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The comparison of anastomosis strength and leakage between double-layer full-thickness and single-layer extramucosal intestine anastomosis

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Background: Various intestine anastomosis techniques have been studied and used, but which is best is still debated. In our center, double-layer full-thickness intestine anastomosis was still considered as standard. However, a single-layer extramucosal intestine anastomosis has shown favorable results. This study created an anastomotic model to compare the anastomosis strength and leakage between double-layer full-thickness and single-layer extramucosal intestine anastomosis.

Methods: This experimental study was performed in 20 randomized healthy male pigs, to be included either in Group A (Single-layer extramucosal intestine anastomosis) or Group B (Double-layer full-thickness intestine anastomosis). Enterotomy followed by an end-to-end anastomosis suture was performed in the jejunum. Fourteen days after the operation, any anastomosis leakage and its location was documented. The anastomosis strength was evaluated using manometry. Data were compared between groups using the Mann–Whitney *U* and Fischer Exact test, considering a significance level of $P < 0.05$.

Results: The overall mean intraluminal anastomotic bursting pressure was 4,257 ± 1,185. Group A had a higher intraluminal anastomotic bursting pressure but was not statistically significant compared to group B (4.726 ± 0.952 vs. 3.787 ± 1.252 kilopascals, $P = 0.063$). One leakage (5%, antimesenteric area) occurred in Group A and three leakages (15%, antimesenteric and mesenteric area) occurred in Group B. However, statistical analysis with Fischer exact showed no significant difference of leakage rate between those groups ($P = 0.291$).

Conclusions: The anastomosis strength and leakage did not differ significantly between the single-layer extramucosal intestine anastomosis group and the double-layer full-thickness anastomosis group. However, the location of leakage was most common in the antimesenteric area in the double-layer full-thickness anastomosis group.

Keywords: all layer, end-to-end, experimental study, seromuscular, surgical anastomosis

Introduction

Intestinal anastomosis is one of the most common surgical procedures performed in emergency and elective settings when benign and malignant gastrointestinal tract lesions require resection[1,2]. Various intestine anastomosis techniques have been studied and used, but which is best is still debated. The principles of a good anastomosis are: adequate exposure or access; proper blood supply from the proximal and distal intestine; preventing fecal contamination or sepsis; suturing; using appropriate staplers to place and bring together all intestinal wall layers without any anastomosis tension; preventing obstruction in the distal intestine due to lumen narrowing; good nutritional condition of the patient; and adequate preparation for colon surgery in cases of elective surgery[3,4].

Bowel healing knowledge has increased due to several long-term studies and a greater understanding of the effects of local, systematic, and demographic factors on healing intestinal anastomoses. The most common systemic factors include the

HIGHLIGHTS

- The anastomosis technique is one factor in elective and emergency surgery success.
- Various surgical techniques have been used, but which is best is still debated.
- This study compared all-layer and seromuscular suture strengths in end-to-end pig intestine anastomoses.
- The anastomosis strength and leakage did not differ significantly between the single-layer extramucosal intestine anastomosis group and the double-layer full-thickness anastomosis group.
- The location of leakage was most commonly the antimesenteric area in the double-layer full-thickness anastomosis group.

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patient's nutritional status (e.g. anemia, hypoalbumin, and hyperbilirubinemia), circumstances (e.g. peritoneal contamination, high or low anastomosis, and anastomosis technique), and demographics (e.g. patient age and sex)[5,6].

Full-thickness anastomosis, in which the mucosa and seromuscular layers are completely sutured, increases mucosal strangulation risk due to submucosal vascular plexus damage. However, in the extramucosal anastomosis, only the seromuscular intestine's seromuscular layer is sutured. This technique connects the intestine's strongest layer (submucosa) and causes minimal damage to the submucosal vascular plexus, anatomically preserving it and reducing necrosis risk[7]. Knowing the strength of the anastomotic suture technique will assist in selecting the best technique for surgical use. Therefore, this study created an anastomotic model to compare the anastomosis strength and leakage between double-layer full-thickness and single-layer extramucosal intestine anastomosis.

Methods

This experimental study has been approved by Hasanuddin University's Animal Research Ethics Committee (approval number: 520/UN04.6.4.5.31/PP36/2022) and performed according to the Animal Research: Reporting of In Vivo Experiments (ARRIVE) Guidelines[8]. This study was conducted in September 2022 in the Research Animal Laboratory, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia. By using the Federer formula for minimal samples count $((n-1)(t-1) \geq 15$, while t was the number of group, thus $n \geq 15$, by additional of 20% drop out risk, so that $n = 20$, where 10 samples in each arms), 10 resected pig intestines in each group receiving either all-layer or seromuscular suturing techniques were compared.

