学　位　論　文

Endoscopic ligation with O-ring closure for mucosal defects after rectal endoscopic submucosal dissection: A feasibility study (with video)

香川大学大学院医学系研究科

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多田　尚矢

Endoscopic ligation with O-ring closure for mucosal defects after rectal endoscopic submucosal dissection: A feasibility study (with video)

Naoya Tada, MD, Hideki Kobara, MD, PhD, Noriko Nishiyama, MD, PhD, Kazuhiro Kozuka, MD, PhD, Takanori Matsui, MD, PhD, Taiga Chiyo, MD, PhD, Nobuya Kobayashi, MD, PhD, Tatsuo Yachida, MD, PhD, Shintaro Fujihara, MD, PhD, Tsutomu Masaki, MD, PhD.

Department of Gastroenterology and Neurology, Faculty of Medicine, Kagawa University, Kagawa, Japan

Short Title: Endoscopic ligation with O-ring closure for rectal defect

Correspondence author: Naoya Tada, MD

Department of Gastroenterology and Neurology, Faculty of Medicine, Kagawa University, 1750-1 Ikenobe, Miki, Kita, Kagawa 761-0793, Japan

Tel: +81-87-891-2156; Fax: +81-87-891-2158

E-mail: tada.naoya@kagawa-u.ac.jp

Number of Tables: 4 (Supplementary Table:1)

Number of Figures: 14

Word count: 3,441 words (Abstract 236 words, Body text 3,205 words)

Keywords: Rectal endoscopic submucosal dissection, Mucosal defect closure, Postoperative adverse events

Abstract

Introduction: Mucosal defect closure after colorectal endoscopic submucosal dissection (ESD) may prevent post-ESD adverse events. Delayed bleeding is a particular concern in the rectum due to the presence of numerous blood vessels. However, rectal defect closure often fails due to the thick rectal wall. This study aimed to examine the feasibility of our newly developed endoscopic ligation with O-ring closure (E-LOC) for defects after rectal ESD.

Methods: This was a prospective observational study conducted at single institution. After excluding two patients with tumors mostly extending into the anal canal, the study cohort comprised 30 consecutive patients who underwent ESD of rectal neoplasms between July 2020 and July 2021. E-LOC using an endoscopic variceal ligation device was performed for closing mucosal defects after rectal ESD. The primary outcome was the complete closure rate. The secondary outcomes were the delayed bleeding rate, E-LOC procedure time, sustained closure rates on postoperative day (POD) 3, and E-LOC-associated complications.

Results: Complete closure of the defect (median defect size 29.0 mm) was successfully achieved in 24 cases (80%). Delayed bleeding occurred in one case with incomplete closure (3.3%). The median E-LOC procedure time was 25.5 minutes (interquartile range,

20.0–30.0 minutes). The sustained closure rates were 83.3% (20/24) on POD 3 in the 24 cases with complete closure. No E-LOC-associated complications occurred.

Discussion/Conclusions: E-LOC was feasible for defect closure after rectal ESD, and probably led to a decreased incidence of delayed bleeding.

Introduction

Endoscopic submucosal dissection (ESD) has been established as a curative treatment for colorectal and upper gastrointestinal epithelial neoplasms [1-4]. ESD enables en bloc resection, regardless of the lesion size. However, colorectal ESD results in various complications, such as delayed bleeding (DB) and delayed perforation (DP). The reported incidences of DB and DP in the colorectal region are 1.5%–3.7% and 0.1%–0.4%, respectively [1,3-5]. The rectum has a higher DB rate than other parts of the colon because of the abundant submucosal vessels in the lower rectum [6-9]. The efficacy of defect closure after colorectal endoscopic resection in preventing DB has been described in several clinical settings [10-13]. However, anatomically, the rectum has a particularly thick wall. Therefore, it is more difficult to approximate the mucosal defect in the rectum than in other parts of the colon [14]. We developed a novel defect closure method using an O-ring and nylon thread (endoscopic ligation with O-ring closure; E-LOC), which potentially enables effective closure of the mucosal defect after ESD [15,16].

The present study aimed to evaluate the feasibility and safety of E-LOC after rectal ESD.

Materials and Methods

Patients

This was a prospective observational study conducted at a single institution. We enrolled 32 consecutive patients with rectal neoplasms who underwent rectal ESD between July 2020 and July 2021. The inclusion criteria for colorectal ESD were determined based on the Japan Gastroenterological Endoscopy Society guidelines.[3] There were no assigned limits regarding the lesion size. The inclusion criteria were: superficial neoplasms of ≥20 mm, or <20 mm with the non-lifting sign, including residual neoplasms after endoscopic mucosal resection or small neuroendocrine tumors with a diameter of <1 cm. In addition, rectal ESD was indicated when the micro-surface and vascular pattern of superficial neoplasms obtained using image-enhanced endoscopy satisfied the diagnostic criteria of early carcinoma. Histological confirmation of carcinoma in preoperative biopsy was not mandatory. The following lesions were excluded: i) lesions with suspected deep submucosal invasive adenocarcinoma (submucosal invasion depth >1000 μm), ii) lesions covering >10% of the total circumference of the anal canal. Patients with suspected submucosal invasive carcinoma or neuroendocrine tumor underwent chest, abdominal, and pelvic CT and/or endoscopic ultrasonography before ESD to check for metastases and determine the clinical stage. The patients' baseline characteristics and treatment outcomes were analyzed. All patients provided written informed consent for the

procedure to be performed. No patient refused E-LOC of the defect after rectal ESD during the study period. This study protocol was approved by the Ethics Committee of Kagawa University (approval no. 2020-042, approval date July 9th, 2020) and was performed in accordance with the Declaration of Helsinki.

Rectal ESD procedure

Patients were treated either under sedation with pentazocine and midazolam or without sedation, depending on the patient's preference or at the operator's discretion. ESD procedures were performed using a video endoscope (GIF-Q260J or GIF-H260Z; Olympus) with CO_2 insufflation during the procedure. A DualKnife (KD-650Q; Olympus) was used for all ESD procedures, and an IT knife-nano (Olympus) and a Coagrasper were used as appropriate for each lesion at the operator's discretion. The electrosurgical unit was a VIO 300D (ERBE, Elektromedizin, Tübingen, Germany). The electrical power settings for the DualKnife were: forced-coagulation mode effect 4, 40 W, for mucosal marking; endo-cut 1 mode effect 2, duration 3, interval 3, for mucosal incision; and swift-coagulation mode effect 2, 50 W, for submucosal dissection. The setting for the Coagrasper was soft-coagulation mode effect 4, 50 W. The ESD strategy and the use of a countertraction device depended on the discretion of each endoscopist.

Visible vessels on the post-ESD mucosal defect were routinely coagulated after complete resection to reduce the risk of DB. All ESD procedures were performed by a single endoscopist who had experienced colorectal ESD in more than 20 cases.

