and leakage. A review of the literature showed that the percentage of leakages and hemorrhages in LSG is about 1.2 and 3.6%, respectively [32]. Therefore, LGCP was first introduced by Talebpour et al. [33] in 2007. This procedure reduces gastric volume by multiple rows of sutures instead of partial gastric resection. Thus, the risk of bleeding or leakage might be reduced. As for weight reduction in LGCP, previous studies [12–21] showed that a 42.1–58.8% excess weight loss was obtained at

Table 1 Population description

	All patients			Propensity-matched patients		
	LGCP	LSG	*p*	LGCP	LSG	*p*
N	53	207		48	48	
Gender, *n* (%)						
Male	13 (24.5)	122 (58.9)	0.028	13 (27.1)	15 (31.3)	0.657
Female	40 (75.5)	85 (41.1)		35 (72.9)	33 (68.8)	
Age (years)	35.00 ± 10.11	34.50 ± 9.76	0.740	35.42 ± 9.94	32.42 ± 9.00	0.125
Body mass index (kg/m^2)	34.42 ± 5.02	40.08 ± 6.65	0.000	34.79 ± 5.11	35.49 ± 5.55	0.522
Weight excess (kg)	34.13 ± 15.90	50.92 ± 20.51	0.000	35.33 ± 16.18	38.53 ± 18.42	0.368
Comorbidity						
Hypertension, *n* (%)	19 (35.8)	96 (46.4)	0.164	19 (39.6)	21 (43.8)	0.683
Hyperglycemia, *n* (%)	6 (11.3)	61 (29.5)	0.001	6 (12.5)	4 (8.3)	0.509
Hyperuricemia, *n* (%)	4 (7.5)	14 (6.8)	0.842	4 (8.3)	8 (16.7)	0.222
Coronary artery disease, *n* (%)	0 (0)	8 (3.9)	0.004	0 (0)	0 (0)	
Fatty liver, *n* (%)	49 (92.5)	198 (95.7)	0.342	45 (93.8)	45 (93.8)	1.000
Osteoarthritis, *n* (%)	5 (9.4)	6 (2.9)	0.127	3 (6.3)	2 (4.2)	0.650
Biochemical exam						
HbA1c (%)	5.73 ± 1.28	6.23 ± 1.34	0.016	5.76 ± 1.33	5.56 ± 0.56	0.354
Glucose AC (mg/dL)	103.7 ± 38.60	110.4 ± 36.63	0.245	105.26 ± 40.30	98.55 ± 25.24	0.336
Insulin AC (mg/dL)	15.7188 ± 12.88	23.288 ± 17.44	0.001	16.00 ± 13.27	17.23 ± 13.73	0.671
C-peptide (mg/dL)	3.3078 ± 1.81	4.0898 ± 1.983	0.012	3.39 ± 1.85	3.05 ± 1.22	0.320
Cholesterol (mg/dL)	195.68 ± 3.10	190.98 ± 37.68	0.434	196.44 ± 44.27	193.45 ± 42.67	0.738
HDL-c (mg/dL)	44.40 ± 9.38	42.10 ± 8.47	0.088	44.40 ± 9.66	45.13 ± 8.46	0.696
Triglyceride (mg/dL)	131.83 ± 69.26	150.37 ± 82.13	0.133	133.21 ± 70.43	139.85 ± 75.79	0.659
LDL-c (mg/dL)	125.01 ± 36.42	121.62 ± 33.52	0.522	125.64 ± 37.78	122.22 ± 40.05	0.669
Uric acid (mg/dL)	5.97 ± 1.48	6.42 ± 1.55	0.058	6.09 ± 1.45	6.48 ± 1.80	0.260
Creatinine (mg/dL)	0.68 ± 0.17	0.72 ± 0.24	0.338	0.69 ± 0.18	0.72 ± 0.20	0.386
e-GFR (mg/dL)	109.60 ± 22.77	112.11 ± 24.37	0.508	109.75 ± 22.91	107.49 ± 23.14	0.637

Data are expressed as the mean ± standard deviation, or *n* (%), as appropriate. We inferred statistical significance for $p < 0.05$

LGCP, laparoscopic greater curvature plication; *LSG*, laparoscopic sleeve gastrectomy; *HbA1c*, hemoglobin A1c; *HDL-c*, high-density lipoprotein cholesterol; *LDL-c*, low-density lipoprotein cholesterol; *e-GFR*, estimated glomerular filtration rate

12 months after surgery. In our study, a comparable outcome of 48.02% was noted. Patients of both groups receiving surgery achieved high body weight loss and most importantly, no major perioperative complication or reoperation was noted.

