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Use of the LigaSure for Closing Peritoneal Defects in an Experimental Rat Model

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Abstract

Background: There has not been an absolute consensus over the routine closure of peritoneal defect (PD) during laparoscopic totally extraperitoneal inguinal hernia repair (TEP). Pretied sutures, endoscopic stapling, and suturing are surgical techniques for closing PDs. Moreover, we observed that we could close small PDs during the TEP procedure by sealing with the LigaSure (LS).

Objectives: The present study aimed to evaluate the necessity of closure PDs under a polypropylene mesh and the early intraperitoneal inflammatory, fibrotic, and adhesional effects of sealing PDs with the LS in an experimental rat model.

Methods: A total of 35 male rats were assigned to five groups. 1- *Control group*: mesh was not used, and the peritoneum was left open; 2- *Mesh group*; mesh was placed directly on the PD without repairing, and three peritoneal repairing methods; 3- *Stapling group:* PD was repaired with metal clips; 4- *Suture group*: PD was repaired with Vicryl sutures; and 5- *LigaSure group:* PD was closed with the LS. Rats were sacrificed on the postoperative 14th day. Adhesion scores, fibrosis, and inflammation scores were compared between all groups.

Results: All rats completed the 14 days of follow-up without complication. The Mesh group had significantly higher adhesion scores than the other groups ($P<0.001$). Nonetheless, no significant difference was observed between peritoneal repairing methods ($P=0.696$). Fibrosis and inflammatory scores were similar in peritoneal repairing methods ($P=0.394$ and $P=0.112$, respectively).

Conclusion: The direct contact of foreign bodies with the intra-abdominal organs increases the risk of adhesion; therefore, the remaining PDs under the polypropylene mesh should be repaired. Sealing PDFs with LS is a simple method that does not increase the inflammatory response, fibrosis, and the risk of adhesion formation.

Keywords: Inguinal hernia, LigaSure, Peritoneal tear, TEP

1. Background

There has not been a consensus over closing peritoneal defects (PD) during laparoscopic totally extraperitoneal hernia repair (TEP). In about 7% of cases, the surgical overview is lost due to the pneumoperitoneum tending effect. Intraperitoneal CO_2 decompression with the Veress needle is often sufficient to continue the operation. Only a few of them need conversion to laparoscopic transabdominal preperitoneal hernioplasty or open surgery. Although guidelines recommend that PDs be closed whenever feasible to prevent adhesions, some surgeons believe routine closure of the PDs is not necessary (1–3).

The management of PDs with a laparoscopic approach to complete TEP inguinal hernioplasty is fundamental. Pretied suturing, loop ligation, endoscopic stapling, and endoscopic suturing are common techniques for closing PDs; nonetheless, they prolong the operation time and require surgical experience. Pretied suturing, loop ligation, and endoscopic stapling are safer and faster than endoscopic suturing; however, they may be inadequate to close large defects. Endoscopic suturing needs surgical experience to perform the procedure in a limited space and may pose vascular and visceral injury (4).

Vessel-sealing devices have been widely used in many fields of surgery (5); nonetheless, they are not routinely used in the TEP procedure. LigaSure may be useful for the dissection of patients with dense adhesions or for requiring rapid intervention for vascular injury.

2. Objectives

Moreover, we observed that we could close small PDs during the TEP procedure by sealing with the LigaSure. In light of the present study, the present study aimed to evaluate the necessity of closure PDs under a polypropylene mesh and the early inflammatory and fibrotic effects that may cause subsequent intraperitoneal adhesions of sealing PDs with the LS in an experimental rat model.

3. Methods

3.1. Study design and samples

The animal ethics committee statistics department determined the minimum number of animals required for group comparisons, and 35 Male Wistar-Albino® rats weighing 370-480 grams (mean 410 grams) were randomly assigned to five groups (n=7 in each group). All rats were housed under standard laboratory conditions at room temperature with a 12 h light/12 h dark cycle and allowed to have ad libitum food and water before and after surgery. During the experimental procedure, the animals were individually placed in cages and kept at room temperature (22°C). All surgical procedures were performed under sterile conditions.

3.2. Ethical considerations

The University Ethics Committee approved the experimental protocol was in compliance with the Declaration of Helsinki (6). All the experimental protocols were carried out under the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences (7).

3.3. Materials

A polypropylene mesh (POLYMESH Polypropylene Mesh, Betatech®, Turkey), a vessel sealing device (Covidien Valleylab LigaSure® Tissue Fusion Laparoscopic Instrument, USA), a metal clip (Hemoclip® Metal Ligation System, Weck®, USA), a fast-absorbable multifilament polyglactin suture (Vicryl 4/0, Ethicon Inc; Johnson&Johnson, USA), and a nonabsorbable silk suture (Perma-Hand*Silk 3/0, Ethicon Inc; Johnson&Johnson, USA) were obtained commercially.