Twenty domestic Healthy male pigs, 4 months old, weighing 36–42 kg, obtained from our previous laparoscopy workshop were habituated in separate adjacent pens and acclimated to their environment for 14 days. Prior to surgery, all pigs were put on nil per oral for at least 12 h and water access were prohibited for at least 2 h. A fentanyl transdermal patches (1 g/kg) were applied along the dorsal midline in the mid-thoracic area at least 12 h before surgery to provide perioperative analgesia. Xylazine (1–2 mg/kg dosage, IM; Xyla), along with midazolam (0.1–0.2 mg/kg dosage, IM) and ketamine (12 mg/kg dosage, IM), were used as premedication. Then, general anesthesia with isoflurane (range 1–5%) through an endotracheal tube was carried out.

In this trial, a single-blinded operator did the laparotomy. All pigs were randomized to be included either in Group A (Single-layer extramucosal intestine anastomosis) or Group B (Double-layer full-thickness intestine anastomosis). The randomization was done in sealed envelope to reduce any bias. Each pigs were positioned and aseptically prepped. By midline laparotomy, the jejunum was exteriorized, while preserving 50 cm of jejunum from the Treitz ligament. Single interrupted sutures of #3–0 PDS (Johnson & Johnson, Inc.) were inserted each at the mesenteric and antimesenteric borders for stabilization. Then, a transverse enterotomy was performed at a 90 degree angle. In 25 cm from the first transection area, a transverse enterotomy was also performed at a 90 degree angle. The separate part of the jejunum was joined back together by using either all-layer or seromuscular sutures based on the stated group from randomization.

In group A, two stay sutures were placed, each in mesenteric and antimesenteric border. The first bowel suture is performed on the medial anterior side interrupted with a 3–0 absorbable suture. Sutures take only the serous and muscular portions leaving the mucosa unstitched. Sutures are carried medial to the respective antimesenteric and mesenteric borders. Then, the bowel is reversed with the aid of the stay suture and the all-layer suture is repeated on the posterior side, interrupting from the medial side. In group B, two stay sutures were placed, each in mesenteric and antimesenteric border. Intestinal sutures covering the serosa to the mucosa are performed with continuous 3–0 absorbable sutures starting from the mesenteric border on the anterior side. Then, the bowel is reversed with the help of the stay suture and the all-layer suture is repeated on the posterior side in a continuous manner. Then, the second layer of interuptted suture was performed encircled the intestine.

The closure of the abdomen was done layer by layer in a simple continuous technique. For analgesia, prior to surgery, pigs were given intramuscular ceftiofur (5 mg/kg dosage; Ceftionel). Meloxicam (0.4 mg/kg, PO, q 24 h 5 days) and fentanyl patches (1 g/kg, TD, 72 h) were used to treat perioperative analgesia. Pigs were observed for clinical signs of pain, appetite, and activity until day 14, at which point the study was terminated.

Fourteen days after the operation, all the pigs were sacrificed, and necropsy investigations were conducted to evaluate the physical appearance of the jejenum and anastomoses as well as the surrounding abdominal cavity. The anastomosis strength was evaluated using manometry (intraluminal bursting pressure). A digital fluid pressure gage (Surgivet V6400 Invasive Blood Pressure Monitor, Smiths Medical PLC) was used to measure intraluminal bursting pressure pressure[9]. Using surgical clamps, the area around the anastomotic site was blocked off, leaving a 12 cm long piece that was centered on the anastomosis. Saline solution was injected into this area using a 14-gage needle and IV line, and a second 14-gage needle was inserted into the opposing side and connected to the pressure monitor. Saline was gradually injected into the lumen while the anastomosis was evaluated for leakage. When a leak was identified, the pressure reading was obtained and was regarded as the highest burst pressure that the anastomotic site for that sample could sustain. The pressure measurement was recorded using a handheld manometer (in kilopascals [kPa]). In addition, leak numbers and locations were recorded. Each result was recorded and documented for later statistical analysis.

Data were statistically analyzed using the SPSS software version 21.0 (IBM Corp.) considering a significance level of $P < 0.05$. Descriptive statistics were showed in tables and numbers. Statistical analysis was performed with the Independent t-test or Mann–Whitney for numeric data and the χ^2 or Fischer exact for categorical data.

Results

In this study, there were 20 pigs that were randomly assigned to group A with single-layer extramucosal anastomosis and group B with double-layer full-thickness anastomosis. After treatment, all the pigs lived until the 14th day. The data in this study were not normally distributed (Shappiro–Wilk test), thus analysis was performed using the test.

In this study, intraluminal anastomotic bursting pressure was carried out using a manometer. The overall mean intraluminal anastomotic bursting pressure was 4257 ± 1185 kPa ranging from 2.16 to 5.72 kPa. Group A had a higher intraluminal anastomotic bursting pressure but was not statistically significant compared to the group B (4.726 ± 0.952 vs. 3.787 ± 1.252 kPa, $P = 0.063$) (Table 1).

In this study, as many as four anastomosis leakages were observed in 20 intestine anatomosis (20%). One leakage (5%) occurred in Group A and three leakages (15%) occurred in Group B. However, statistical analysis with Fischer Exact showed no significant difference of leakage rate between those groups ($P = 0.291$) (Table 2). Furthermore, in Group A, the anastomosis leakage occurred in the antimesenteric area, while in Group B, one anastomosis leakage occurred in the antimesenteric area and two anastomosis leakages occurred in the antimesenteric area.