E-LOC procedure

The schema and preparation of E-LOC are shown in Figure 1 and 2, respectively. After complete ESD of a 25-mm-diameter laterally spreading tumor in the lower rectum (Figure 3A), the defect after rectal ESD (Figure 3B) was closed using the following E-LOC procedure (Supplementary viedo) [15,16]. First, a 2-cm-diameter 3-0 surgical nylon loop was fixed at the two lateral edges of the defect by hemoclips (HX-610-090; Olympus, Tokyo, Japan), and/or another hemoclip was applied to the muscle layer (Figure 3C). To close the center of the whole defect, E-LOC with the clips anchored on the three mucosa-muscle-mucosa points was performed at least once to prevent the creation of a submucosal pocket by mucosal suturing. Next, a Coagrasper (FD-411QR; Olympus) or a clip applicator (ZP-S195S; Zeon Medical, Tokyo, Japan) was used to pull the loop into the cap of an endoscopic variceal ligation device (MD-48720U; Sumius, Tokyo, Japan) (Figure 3D). Then, these deployed hemoclips were captured into the cap (Figure 3E), and an O-ring was fired around them (Figure 3F). This approximated the defect, and was

defined as the first completion of the E-LOC procedure. The extra nylon loop was excised using the Loop cutter (FS-5L-1; Olympus, Tokyo, Japan). This procedure was repeated from one end of the defect to the other (Figure 3G). One O-ring was used for every 1 cm length of defect; for example, three O-rings were used for a 3-cm-long defect. When an exposed mucosal defect appeared in the gap between the deployed O-rings, additional clips were applied to close the defect and achieve complete closure (CC). Finally, the defect was closed completely (Figure 3H). The closure of 90% or more of the maximum defect length was defined as CC, closure of 50% to 90% was defined as partial closure (PC), and closure of less than 50% was defined as unable to perform closure (UC). All E-LOC procedures were performed by a single operator among six endoscopists who had experienced E-LOC in more than two cases.

Exclusion criteria for E-LOC

Lesions that extended to the anal canal and involved more than 10% of the whole circumference of the anal canal were excluded. The reasons for the exclusion of these lesions were: the difficulty in achieving complete defect closure in the anal canal because of the narrow lumen, and the increase in patient discomfort after hemoclip closure in the anal canal that is innervated by somatosensory nerves and is highly sensitive to painful

stimuli.

Clinical pathway for rectal ESD and follow-up endoscopy

The patients were instructed to stay in bed until the next morning after the rectal ESD. They were then allowed to move freely and drink from the day after the ESD. From day 2 after rectal ESD, the patients were started on a liquid diet, and the form of the diet was increased each day. The patients did not take any additional medications, such as laxatives, after undergoing rectal ESD. Patients with no complications were discharged on day 8. Follow-up rectal endoscopy was performed on postoperative day (POD) 3 to check for wound dehiscence and post-ESD DB and DP.

Study outcomes

The primary outcome was the CC rate achieved by E-LOC. Complete defect closure was defined as successful closure that covered more than 90% of the maximum length of the wound surface. PC and UC were defined as incomplete closure. The secondary outcomes were the incidence of DB (defined as evident hematochezia that required endoscopic hemostasis within 14 days after ESD), incidence of DP (defined as perforation within 14 days after ESD), procedure time of E-LOC (defined as the time between the

fixing of the first hemoclip and the release of the last O-ring), sustained closure rate on POD 3 (defined as maintaining the mucosal defect closure of more than 90% in cases with CC), and E-LOC-associated complications. To evaluate the clip durability, the wound dehiscence rates on POD 3 were analyzed in cases with CC and PC (defined as wound dehiscence from CC to PC or UC, and from PC to UC).

Statistical analysis

Continuous variables were presented as medians with 25th and 75th percentiles. Categorical variables of patients with complete versus incomplete defect closure were compared using Fisher's exact test, Wilcoxon signed-rank test or Mann-Whitney U test. $P < 0.05$ was considered statistically significant. All statistical analyses were conducted using JMP Pro 15.1.0 (SAS Institute Inc., Cary, NC, USA).

Results

In accordance with the exclusion criteria, two patients were excluded because they had tumors involving more than half circumference around the anal canal, which led to concerns about possible post-closure stenosis and pain and discomfort around the anus. The final study cohort comprised 30 patients who underwent E-LOC for a mucosal defect

after rectal ESD. The flowchart of patient enrollment is shown in Figure 4. The CC rate was 80% (24/30). The location of the lesion was the upper rectum in five cases, the middle rectum in six cases, and the lower rectum in 19 cases (including the anal canal in three cases). The baseline characteristics of the patients and lesions are shown in Table 1.

The outcomes of rectal ESD are summarized in Table 2. The median lesion size and resected specimen size (based on histologic assessment) were 19.0 mm (interquartile range (IQR), 8.5–26.5 mm) and 29.0 mm (IQR, 23.5–31.8 mm), respectively. En bloc resection and complete ESD of all lesions were successfully achieved without complications. The total curative resection rate was 56.7%; the curative resection rate was 72.7% for 22 superficial neoplasms and 12.5% for eight neuroendocrine tumors. Most cases with neuroendocrine tumors except one had lymphovascular invasion. The degree of submucosal fibrosis was classified as F0 (no fibrosis), F1 (mild fibrosis), and F2 (severe fibrosis) in accordance with previous reports [17].

The outcomes of E-LOC are summarized in Table 3. E-LOC achieved CC of the defect in 80% of cases (24/30). The CC rates based on the defect size were 86.7% (13/15) for lesions <30 mm and 73.3% (11/15) for lesions ≥30 mm. The median procedure time of E-LOC was 25.5 minutes (IQR, 20.0–30.0 minutes). There was no post-ESD DP. DB occurred in one patient with renal hemodialysis who had incomplete closure after E-LOC.

Thus, the DB rate was 3.3%. Among the 24 cases with CC, the sustained closure rates were 83.3% (20/24) on POD 3. Among 27 cases (including 24 with CC and three with PC), the wound dehiscence rates were 22.2% (6/27) on POD 3. No E-LOC-associated complications occurred. A comparative analysis of the CC vs. incomplete closure groups is shown in Table 4. While the incidence of DB did not significantly differ between groups, there was a significantly higher prevalence of low rectal tumors involving the anal canal in the incomplete closure group than in the CC group (P=0.0049). The degree of submucosal fibrosis did not significantly differ between the complete and incomplete closure groups. The defect size per single muscle layer clip was similar in the closure and incomplete closure groups (20 mm vs. 19.5 mm, P>0.05). The CC rate was 71.4% (5/7) for lesions extending across the rectal fold. The prevalence of a lesion extending across the rectal fold did not significantly differ between the complete and incomplete closure groups (20.8% vs. 33.3%, P>0.05). A representative case with sustained CC by E-LOC is shown in Figure 5.

Discussion

The present study found that E-LOC achieved an acceptable successful closure rate of 80% for large rectal defects, without causing complications.

The clinical question of whether prophylactic closure of the mucosal defect after colorectal ESD is efficacious is still unclarified.