3.4. Surgical procedure

Before the surgery, all animals were weighed, and the results were recorded. The procedure is performed under sterile conditions. Rats were anesthetized with an intramuscular injection of ketamine hydrochloride (5 mg/kg, Ketalar; Parke-Davis, Morris Plains, NJ) and Xylazine (0.8-1.3 ml/kg, Rompun; Bayer, Istanbul), and 1 mg/kg intramuscular cefazolin (120 mg/kg) was administered via intraperitoneal. The rats were shaved and prepared with povidone-iodine. Laparotomy was performed with a four-centimeter right median incision. After two centimeters of PD had occurred, peritoneal dissection was made around the defect in about a 4X4 centimeter square area. ***Group 1*** *(Control group):* Mesh was not used, and the peritoneum was left open. ***Group 2*** *(Mesh group):* Mesh was placed directly on the PD without repairing. ***Group 3*** *(Stapling group):* Mesh was placed on the peritoneum after PD was repaired with metal clips. ***Group 4*** *(Suture group):* Mesh was placed on the peritoneum after PD was repaired with Vicryl sutures. ***Group 5*** *(LigaSure group):* Mesh was placed on the peritoneum after PD was closed with the LigaSure (Figure 1 & 2). Mesh was not fixated in any of the groups, and muscular layers were closed with mattress suture of Vicryl 4/0. The skin was closed with a simple interrupted suture of Perma-Hand Silk 3/0.

Surgeons who were blinded to the groups sacrificed rats on the postoperative 14th day. Overdose anesthesia was used, causing immediate cardiac arrest. The abdomen was opened with the left median incision.

3.5. Macroscopic and microscopic analysis

Three surgeons (K.U., H.O., and F.D.) determined the macroscopic adhesion of each rat independently with the adhesion severity scoring system (Table 1). In case of a mismatch in the results, the median score was accepted. Specimens were fixated in a buffered formaldehyde solution of 10%. An experienced pathologist (EY) who was blinded to the methods examined all the specimens. Tissues were dehydrated and embedded in paraffin for light microscopy. Slices were cut 5-10 µm thick and were stained with hematoxylin-eosin; thereafter, they were determined for fibrosis and inflammation with the histopathologic scoring system (Table 2 & 3).

Table 1. Adhesion severity scoring scale

Score	Macroscopic Finding
0	No adhesion
1	Filmy adhesions easily separable with blunt dissection
2	Mild to moderate adhesions with a freely dissectible plane
3	Moderate to dense adhesion with difficult dissection
4	Non-dissectible plane

Table 2. Histopathologic evaluation of fibrosis and inflammation

	Score	Definition
Fibrosis	0	None
Score	1	Minimal, loose
	2	Moderate
	3	Florid, dense
İnflammation	0	None
Score	1	Giant cells, occasional lymphocytes, and plasma cells
	2	Giant cells, plasma cells, eosinophils, neutrophils
	3	Many inflammatory cells, microabscesses

3.6. Statistics

The data were analyzed in SPSS (version 23). Normality was determined using the Shapiro-Wilk test. The differences between groups were compared using the Kruskal-Wallis test. Post-hoc analysis was performed with the Tamhane test. A p-value of less than 0.05 was considered statistically significant.

4. Results

All rats completed the 14 days of follow-up. Throughout the study, no postoperative complications were observed. A severe adhesion of the small bowels on the operation field was detected in the *Mesh group* (G2), in which the mesh showed a direct relationship with visceral organs (Figure 3a). Blunt dissection was not sufficient to release these adhesions in three rats, and two of them had a small bowel injury during the sharp dissection. Nonetheless, two rats in the Suture group (G4) had adhesions on the intraperitoneal suture section that could simply be dissected with blunt dissection (Figure 3b). When the adhesion scores were evaluated, a significant difference was found between the *Mesh group* (G2) and the other groups (G1, G3, G4,