Table 2 Early postoperative complications

	All patients			Propensity-matched patients		
Complications	LGCP	LSG	*p*	LGCP	LSG	*p*
N	53	205		48	48	
Nausea, *n* (%)	3 (5.7)	7 (3.4)	0.452	2 (4.2)	2 (4.2)	1.000
Vomiting, *n* (%)	19 (35.8)	42 (20.5)	0.034	18 (37.5)	11 (22.9)	0.122
Abdominal pain, *n* (%)	6 (11.3)	15 (7.3)	0.333	5 (10.4)	6 (12.5)	0.752
Stenosis, *n* (%)	1 (1.9)	0 (0.0)	0.322	0 (0)	0 (0)	
Operative time, (min)	193.49 ± 43.9	167.26 ± 49.04	0.000	192.23 ± 42.9	168.10 ± 57.6	0.022
Length of stay, (days)	4.23 ± 1.2	5.07 ± 5.36	0.258	4.15 ± 0.9	4.40 ± 0.8	0.158

Data are expressed as the mean ± standard deviation, or *n* (%), as appropriate. We inferred statistical significance for $p < 0.05$

LGCP, laparoscopic greater curvature plication; *LSG*, laparoscopic sleeve gastrectomy

Table 3 Difference in BMI, %EBMIL, %EWL, and %TWL between matched LGCP and LSG groups

Group	Value at:			
	1 month	3 months	6 months	12 months
LGCP				
N (%)	45 (93.7)	38 (79.2)	29 (60.4)	23 (47.9)
BMI, kg/m^2, mean ± SD	31.22 ± 4.62	29.88 ± 4.11	28.67 ± 4.05	28.53 ± 3.52
%EBMIL, mean ± SD	29.37 ± 11.71	39.59 ± 12.60	46.51 ± 18.84	47.98 ± 20.22
%EWL, mean ± SD	29.46 ± 11.67	39.67 ± 12.58	47.4 ± 19.30	48.02 ± 20.17
%TWL, mean ± SD	9.96 ± 2.78	13.53 ± 3.44	15.51 ± 5.49	16.27 ± 6.05
LSG				
N (%)	48 (100)	44 (91.7)	36 (75)	24 (50)
BMI, kg/m^2, mean ± SD	31.53 ± 4.82	29.53 ± 4.39	27.89 ± 3.90	27.90 ± 5.03
%EBMIL, mean ± SD	32.24 ± 13.19	47.42 ± 12.46	58.24 ± 18.84	65.58 ± 25.58
%EWL, mean ± SD	33.12 ± 12.13	47.36 ± 12.95	57.97 ± 19.28	66.08 ± 25.42
%TWL, mean ± SD	11.43 ± 2.30	16.68 ± 3.36	19.93 ± 5.24	23.76 ± 5.30
BMI *p* value	0.752	0.709	0.434	0.621
%EBMIL *p* value	0.271	0.006	0.015	0.012
%EWL *p* value	0.142	0.008	0.032	0.010
%TWL *p* value	0.007	0.000	0.002	0.000

Data are expressed as the mean ± standard deviation, or *n* (%), as appropriate. We inferred statistical significance for $p < 0.05$

LGCP, laparoscopic greater curvature plication; *LSG*, laparoscopic sleeve gastrectomy; *BMI*, body mass index; *%EBMIL*, percentage of excess body mass index loss, *%EWL*, percentage of excess weight loss, *%TWL*, percentage of total weight loss

As for the outcome of body weight loss between LSG and LGCP, Shen et al. [12] reported the comparison of the two groups in a total of 39 patients. The short-term outcome of LGCP was inferior to LSG, but LGCP had a lower self-paid.

Table 4 Difference in blood sugar between matched LGCP and LSG groups

Group	Value at:			
	1 month	3 months	6 months	12 months
LGCP				
N (%)	45 (93.7)	38 (79.2)	29 (60.4)	23 (47.9)
HbaA1c (%), mean ± SD	5.64 ± 1.04	5.30 ± 0.33	5.28 ± 0.33	5.18 ± 0.34
Glucose AC (mg/dL), mean ± SD	92.00 ± 9.32	90.24 ± 6.57	89.42 ± 7.89	90.12 ± 5.95
Insulin AC (mg/dL), mean ± SD	8.04 ± 3.80	8.11 ± 4.25	9.47 ± 5.60	7.84 ± 3.90
C-peptide (mg/dL), mean ± SD	2.21 ± 0.94	2.11 ± 1.06	2.70 ± 1.32	1.75 ± 0.75
LSG				
N (%)	48 (100)	44 (91.7)	36 (75)	24 (50)
HbaA1c (%), mean ± SD	5.39 ± 0.73	5.16 ± 0.48	5.14 ± 0.23	4.96 ± 0.26
Glucose AC (mg/dL), mean ± SD	91.39 ± 22.86	89.31 ± 12.33	87.45 ± 7.68	87.84 ± 9.44
Insulin AC (mg/dL), mean ± SD	9.84 ± 6.68	8.98 ± 6.70	8.24 ± 4.04	7.54 ± 3.04
C-peptide (mg/dL), mean ± SD	2.81 ± 2.29	2.35 ± 1.26	2.17 ± 0.88	1.99 ± 0.74
HbA1c *p* value	0.322	0.249	0.155	0.076
Glucose AC *p* value	0.875	0.694	0.338	0.400
Insulin AC *p* value	0.284	0.635	0.487	0.836
C-peptide *p* value	0.282	0.555	0.222	0.508