A recent randomized controlled trial (RCT) [18] and several meta-analyses [19-21] revealed that prophylactic closure after polypectomy reduces DB in the right side of the colon. Furthermore, several retrospective studies [12,22] and a meta-analysis [23] showed the efficacy of prophylactic closure in preventing DB after colorectal ESD. The need for prophylactic closure may be influenced by the procedural differences between polypectomy and ESD because of the differences in the density and thickness of the vessels in the dissected layer. In contrast, a RCT [24] that assessed the efficacy of defect closure in preventing post-coagulation syndrome after colorectal ESD found no significant effect regarding DB. However, because DB was not set as a primary outcome in the previous studies, there are concerns about the sample size calculations. Considering the low rate of DB occurrence and the costs associated with defect closure, it may be beneficial to evaluate the clinical significance of defect closure in patients at high risk of DB. Thus, a large-scale multicenter RCT of a reliable closure technique in patients at high risk of DB is needed to reveal the clinical significance of colorectal post-ESD defect closure in preventing DB.

It is widely acknowledged that the rate of DB after rectal ESD (7.9%–8.9%) is higher

than ESD in other parts of the colon (1.2%–1.5%) [7, 25]. A previous study reported that 14 of 509 lesions developed DB after colorectal ESD [7]. Eight of these 14 lesions (57.1%) were in the rectum (P<0.001), resulting in a DB rate after rectal ESD of 8.9% (8/90) [7]. In our experience of colorectal ESD between January 2012 and March 2019, DB occurred in 16 of 407 lesions (3.9%), and 75% (12/16) of the lesions with DB were found in the rectum (P<0.001). Prophylactic closure of the rectal mucosal defect after ESD was not performed in all cases. In our retrospective cohort, 105 cases that met the inclusion criteria were extracted as a historical control group (non-closure group). Although there was no significant difference in the DB rate between the historical control and E-LOC groups (11.4% vs. 3.3%), the rate tended to be lower in the E-LOC group (Supp File Table S1). A previous study found that defect closure was most difficult in the lower rectum due to the wall thickness, leading to a low CC rate of 33.3% (1/3) [14]. Mucosal closure methods may lead to premature mucosal dehiscence because of the formation of mucosal bridges and cavities [26]. Therefore, because of the high DB rate and anatomical characteristics of the rectum, we focused on the closure ability and durability of E-LOC in the rectum.

Several closure methods for colorectal ESD have recently been developed [14,27-31]. The reported CC rate of ligation using the double-loop clips technique for defect

closure after colorectal ESD is 88.5% (23/26) [14]. Our study also showed similar successful closure rate of 80% (24/30). In the analysis of risk factors for failure, the previous study found that defect closure in the lower rectum was the most difficult due to the wall thickness, giving a low CC rate of 33.3% (1/3) [14]. In contrast, the E-LOC procedure performed in the present study achieved a high CC rate of 73.7% (14/19) for lesions in the lower rectum. However, in the overall cohort, there were six incomplete closure cases, comprising PC in three cases and UC in three cases. Of these incomplete closure cases, three lesions involved the anal canal, one was a recurrent lesion with severe massive fibrosis after transanal endoscopic microsurgery, one lesion was close to the orifice of the anal canal, and one lesion was located behind the folds of the middle Houston valve. As shown in Table 3, the rate of successful CC significantly differed between the lesions involving the anal canal vs. the lesions not involving the anal canal (P=0.0049), although the present study assigned exclusion criteria related to the anal canal. In addition, three cases with a defect larger than 50 mm were analyzed. The maximum size of CC was 56mm. Although CC was achieved in two of these three cases, one case with 86 mm lesion extending into the anal canal was PC. Consequently, E-LOC showed a limited ability to close defects in the anal region. A representative case with incomplete closure by E-LOC is shown in Figure 6.

In our study, DB occurred on POD 5 in one patient who had UC due to severe fibrosis in the submucosa and the muscle layer after transanal endoscopic microsurgery. Massive bleeding with a decreased hemoglobin concentration occurred, requiring endoscopic hemostasis and blood transfusion. The scarring of the muscle layer made it difficult to approximate the defect, resulting in UC. This patient was also receiving hemodialysis and single antiplatelet medication, which are high risk factors for DB. This case may indicate the importance of complete and sustained defect closure in patients at high risk of DB.

The onset of DB is most frequently within 7 days after colorectal ESD [3], and the median time from colorectal ESD to the onset of DB is reportedly 2 days (range, 1–14 days) [11]. Therefore, maintenance of the defect closure for the first few days after ESD might be important in preventing DB. However, few studies have examined the sustained closure rate after post-ESD defect closure. One study reported that defect closure using an endoscopic hand-suturing technique after colorectal ESD (five lesions in the colon and six in the rectum) was successful in 73% of cases (8/11) and sustained in 64% (7/11) on POD 3–4 [29]. In our study, the sustained closure rates were 83.3% (20/24) on POD 3 in the 24 cases with CC. Similarly, the wound dehiscence rates were 22.2% (6/27) on POD

3 in the total 27 cases (24 with CC and three with PC). The overall cost of E-LOC is approximately 195USD, comprising a median of eight clips (68USD) and a single endoscopic variceal ligation device (127USD). Although this cost is relatively low, the cost-effectiveness of defect closure after rectal ESD may limit its indication to patients at high risk of DB. E-LOC has another advantage in the management of a large perforation site during gastric ESD [32] and full-thickness resection [33]. Approximation of the large defect by E-LOC minimizes the air leakage and facilitates the consecutive over-the-scope clip (Ovesco Endoscopy) closure. Despite the disadvantage of E-LOC regarding scope reinsertion in the proximal colon, it has the potential ability to handle large colorectal perforations.

Our study has several limitations. First, this was an observational study with a single arm and a small number of cases treated in a single institution. Future prospective studies with a larger sample size comparing the E-LOC group with the non-closure group in high-risk cases of DB is necessary to determine the effectiveness of this method for preventing post-operative adverse events. Second, this study excluded lesions that covered more than 10% of the whole circumference of the anal canal. However, the closure method for lesions in the anal canal should be selected carefully because of the risks of discomfort and anal stenosis. Third, the tumor heterogeneity may have influenced the ESD treatment

outcomes, such as the curative resection rate. Fourth, as the present study included neuroendocrine tumors <10 mm in size, the small defect size was a concern. We applied the pocket creation method with sufficient margins for neuroendocrine tumors, thus leading to large defects of >20 mm. On the basis of our experiences before starting the study, neuroendocrine tumors were not excluded, resulting in a median defect size of 23.1 mm (range: 15–30 mm). Further study is required to clarify the efficacy of E-LOC compared with endoclip closure for large defects.

Conclusions

The E-LOC technique is feasible, safe, and potentially effective for reducing DB after rectal ESD.

Acknowledgments

We thank Kelly Zammit, BVSc, from Edanz (https://jp.edanz.com/ac) for editing a draft of this manuscript.

Disclosure statement

All authors have no conflicts of interest to disclose.

Funding sources

This work is not funded.

Statement of Ethics

This study protocol was approved by the Ethics Committee of Kagawa University (approval no. 2020-042, approval date July 9th, 2020) and was performed in accordance with the Declaration of Helsinki. All patients provided written informed consent for the procedure.