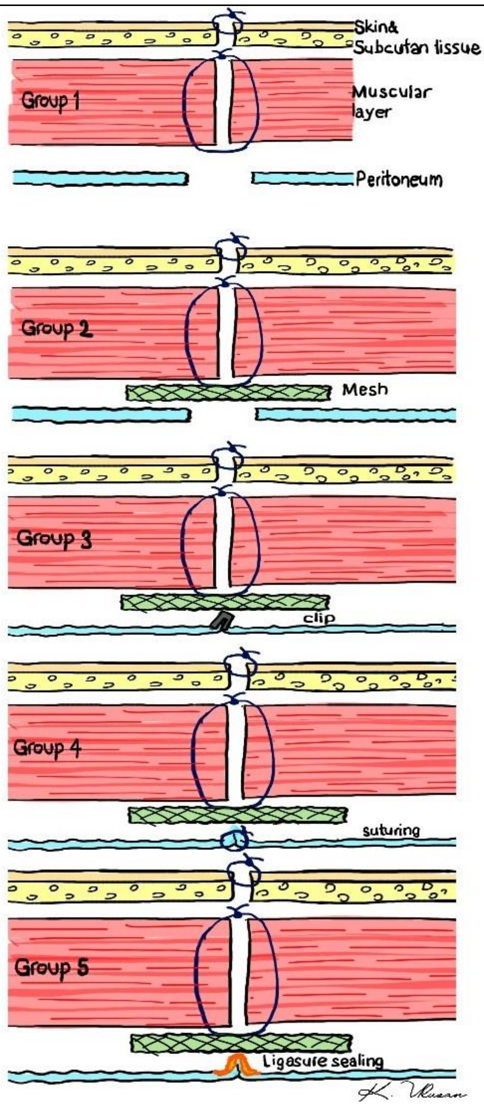

Figure 1. Drawings showing the surgical procedures performed on the groups on the axial view of the anatomy of the anterior abdominal wall (Illustrations by Kivilcim Ulusan). Group 1 (Control group), Group 2 (Mesh group), Group 3 (Stapling group), Group 4 (Suture group), and Group 5 (LigaSure group)

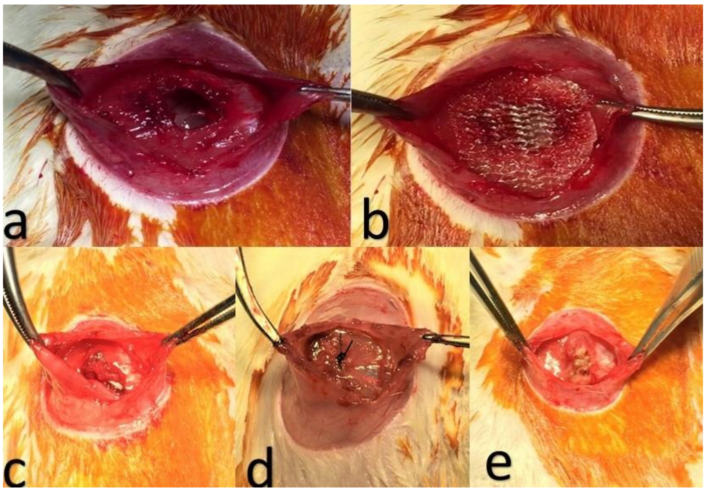

Figure 2. Intraoperative view of the procedures applied to the groups. **a.** ***Group 1*** *(Control group)*, ***b****.* ***Group 2*** *(Mesh group)*, ***c. Group 3*** *(Stapling group)*, **d.** ***Group 4*** *(Suture group)*, ***e. Group 5*** *(LigaSure group)*

G5) (P<.001). However, no significant difference was detected between peritoneum repairing methods (G3, G4, G5) (P=.696). In histologic evaluation, fibrosis scores had no significant difference among

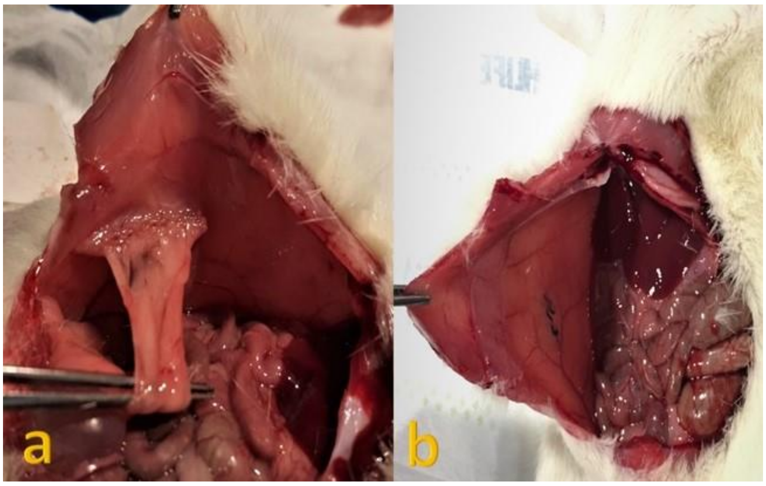

Figure 3. Dissection images of the groups on the postoperative 14th day. **a.** There is a severe adhesion of intestinal loops and an omentum onto the mesh in the *Mesh group* (Group 2), which cannot be separated by blunt dissection, **b.** There is no adhesion in the *Suture group* (Group 4)