Data are expressed as the mean ± standard deviation, or *n* (%), as appropriate. We inferred statistical significance for $p < 0.05$

LGCP, laparoscopic greater curvature plication; *LSG*, laparoscopic sleeve gastrectomy; *HbA1c*, hemoglobin A1c

The economic benefits may influence the clinical decision-making of patients, especially in developing countries. But in Taiwan, with the National Health Insurance, the reimbursements for the two procedures are similar. In the year following Shen et al. [12], Tamer et al. [13] proposed a larger comparison between LGCP and LSG; 140 patients were enrolled and a better reduction in body weight with LSG was noted in the short-term follow-up. However, the baseline clinical characteristics showed a statistically significant lower BMI in the LGCP group. This might be a potential bias of the surgical results. Regular follow-ups were relatively difficult in most patients, regardless of whether they were morbidly obese. Thus, long-term outcomes were lacking in the prior study. Grubnik et al. [14] reported on a multicenter prospective randomized trial that began in 2010 with a total of 54 morbidly obese patients being allocated to either the LGCP group or the LSG group. Equal effectiveness was noted for both groups in short-term follow-ups, but 2-year follow-ups showed better outcomes in the LSG group. Although the baseline clinical characteristics were similar between the two groups, the patient number was relatively small in this study.

Two meta-analyses comparing LGCP to LSG have been published in the past few years. One was published by Tang et al. [20] in 2015. LSG was found to be better in %EWL at follow-ups of 3, 6, and 12 months, and it was even associated with fewer adverse event. However, there were no differences in operation time and the resolution of obesity-related comorbidities. Ye et al. [21] also reported similar results. Shorter postoperative hospital stays and greater excess weight loss at 3-year follow-ups were seen in the LSG group as compared to the LGCP group. But even with the relatively strong evidence of these meta-analyses, the comparison of the baseline data and comorbidities was still ignored in these studies. Thus, there might be a selection bias in the study results. Comparison of the two procedures based on propensity-matched analysis was lacking.

In our study, a total of 260 patients were enrolled between January 2012 and December 2015, of whom 53 underwent LGCP and 207 underwent LSG. As noted also in prior studies, the population characteristics of the LGCP group showed a lower mean BMI and fewer comorbidities compared to the LSG group. The female gender was also dominant in the LGCP group, but it was not dominant in the LSG group. The baseline difference between the two groups was the most mentioned aspect of the previous study that might influence the surgical outcomes of the two procedures. For surgeons, LGCP was first recommended for patients with lower body mass index and fewer comorbidities. In order to minimize baseline demographic and selection bias such as gender, age, body mass index, and pre-operative comorbidity, we matched the two groups based on clinical characteristics and finally 96 patients were enrolled in our study with 48 patients in each group. After matching, the baseline data showed no significant difference in BMI, comorbidities, and other clinical characteristics between the two groups.

In our study, the operative time was longer in the LGCP group. This can be explained by the improvement of endostapling devices and the fact that LSG uses fewer laparoscopic sutures. The LSG group showed significantly greater decreases in excess body weight than the LGCP group at 3 months, 6 months, and 12 months after surgery. In the general concept of current bariatric surgery, LSG and LGCP utilize gastric volume restriction to achieve body weight reduction. Furthermore, in the concept of change in endocrine pattern, LSG further reduces the secretion of several known hormones, such as ghrelin, that are proven to be related to obesity and body weight gain [34]. The results in our study may further reveal the clinical benefits of LSG in body weight reduction. Perioperative complications were not significantly different between the LGCP and LSG groups.

There are limitations in our study including the bias due to the retrospective study design, lack of longer follow-up for the assessment of weight loss and comorbidity improvement, and lack of data about patient satisfaction. A longer follow-up and prospective randomized trials are needed to further investigate the effectiveness of the different kinds of bariatric surgeries.

Conclusion

LGCP and LSG are both feasible and safe procedures for surgical weight reduction. In short-term follow-ups, LSG demonstrates a better excess body weight reduction and has perioperative complications similar to LGCP.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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