Author Contributions

Concept and design: Naoya Tada, Hideki Kobara and Noriko Nishiyama; drafting of the article:Naoya Tada; performing the endoscopic procedures: Naoya Tada, Hideki Kobara, Noriko Nishiyama, Taiga Chiyo, Nobuya Kobayashi and Tatsuo Yachida; analysis and interpretation of the data: Naoya Tada, Kozuka Kazuhiro, Takanori Matsui, Taiga Chiyo, Nobuya Kobayashi, Tatsuo Yachida and Shitaro Fujihara; critical revision of the article

for important intellectual content: Hideki Kobara; final approval of the manuscript, Tsutomu Masaki. All authors have read and agreed to the published version of the manuscript

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

References

1. Saito Y, Uraoka T, Yamaguchi Y, Hotta K, Sakamoto N, Ikematsu H, et al. A prospective, multicenter study of 1111 colorectal endoscopic submucosal dissections (with video). Gastrointest Endosc. 2010 Dec;72(6):1217-1225.
2. Niimi K, Fujishiro M, Kodashima S, Goto O, Ono S, Hirano K, et al. Long-term outcomes of endoscopic submucosal dissection for colorectal epithelial neoplasms. Endoscopy. 2010 Sep;42(9): 723-729.
3. Tanaka S, Kashida H, Saito Y, Yahagi N, Yamano H, Saito S, et al. Japan Gastroenterological Endoscopy Society guidelines for colorectal endoscopic

submucosal dissection/endoscopic mucosal resection. Dig Endosc. 2020 Jan;32(2): 219-39.

4. Boda K, Oka S, Tanaka S, Nagata S, Kunihiro M, Kuwai T, et al. Clinical o utcomes of endoscopic submucosal dissection for colorectal tumors: a large mu lticenter retrospective study from the Hiroshima GI Endoscopy Research Group. Gastrointest Endosc.2018 Mar;87(3): 714-722.
5. Oka S, Tanaka S, Kanao H, Ishikawa H, Watanabe T, Igarashi M, et al. Current status in the occurrence of postoperative bleeding, perforation and residual/local recurrence during colonoscopic treatment in Japan. Dig Endosc. 2010 Oct;22(4): 376-380.
6. Ogasawara N, Yoshimine T, Noda H, Kondo Y, Izawa S, Shinmura T, et al. Clinical risk factors for delayed bleeding after endoscopic submucosal dissecti on for colorectal tumors in Japanese patients. Eur J Gastroenterol Hepatol. 20 16 Dec;28(12): 1407-1414.
7. Okamoto K, Watanabe T, Komeda Y, Kono T, Takashima K, Okamoto A, et al. Risk Factors for Postoperative Bleeding in Endoscopic Submucosal Dissection of Colorectal Tumors. Oncology. 2017 Dec; 93: 35-42.
8. Seo M, Song EM, Cho JW, Lee YJ, Lee B, Kim JS, et al. A risk-scoring model for the prediction of delayed bleeding after colorectal endoscopic submucosal dissection.

Gastrointest Endosc. 2019 May; 89(5): 990-998. e2.

9. Santos JB, Nobre MRC, Oliveira CZ, Safatle-Ribeiro AV, Kawaguti F, Martins B, et al. Risk factors for adverse events of colorectal endoscopic submucosal dissection: a systematic review and meta-analysis. Eur J Gastroenterol Hepatol. 2021 Dec; 33: e33-e41

10. Zhang Q, Han B, Xu J, Gao P, Shen Y. Clip closure of defect after endoscopic resection in patients with larger colorectal tumors decreased the adverse events. Gastrointest Endosc. 2015 Nov; 82(5): 904-909.

11. Matsumoto M, Fukunaga S, Saito Y, Matsuda T, Nakajima T, Sakamoto T, et al. Risk factors for delayed bleeding after endoscopic resection for large colorectal tumors. Jpn J Clin Oncol. 2012 Nov; 42(11): 1028-1034.

12. Ogiyama H, Tsutsui S, Murayama Y, Maeda S, Satake S, Nasu A, et al. Prophylactic clip closure may reduce the risk of delayed bleeding after colorectal endoscopic submucosal dissection. Endosc Int Open. 2018 May; 6(5): E582-E588.

13. Liaquat H, Rohn E, Rex D. Prophylactic clip closure reduced the risk of delayed postpolypectomy hemorrhage: Experience in 277 clipped large sessile or flat colorectal lesions and 247 control lesions. Gastrointest Endosc. 2013 Mar; 77(3): 401-407.

14. Abiko S, Yoshida S, Yoshikawa A, Harada K, Kawagishi N, Sano I, et al. Feasibility of a new ligation using the double-loop clips technique without an adhesive agent for ulceration after endoscopic submucosal dissection of the colon (with video). Gastrointest Endosc. 2020 Aug; 92(2): 415-421.

15. Nishiyama N, Kobara H, Kobayashi N, Chiyo T, Tada N, Kozuka K, et al. Efficacy of endoscopic ligation with O-ring closure for prevention of bleeding after gastric endoscopic submucosal dissection under antithrombotic therapy: a prospective observational study. Endoscopy. 2022 Feb. Online ahead of print

16. Tada N, Kobara H, Nishiyama N, Masaki T. Endoscopic ligation with O-ring closure for a large artificial defect after rectal endoscopic submucosal dissection. Dig Liver Dis. 2022 Jan; 54(1): 142-143

17. Matsumoto A, Tanaka S, Oba S, Kanao H, Oka S, Yoshihara M, et al. Outcome of endoscopic submucosal dissection for colorectal tumors accompanied by fibrosis. Scand J Gastroenterol. 2010 Nov; 45(11): 1329 -1337.

18. Pohl H, Grimm IS, Moyer MT, Hasan MK, Pleskow D, Elmunzer BJ, et al. Clip closure prevents bleeding after endoscopic resection of large colon polyps in a randomized trial. Gastroenterology. 2019 Oct;157(4):977-984.

19. Spadaccini M, Albeniz E, Pohl H, Maselli R, Chandrasekar VT, Correale L, et al.

Prophylactic clipping after colorectal endoscopic resection prevents bleeding of large, proximal polyps: Meta-analysis of randomized trials. Gastroenterology. 2022 Jul;159(1):148-158.