the groups (P=.455). Nevertheless, the inflammatory score had a significant difference between groups (G1, G2, G3, G4, G5) (P=.002). In subgroup analysis, the *Mesh group* (G2) had significantly high scores than the *Control group* (G1) and the *LigaSure group* (G5) (P=0.004 and P=0.046, respectively) (Figure 4). Fibrosis and inflammatory scores were similar between the peritoneal repairing methods (G3, G4, G5) (P=0.394 and P=0.112, respectively). (Table 4).

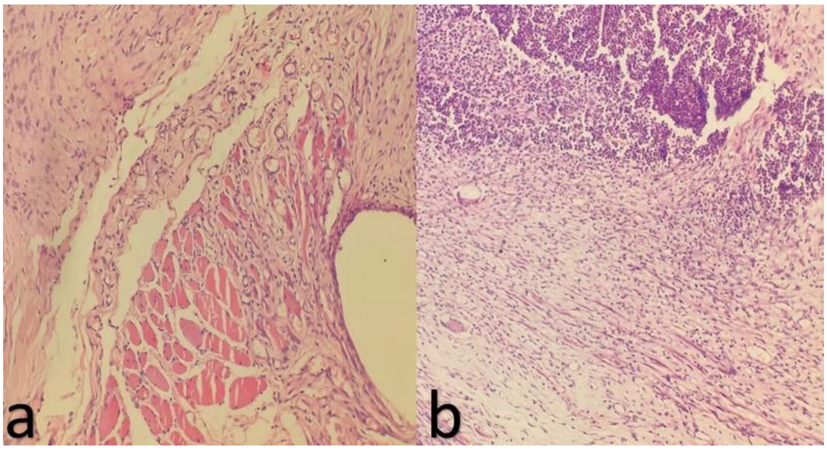

Figure 4. Histological observations **a.** Severe, neutrophil-rich mixed-type inflammatory cell infiltration and microabscess formation (top), giant cell (bottom left), and intense fibroblastic activity (bottom). In this example, both fibrosis and inflammation scores were evaluated as 3. *Mesh group* (Group 2), hematoxylin-eosin (x200). **b.** Inflammatory cells consist of sparse plasma cells, lymphocyte infiltration (bottom right), and mild fibroblastic activity (top left). In this example, both fibrosis and inflammation scores were evaluated as 1. *LigaSure group* (Group 5), hematoxylin-eosin (x200)

Table 3. Adhesion severity, fibrosis, and inflammation scores of the rats in the groups

		R1	R2	R3	R4	R5	R6	R7
Control group	Fibrosis	1	1	1	3	2	1	1
	Inflammation	1	2	1	3	2	3	2
	Adhesion	0	0	0	0	0	0	0
Mesh group	Fibrosis	3	2	1	1	2	2	2
	Inflammation	1	1	1	1	1	1	1
	Adhesion	3	3	3	3	3	3	3
Stapling group	Fibrosis	1	1	2	2	2	2	2
	Inflammation	1	1	2	1	2	2	2
	Adhesion	0	0	0	1	1	0	0
Suture group	Fibrosis	1	3	2	1	2	2	2
	Inflammation	2	3	2	2	3	2	1
	Adhesion	0	0	0	0	0	3	2
LigaSure group	Fibrosis	1	2	2	2	1	1	1
	Inflammation	1	1	2	2	1	1	1
	Adhesion	0	0	0	0	0	0	1

Table 4. Comparison of the Adhesion severity, Fibrosis, and Inflammation scores of the groups

Group	Groups	Adhesion Scores	Fibrosis Scores	Inflammatory Scores
Control group	Mesh group	<0.001	0.729	0.025
	Suture group	0.231	0.729	0.008
	Stapling group	0.911	0.921	0.378
	LigaSure group	0.993	10.000	0.651
Mesh group	Control group	<0.001	0.729	0.025
	Suture group	<0.001	1.000	0.991
	Stapling group	<0.001	0.994	0.651
	LigaSure group	<0.001	0.729	0.378
Suture group	Control group	0.231	0.729	0.008
	Mesh group	<0.001	1.000	0.991
	Stapling group	0.703	0.994	0.378
	LigaSure group	0.443	0.729	0.179
Stapling group	Control group	0.911	0.921	0.378
	Mesh group	<0.001	0.994	0.651
	Suture group	0.703	0.994	0.378
	LigaSure group	0.993	0.921	0.991
LigaSure group	Control group	0.993	1.000	0.651
	Mesh group	<0.001	0.729	0.378
	Suture group	0.443	0.729	0.179
	Stapling group	0.993	0.921	0.991