Discussion

Until now, the intestinal anastomosis technique has many variations with the advantages and disadvantages of each technique. Many observational studies, RCTs, and even meta-analyses have compared bowel suture techniques; however, no single report can claim that certain bowel suture techniques are better than others. In our center in Makassar, Indonesia, double-layer full-thickness anastomosis had become the local standard and has traditionally been considered safer than others techniques. However, recent studies had shown numerous advantages by using seromuscular anastomosis even single-layer due to faster to be performed, prevent intestinal lumen narrowing, better anastomosis strength, and faster postoperative return of normal bowel function and oral intake, and flatus and stool presence[1]. Thus, in this study, the authors will compare the double-layer full-thickness or single-layer extramucosal intestine anastomosis technique, in which full-thickness suturing involves all intestine layers including the mucosa, while extramucosal suturing only suture the seromucular layer leaving the mucosa layer free.

The results of this study showed that seromuscular intestine anatomosis showed higher resistance to pressure than double-layer full-thickness intestine anastomosis. The rate of anastomosis leakage was found to be higher in double-layer full-thickness than single-layer extramucosal group. However, the comparison results were not statistically significant as the seromuscular suture yielded only a 1 kPa increase in resistance compared to the all-layer technique.

The double-layer anastomosis full-thickness technique provides good adhesion because all intestinal tissue layers are sutured. However, seromucular or extramuscular anastomosis can provide good tissue adaptation[10]. The full-thickness anastomosis technique increased the damage of mucosal and submucosal vascular plexus through suture. Differ with the extramucosal anastomosis technique, which only connected the intestine's strongest layer (muscularis in submucosa), preserving the submucosal vascular plexus, thus reduce the risk of bowel ischemia[7,11]. Other theory about leaving the mucosa free was that the mucosa heals quickly and a water shield forms within 24 h[1].

Table 1

Intraluminal anastomotic bursting pressure in groups A and B

Group	Mean pressure (kPa)	SD	Mean rank	*P*
A (Seromuscular)	4.726	0.952	12.95	0.063*
B (All-layer)	3.787	1.252	8.05	

*Note: Mann–Whitney test.

Table 2

The location and number of anastomosis leakage in groups A and B

Group	Number of leakage	Location of anastomosis leakage		*P*
		Antimesenteric	Mesenteric	
A (Seromuscular)	1	1	0	0.291*
B (All-layer)	3	2	1	

*Note: Fischer exact test.

The full-thickness suture; however, tend to narrow the lumen, especially in the early phase when postoperative inflammation began. In addition, continuous sutures impede the blood supply to the cut end, which is unfavorable except in well-vascularized areas. Interestingly, we found that the anastomosis leakage in the double-layer full-thickness group was commonly found in the mesenteric area, while no anastomosis leakage in the mesenteric area was found in the single-layer extramucosal group.

We recommend the use of single-layer extramucosal intestine anastomosis for several evidence-based reasons: no damage to the submucosal vascular plexus; no foreign-body inflammation in the mucosal layer, leaving the mucosa to heal faster; easier adjustment of the intestine diameter in anastomosis; and lower incidence of leakage if any of the stitches break. In addition, the novelty in this study is in emphasizing that not only sutures take just the serous and muscular portions, leaving the mucosa unstitched, but also that sutures are carried first from the medial to the respective antimesenteric and mesenteric borders. In usual practice, suturing is performed from peripheral to central, but the authors recommend fixating the intestine centrally for better practice.

In this study, anastomosis was only performed on the jejunum, considering that this is the part of the intestine most prone to leaks. In the future, a comparison of all-layer and seromuscular anastomosis techniques can be tried on other parts of the intestine. Therefore, human-based multicenter studies are needed to assess anastomotic technique use and determine which is best for clinical use.

Conclusions

The anastomosis strength and leakage did not differ significantly between the single-layer extramucosal intestine anastomosis group and the double-layer full-thickness anastomosis group. However, the location of leakage was most common in the antimesenteric area in double-layer full-thickness anastomosis group.

Ethical approval

Ethical approval for this study was provided by the Ethical Committee Faculty of Medicine, Hasanuddin University, makassar Indonesia, number: 520/UN4.6.4.5.31/PP36/2022 on 16 September 2022.

Consent

NA.

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Author contribution

W.S., R.L., D.U., M.R.J., A.A.M., A.A., J.H., and M.F. wrote the manuscript and participated in the study design; W.S., R.L., D.U., M.R.J., A.A.M., A.A., J.H., and M.F. drafted and revised the manuscript; W.S., D.U., M.R.J., A.A.M., A.A., and M.F. performed head trauma treatment and surgery; W.S. and J.H. performed bioinformatics analyses and revised the manuscript. All authors read and approved the final manuscript.

Conflicts of interest disclosure

The authors declare that they have no financial conflict of interest with regard to the content of this report.

Research registration unique identifying number (UIN)

Name of the registry: not applicable.
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NA.

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