20. Chen B, Du L, Luo L, Cen M, Kim LL. Prophylactic clips to reduce delayed polypectomy bleeding after resection of large colorectal polyps: a systematic review and meta-analysis of randomized trials. Gastrointest Endosc. 2021 Apr;93(4):807-815.
21. Yang TC, Wu YH, Lee PC, Chang CY, Lu HS, Chen YJ, et al. Prophylactic clipping after endoscopic mucosal resection of large nonpedunculated colorect al lesions: A meta-analysis. J Gastroenterol Hepatol. 2021 Jul; 36: 1778-1787.
22. Miyakawa A, Kuwai T, Sakuma Y, Kubota M, Nakamura A, Itobayashi E, et al. The efficacy of prophylactic clip closure of mucosal defects after colorec tal endoscopic submucosal dissection on delayed bleeding. Scand J Gastroente rol. 2021 Oct; 56(10) :1236-1242.
23. Liu M, Zhang Y, Wang Y, Zhu H, Xu H. Effect of prophylactic closure on adverse events after colorectal endoscopic submucosal dissection: A meta-anal ysis. J Gastroenterol Hepatol. 2020 Nov; 35(11): 1869-1877.
24. Nomura S, Shimura T, Katano T, Iwai T, Mizuno Y, Yamada T, et al. A mu lticenter, single-blind randomized controlled trial of endoscopic clipping closur

e for preventing coagulation syndrome after colorectal endoscopic submucosal dissection. Gastrointest Endosc. 2020 Apr;91(4):859-867.

25. Nakajima Y, Nemoto D, Nemoto T, Takahara Y, Aizawa M, Utano K, et al. Short-term outcomes of patients undergoing endoscopic submucosal dissection for colorectal lesions. DEN open. 2022 Jun;3(1):e136. doi: 10.1002/deo2.136.
26. Akimoto T, Goto O, Sasaki M, Mizutani M, Tsutsumi K, Kiguchi Y, et al. Endoscopic hand suturing for mucosal defect closure after gastric endoscopic submucosal dissection may reduce the risk of postoperative bleeding in patien ts receiving antithrombotic therapy. Dig Endosc. 2022 Jan; 34(1): 123-132.
27. Akimoto T, Goto O, Sasaki M, Ochiai Y, Maehara T, Fujimoto A, et al. "Hold-and-drag" closure technique using repositionable clips for large mucosal defects after colonic endoscopic submucosal dissection. Endosc Int Open. 2016 Oct; 4(10): E1068-E1072.
28. Kato M, Takeuchi Y, Yamasaki Y. Line-assisted complete closure of a large colorectal mucosal defect after endoscopic submucosal dissection. Dig Endsc. 2016 Sep; 28(6) :685-689.
29. Abe S, Saito Y, Tanaka Y, Ego M, Yanagisawa F, Kawashima K, et al. A novel endoscopic hand-suturing technique for defect closure after colorectal endoscopic

submucosal dissection: A pilot study. Endoscopy. 2020 Sep; 52(9): 780-785.

30. Otake Y, Saito Y, Sakamoto T, Aoki T, Nakajima T, Toyoshima N, et al. New closure technique for large mucosal defects after endoscopic submucosal dissection of colorectal tumors (with video). Gastrointest Endosc. 2012 Mar; 75(3): 663-667.

31. Fujii T, Ono A, Fu K. A novel endoscopic suturing technique using a specially designed so-called "8-ring" in combination with resolution clips (with videos). Gastrointest Endosc. 2007 Dec; 66(6): 1215-1220.

32. Kobayashi N, Kobara H, Nishiyama N, Masaki T. Muscle layer closure involving lesser omentum by endoscopic ligation with O-ring closure for large gastric perforation. 2021 Dec;53(12):1662-1663.

33. Kobara H, Nishiyama N, Fujihara S, Tada N, Kozuka K, Matsui T, et al. Traction-assisted endoscopic full-thickness resection followed by O-ring and over-the-scope clip closure in the stomach: an animal experimental study. Endosc Int Open. 2021 Jan;9(1):E51-E57.

Figure legends

Figure 1.

Schematic illustration of endoscopic ligation with O-ring closure (E-LOC) procedure.

(A) Anchoring of a nylon loop at both lateral edges of the defect by hemoclips, and/or applying another one to the muscle layer. (B) Capturing three deployed clips into the cap of an endoscopic variceal ligation device. (C) Release of the O-ring band, resulting in the approximated defect.

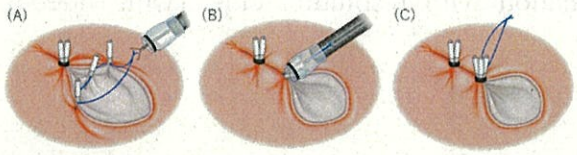


Figure 2.

Preparation for endoscopic ligation with O-ring closure.

(A) The equipment of the method. (B) A nylon loop (2 cm diameter) is created by using 20 ml syringe. (C) After grasping the knot of the loop with a hemoclip, the loop is stored in the clip sheath.

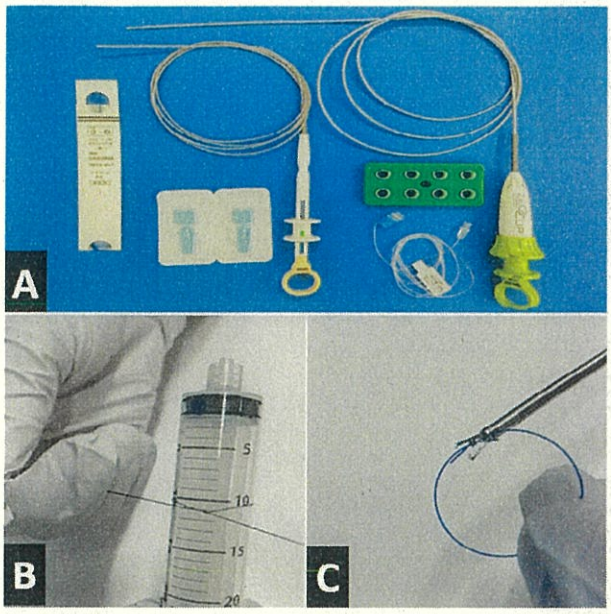
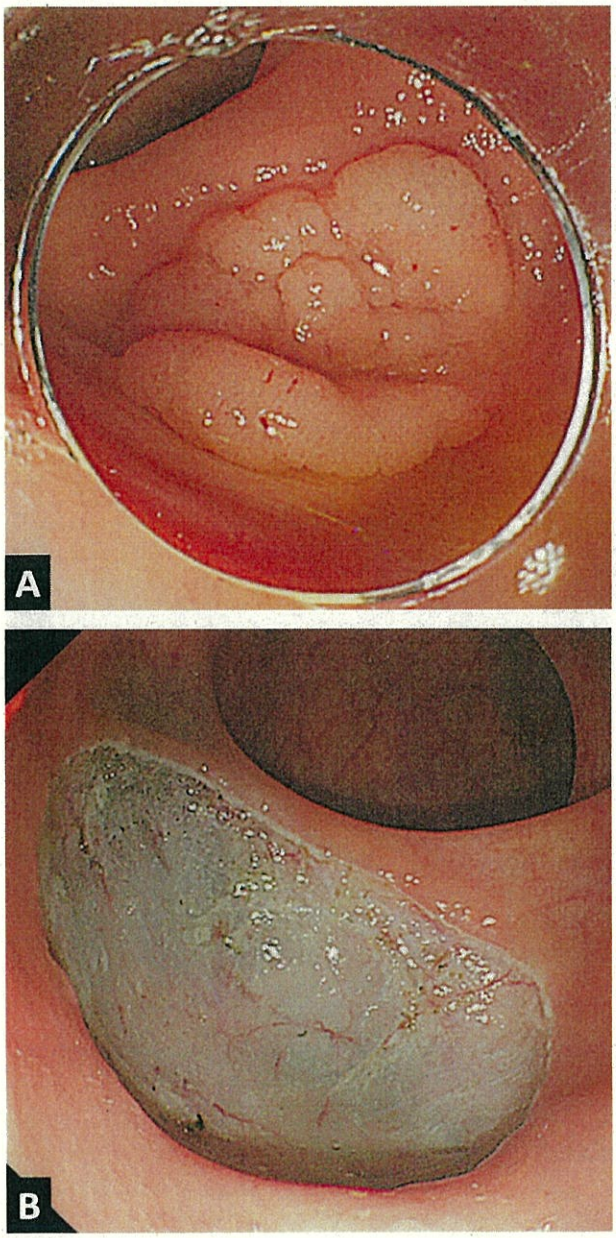
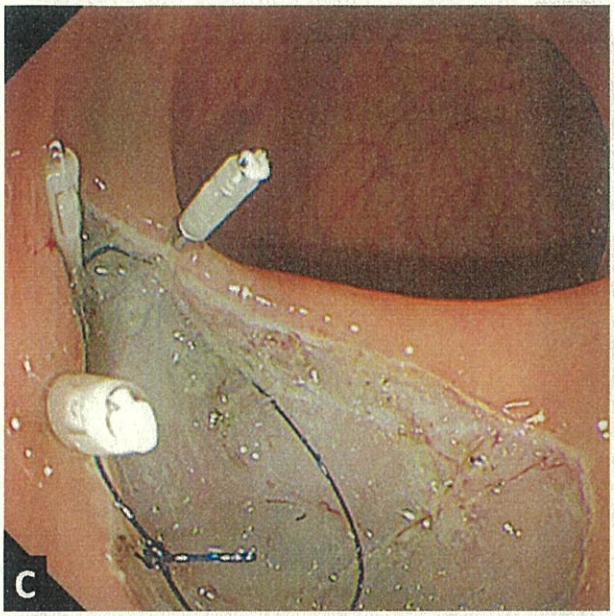


