Original Article
Preliminary study on endoscopic trans-esophageal submucosal tunneling surgery: a new therapeutic approach

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Abstract: Background: Natural orifice transluminal endoscopic surgery (NOTES) provides minimally invasive alternative access to the peritoneal cavity, avoiding abdominal wall incisions. The current study presents a new therapeutic approach, endoscopic trans-esophageal submucosal tunneling surgery (EESTS), aiming to protect overlying mucosa to the maximum extent. Some preliminary explorations were carried out for preoperative localization, surgical positioning, incision approaches of the muscularis propria, and differentiation of endoscopic and standard anatomic images. Methods: In this study, 27 porcine corpses were tested. The developed method was divided into 4 parts. In part 1, 6 pigs were randomly divided into two groups: methylene blue solution group (ML-group) and control group (CT-group). The duration of operation starting from the creation of the tunnel incision up to the entering of the endoscope into abdominal cavity was recorded. In part 2, 9 pigs were randomly divided into three groups, L-group: fixed in the left-lateral position, S-group: fixed in the supine position, and RR-group: fixed in the raised right shoulder position. Difficulties related to the operation and endoscopic view were also recorded. In part 3, 9 pigs were randomly divided into three groups: transverse full-thickness incision group (T-group), longitudinal full-thickness incision (L-group), and progressive longitudinal full-thickness incision group (PL-group). In part 4, EESTS was performed to record and differentiate endoscopic and standard anatomical images. Results: In part 1, duration of the operation in the ML-group (21.67 ± 2.08 minutes) was shorter than that in the CTL-group (15.00 ± 3.00 minutes). In part 2, the RR-group presented with a better entrance site, shorter duration of operation (14.7 ± 1.5 minutes), straight tunnel, appropriate endoscopic vision, and easier operation. In part 3, the PL-group with a 2-cm incision length had proper flexibility of the endoscope and a straight tunnel, which could also be used for future operations. In part 4, the abdominal aorta, left hepatic lobe, inferior vena cava, splenic vein, gastric fundus, and spleen pancreas were observed under different endoscopic conditions. Conclusion: The raised right shoulder position, preoperative localization, and progressive longitudinal full-thickness incision were optimally achieved. In addition, endoscopic images were recorded in all positions. This represents a proper basis for future surgeries.

Keywords: Endoscopic trans-esophageal submucosal tunneling surgery, endoscopic tunneling technique, full-thickness resection, gastrointestinal tract

Introduction

Regarding endoscopy, several studies have concentrated on treating deeper diseases, even diseases outside the gastric wall, with extremely minimally invasive methods [1-3]. Natural orifice transluminal endoscopic surgery (NOTES) consists of several new endoscopic and surgical entryways into the abdominal cavity. It has been basically regarded as one of the potential paths for flexible endoscopy beyond the gastrointestinal wall [4, 5]. Many novel diagnostic approaches have been proposed for animal models, demonstrating appropriate results. These include transgastric peritoneoscopy, gastrojejunoscopy, transvaginal cholecystectomy liver resection, and lymph node biopsy [6-10]. Therefore, surgery from intramural to extramural diseases may be a trend and NOTES may overcome some serious problems in gastroenterology.

However, there are still several unanswered questions about the advantages and disadvantages of NOTES. Through gastric access, NOTES cannot be performed under a sterile environ-
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Thus, incidence of infections and complications of arteriovenous fistulas would be remarkable [11, 12]. Peroral endoscopic myotomy (POEM) is the application of esophageal myotomy to NOTES through the utilization of the submucosal tunneling method. It can protect the overlying gastric mucosa, which can effectively resist bacterial invasion and reduce complications [13-15]. Based on this theory, the investigators originally attempted the transluminal endoscopic surgery procedure via the submucosal tunneling technique, called endoscopic trans-esophageal submucosal tunneling surgery (EESTS).

The present study conducted a preliminary investigation on preoperative localization, surgical positioning, and muscularis propria, aiming to search for an optimal method for entrance of the endoscope into the peritoneal cavity. In addition, several approaches regarding the operation of an endoscope to reach a vision were investigated. This was based on the navigation system for an endoscope and the discrepancy between endoscopic images and standard anatomical images.