5. Discussion

Prosthetic mesh materials are the essential components of laparoscopic hernia operations. The tendency of adhesion formation at the peritoneal side is the main disadvantage that may lead to bowel obstruction and fistula formation. Although antiadhesive materials have been developed, the prevention of the intraperitoneal connection with mesh is more physiological (8). The TEP is one of the best choices in this field; however, there has not been a consensus over the location or size of the PDs that require closure during the TEP procedure.

Peritoneum has a quick recovery, and closure does not appear to provide any benefit during abdominal operations. The PDs cause pneumoperitoneum in 7% of TEP procedures and mostly do not affect the operative course (1). The redundant peritoneum folds upon itself and seals the defect quickly after desufflation. Some surgeons reported that routine closure of PDs is technically challenging and significantly prolongs the duration of the surgery. They did not observe any intraoperative or postoperative complications during the early and long-term follow-ups when they left it open in their series (2,3,9,10).

On the other hand, current guidelines recommend that PDs be closed whenever feasible to prevent potential complications, such as adhesion and internal herniation (1,4). In the present study, no macroscopic or histological difference was found between the group PD (Group 1) and the meshed groups where the peritoneum was repaired (Group 3-5). Nevertheless, adhesion was higher macroscopically and histologically in the meshed group in which PD was left open (Group 2) than in the other groups. Therefore, mesh should be closed if the PD is located under a foreign body, such as polypropylene.

Today, vessel-sealing instruments are widely used in many surgical procedures. These became more critical after the introduction of laparoscopic surgery (11). However, they are not routinely used in laparoscopic hernia procedures. Although these increase the cost, LigaSure may be beneficial in recurrent and complicated cases. Moreover, we can close PD during peritoneal dissection in the TEP procedure.

Pretied suture, loop ligation, endoscopic stapling, and endoscopic suturing are routine surgical techniques for the closure of PDs during the TEP procedure. All of them are equally successful without complications (4). However, some practical solutions that can aid laparoscopic surgery have been identified, such as Extracorporeal Peritoneal Knotting (12) and sealed with bipolar diathermy (13). Apart from technical success, two critical factors affect the intraperitoneal adhesion; (i) material used in peritoneal closure (metal clip, vicryl suture) causing foreign body reaction, (ii) devascularization in the repaired peritoneal region.

Sutures and clips stay on the peritoneum in the pretied suture, loop ligation, and endoscopic stapling techniques. However, the peritoneal side of the suture may cause visceral adhesion (14). Based on the results of the present study, the small bowel adhered to the endoscopic suturing material in two rats. Blunt dissection quickly separated these adhesions, and there was no statistical difference between the other peritoneal closure methods.

Mesothelial cells are generally accepted to be the most critical component in peritoneal repairing (15,16). In the presence of the devascularized peritoneal areas, mesothelial cells are slowly or incompletely colonized on the defect, resulting in physical contact between mesh and viscera and leading to adhesions (17). The peritoneum was intentionally devascularized during the closure of the PD with LigaSure; nonetheless, in our study, PDs were safely repaired with LigaSure without any intraperitoneal adhesion complication in the early duration. However, surgeons must be careful about the risk of damaging the visceral organs due to thermal injury during laparoscopic surgery (11). In the proposed method, since the surgery is open and the field of surgery is less limited compared to the TEP procedure, the possibility of this complication may not be taken seriously. This is a limitation of this new method of closing the PDs, which should be looked at more carefully in human studies.

6. Conclusion

Direct contact of foreign bodies with the intra-abdominal organs increases the risk of adhesion. Therefore, the remaining PDs under the polypropylene mesh should be repaired. The routine use of the Ligasure device in the TEP procedure increases the surgical cost. However, it provides convenience to the surgeon in difficult cases, such as recurrent hernias. Peritoneal defects that occur in cases where the Ligasure is used can be closed simply and safely by sealing with the device.

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None.

Footnotes

Conflicts of Interest: The authors declare that they have no competing interests.
Authors' contributions: HO performed surgical procedures, collected data, and designed the work. KU performed surgical procedures and was mainly responsible for processing data, as well as writing and revision of the whole article. FD performed surgical procedures and collected data. EY performed the pathological examination. Both authors read and approved the final manuscript.
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