Figure 3.

Technical steps of Endoscopic ligation with O-ring closure (E-LOC) from (A) to (H).

The lesion was a 25-mm-diameter laterally spreading tumor in the lower rectum. E-LOC was performed for a 30-mm-diameter mucosal defect after rectal endoscopic submucosal dissection. Complete defect closure was successfully obtained.


A
B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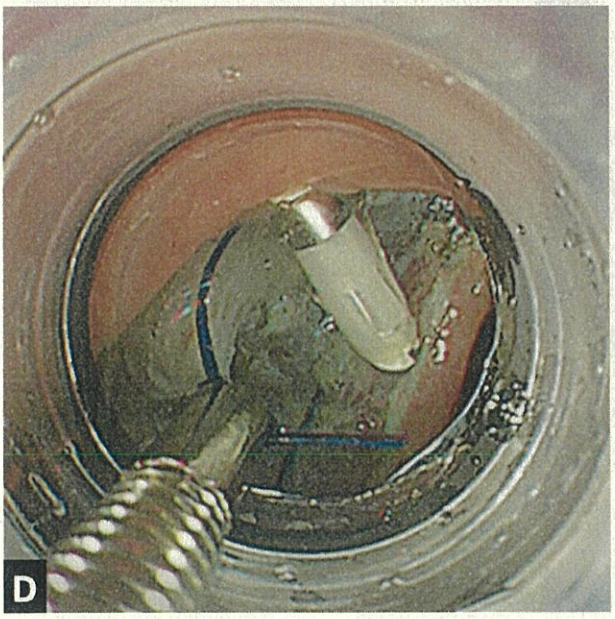
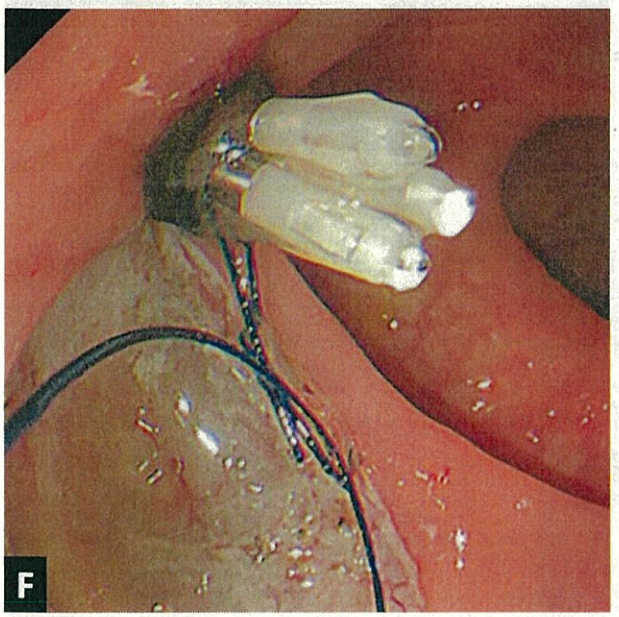

D


E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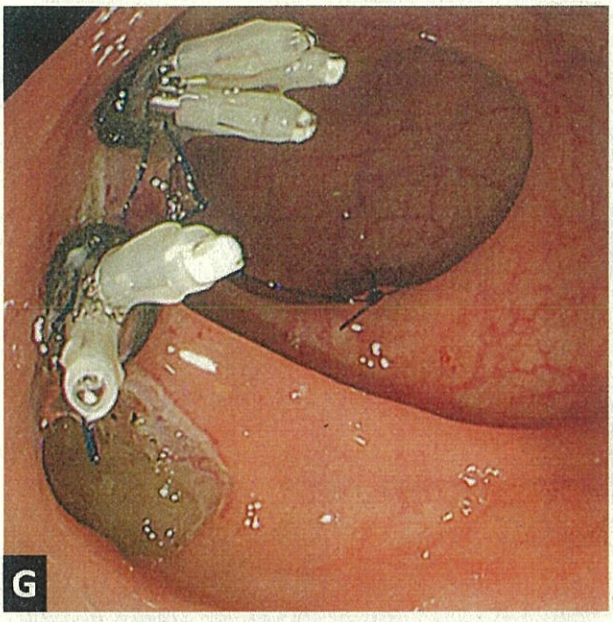
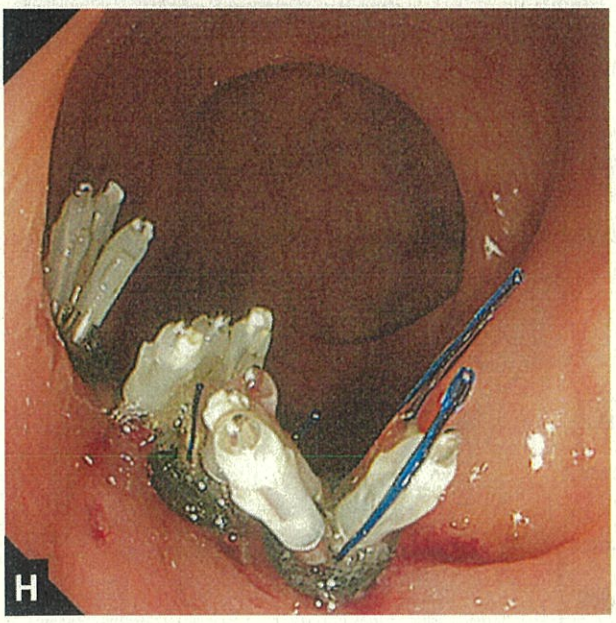

G


H

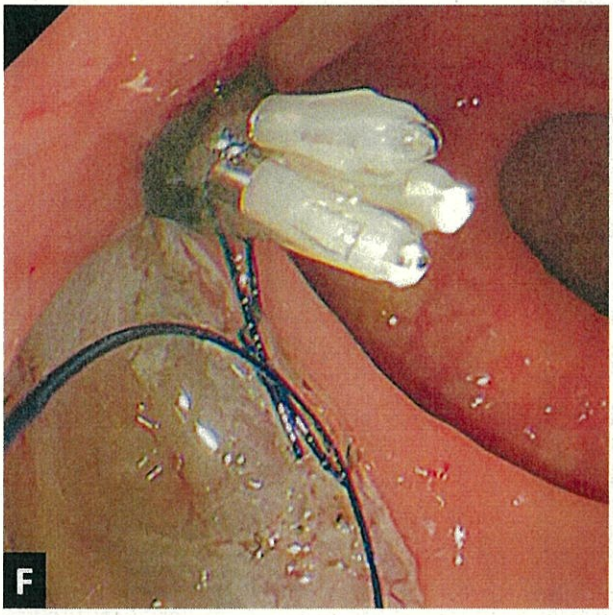


Figure 4.

Flowchart of the present study.

ESD, endoscopic submucosal dissection; E-LOC, endoscopic ligation with O-ring closure.

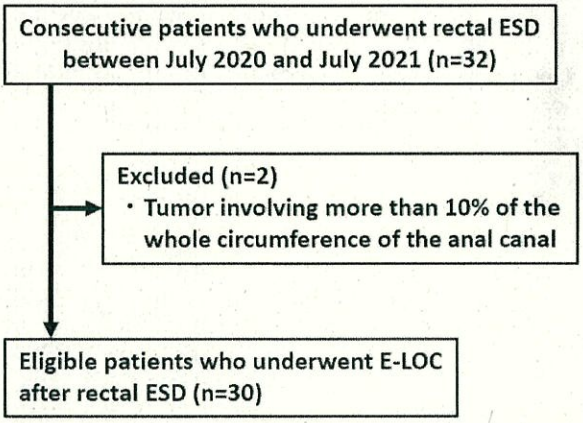


Figure 5.

A representative case with sustained complete closure.

(A) Complete defect closure by endoscopic ligation with O-ring closure in the upper rectum. (B) Sustained closure on day 3 after rectal endoscopic submucosal dissection.

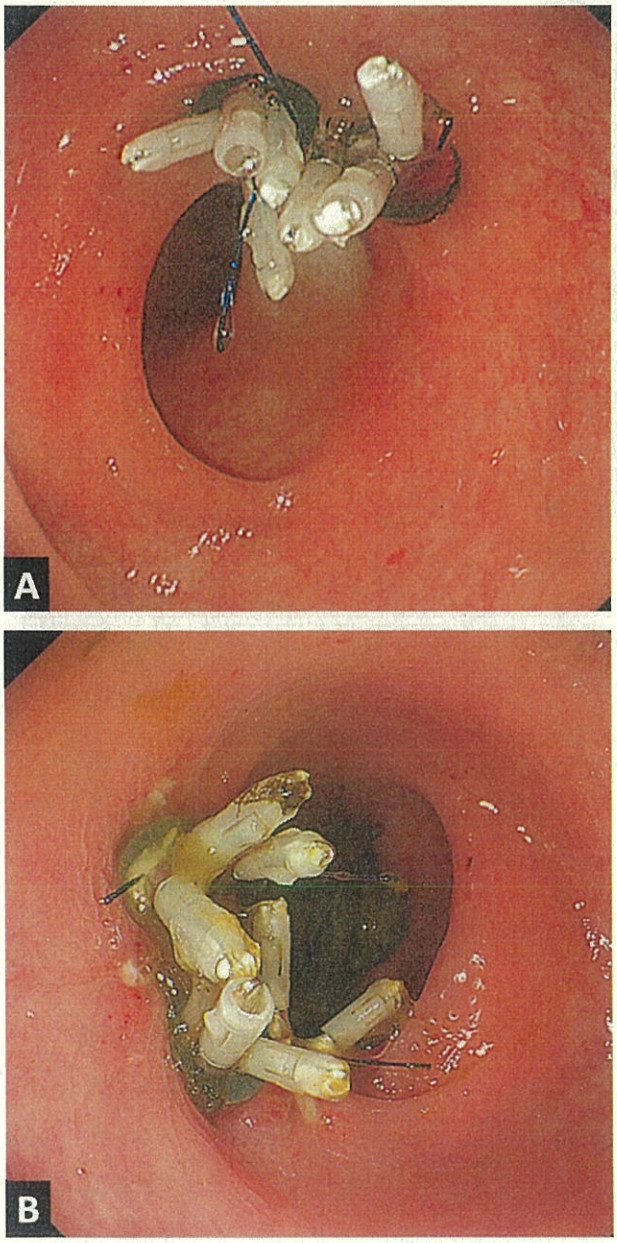


Figure 6.

A representative case with incomplete closure due to the scarring of the muscle layer after

transanal endoscopic microsurgery.

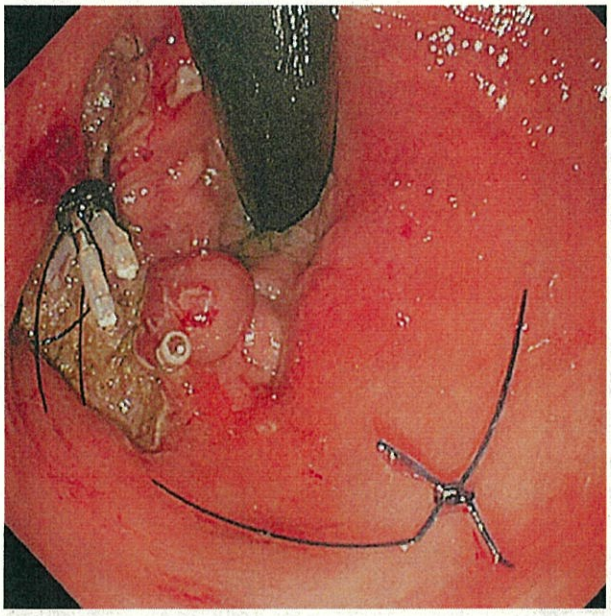


Table 1. Patient and lesion characteristics (n=30)

Age (years), median (IQR)	71 (62.7-76)
Sex (male), n (%)	13 (43.3)
Lesion site, n (%)	
Upper rectum	5(16.7)
Middle rectum	6(20)
Lower rectum	19 (63.3)
Morphology, n (%)	
LST-NG	3 (10)
LST-G	11(36.7)
0-IIa	2 (6.7)
0-IIa+IIc	1 (3.3)
Is, Isp	5 (16.6)
Submucosal tumor-like protrusion	8 (26.7)
Histology, n (%)	
Tubular adenoma	9 (30)
Sessile serrated lesion	1 (0.3)
Tubular adenocarcinoma	12 (40)
-Tis-T1a*	6 (50)

-T1b*	6 (50)
-Lymphovascular invasion	3 (25)
Neuroendocrine tumor	8 (26.7)
-G1	7(87.5)
-G2	1(12.5)
-Lymphovascular invasion	8 (100)
Use of antithrombotic agents, n (%)	4 (13.3)

※LST-NG, Laterally spreading tumor; LST-G, Laterally spreading tumor

* Tis, mucosal lesion; T1a, submucosal invasion < 1000μm; T1b, submucosal invasion ≥ 1000μm

Table 2. Outcomes of rectal ESD (n=30)

Lesion size (mm), median (IQR)	19.0 (8.5-26.5)
Resected specimen size (mm), median (IQR)	29.0 (23.5-31.8)
Degree of submucosal fibrosis, n (%)	
F0 (no fibrosis)	12(40)
F1 (mild fibrosis)	12(40)
F2 (severe fibrosis)	6(20)
En bloc resection, n (%)	30 (100)
R0 resection, n (%)	28 (93.3)
Curative resection, n (%)	16 (53.3)
Intraoperative perforation, n (%)	0 (0)

※ESD, Endoscopic submucosal dissection; IQR, Interquartile range

Table 3. Technical and clinical outcomes of E-LOC (n=30)

Defect closure rate	
Complete closure rate, % (n/N)	80 (24/30)
-Upper rectum	100(5/5)
-Middle rectum	83.3(5/6)
-Lower rectum	73.7 (14/19)
-Defect size < 30 mm	86.7 (13/15)

-Defect size ≥ 30 mm	73.3 (11/15)
Partial closure rate, % (n/N)	10 (3/30)
Unable closure rate, % (n/N)	10 (3/30)
Patients with additional clips, % (n/N)	56.7 (17/30)
Delayed bleeding, % (n/N)	3.3 (1/30)
Delayed perforation, % (n/N)	0 (0/30)
E-LOC procedure time (min), median (IQR)	25.5 (20-30)
Sustained closure rate (POD 3), % (n/N)	83.3 (20/24)
Wound dehiscence rate (POD 3), % (n/N)	22.2 (6/27)

※E-LOC, Endoscopic ligation with O-ring closure; IQR, Interquartile range

Table 4. Comparison of patients with complete and incomplete closure

Characteristics	Complete closure (n=24)	Incomplete closure (n=6)	P value
Age (years), median (IQR)	69.5 (59.0-72.0)	76.5 (69.5-83.8)	.1005*
Sex (male), n (%)	10 (41.6)	3 (50)	.7134*
Lesion site, n (%)			
Upper rectum	5 (20.8)	0 (0)	.5526*
Middle rectum	5 (20.8)	1 (16.7)	1.000*
Lower rectum	14 (58.4)	5 (83.3)	.3717*
Lower rectum involving anal canal	0 (0)	3 (50)	.0049*
Lesion size (mm), median (IQR)	17.0 (8.0-25.0)	23.0 (19.0-50.8)	.0570*
Resected specimen size (mm), median (IQR)	27.0 (22.0-31.3)	31.0 (27.8-57.3)	.0907#
ESD procedure time (min), median (IQR)	38.0 (25.0-55.0)	87.5 (48-155.8)	.1357*
E-LOC procedure time (min), median (IQR)	24.0 (14.8-30.0)	31.0 (23.6-56.6)	.0729#
Degree of submucosal fibrosis, n (%)			
F0 (no fibrosis)	11 (45.8)	1 (16.7)	.2044*
F1 (mild fibrosis)	9 (37.5)	3 (50)	.4555*
F2 (severe fibrosis)	4 (16.7)	2 (33.3)	.3438*
Defect size per muscle layer clip, mm, median (IQR)	20 (10.9-27.0)	19.5 (14.4-29.1)	.7821#

Lesion extending across the rectal fold, n (%)	5 (20.8)	2 (33.3)	.6033*
Delayed bleeding, n (%)	0 (0)	1 (16.7)	.2000*

※IQR, interquartile range; ESD, endoscopic submucosal dissection; E-LOC, endoscopic ligation with O-ring closure.

*Fisher's exact test, #Wilcoxon signed-rank test

Table S1.

Comparison of patient characteristics and delayed bleeding between our historical cohort (Non-closure) and E-LOC.

Variable	Non-closure (n=105)	E-LOC (n=30)	P-value
Age (year), median (IQR)	69 (62-78)	71 (62.7-76)	0.754*
Resection specimen size, median (IQR)	34.0 (25.0-45.0)	29.0 (23.5-31.8)	0.065#
Lesion size, median (IQR)	25.0 (15.0-35.7)	19.0 (8.5-26.5)	0.148#
Lesion site, n (%)			
Upper rectum	22 (21.0)	5 (16.7)	0.797*
Middle rectum	24 (22.8)	6 (20)	0.809*
Lower rectum	59 (56.2)	19 (63.3)	0.535*
Morphology, n (%)			
LST-NG	7 (6.7)	3 (10.0)	0.692*
LST-G	63 (60.0)	11 (36.7)	0.037*
0-IIa	2 (1.9)	2 (6.7)	0.206*
0-IIa+IIc	2 (1.9)	1 (3.3)	0.533*
0-IIa+Is	4 (3.8)	0 (0)	0.575*
0-Is+IIa	2 (1.9)	0 (0)	1.000*

Is, Isp	10 (9.5)	5 (16.6)	0.323*
Ip	1 (1.0)	0 (0)	1.000*
Submucosal tumor-like protrusion	14 (13.3)	8 (26.7)	0.096*
Histology, n (%)			
Neoplasia	88 (81.5)	21 (70.0)	0.482*
Sessile serrated lesion	2 (1.9)	1 (0.3)	0.410*
Neuroendocrine	15 (13.9)	8 (26.7)	0.165*
Resection time, min (IQR)	65.0 (45.0-100)	42.5 (26.7-62.5)	0.169#
Use of antithrombotic agents, n (%)	8 (7.6)	3 (10.0)	0.708*
Delayed bleeding, n (%)	12 (11.4)	1 (3.3)	0.166*

E-LOC, endoscopic ligation with O-ring closure; IQR, interquartile range; LST-NG, laterally spreading tumor - non granular type; LST-G, laterally spreading tumor - granular type

*Fisher's exact test, #Mann-Whitney U test

Video legend

Supplementary video. Mucosal defect closure after rectal endoscopic submucosal dissection using endoscopic ligation with O-ring closure.