Materials and methods

Animals

For experimentation, the 14 male and 13 female porcine corpses were contributed by the Animal Laboratory of Pinggu District Hospital (Beijing, China). The present study was approved by the Ethics Committee of the Animal Facility of Chinese PLA General Hospital.

Study protocol

Investigating preoperative localization: EESTS, using the submucosal tunneling technique, was undertaken as follows: 1) Si pigs were fixed in the left-lateral position; 2) The methyl blue/nor mal saline mixture with concentrations of 1:2 and 1:10000 was prepared, respectively, as shown in Figure 2A. The experimental pigs were randomly divided into two groups: methylene blue solution group (ML-group) and control group (CT-group); 3) In the ML-group, the methylene blue solution (1:2) was injected into submucosal layer in the site of the right posterior wall of the cardia for localization (Figure 2B). In the CT-group, no injection was made; 4) 10-mL methylene blue solution (1:10,000) was injected to form a submucosal cushion in the right posterior wall, which was 5 cm above the esophagogastric junction (EGJ) (Figure 2C); 5) An inverted T-shaped incision was created in the mucosal layer of the esophagus (Figure 2D) in both groups; 6) An endoscopy was introduced into the submucosal space and the submucosa was gently separated from the muscularis propria, creating a submucosal tunnel in both groups. The dark blue localization spot in the cardia should be sought in the ML group (Figure 2E). In the CTL group, the endoscope…
was withdrawn from the submucosal tunnel and entered into the stomach towards the gastric fundus, aiming to observe the tunnel position; 7) A full-thickness incision was created through the muscularis propria and serosa, as presented in Figure 2F. Next, the scope was moved into lesser sac or omental bursa, which is a potential peritoneal space within the abdomen and part of the peritoneal cavity (Figure 2G); 8) The endoscopist carefully identified the other organs of the abdominal cavity, such as the liver, inferior vena cava, abdominal aorta, spleen, and posterior wall of the stomach. The scope was repeatedly entered through the abdominal cavity and the endoscopist contrasted the location of these organs; 9) Necropsy was carried out to observe the incision site, where the scope was entered into the abdominal cavity (Figure 2H); 10) The duration of the operation from submucosal tunnel creation to entrance of the scope into abdominal cavity was recorded.

Assessment of surgical positioning: EESTS was conducted, using the submucosal tunneling technique, as follows: 1) Nine pigs were randomly divided into three groups: left-lateral position (L-group), supine position (S-group), and raised right shoulder position (RR-group) (Figure 3A-C); 2) Steps in the section, “Investigating the preoperative localization” (steps 3-9) were implemented; 3) Challenges related to the operation and endoscopic vision were recorded.
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Incision approaches of muscularis propria: EESTS was conducted, using the submucosal tunneling technique, as follows: 1) Nine pigs were randomly divided into three groups: transverse full-thickness incision (T-group), longitudinal full-thickness incision (L-group), and progressive longitudinal full-thickness incision (PL-group) (Figure 4A-C); 2) The pigs were fixed in the raised right shoulder position; 3) The steps in the section, “Investigating the preoperative localization” (steps 3-6) were implemented; 4) In the T-group, the muscularis propria and serosa were incised in a transverse way using an electric knife. The incision length of the gastric parietal muscle wall was 1.5 cm, as depicted in Figure 4A. In the L-group, the incision was made in a longitudinal manner. The incision length of the gastric parietal muscle wall was 1.5-2.0 cm (Figure 4B). In the PL-group, the incision was made in the progressive longitudinal approach. In addition, an incision was progressively made from 1.2 cm in the oral margin of the preoperative location to the distal margin. The depth of incision was from the circular muscle layer to the longitudinal muscle layer. A full-thickness incision was made by incising the serosa. The scope was entered through the lesser omental sac. The incision length of the gastric parietal muscle wall was 2.0 cm, as shown in Figure 4C; 5) Status of the endoscope, operational challenges, flexibility of the endoscope, and experiences and difficulties related to endoscopic vision were recorded.

Table 1. Comparing incision sites and duration of operation between ML- and CT-groups (mean ± SD)

<table>
<thead>
<tr>
<th>Group</th>
<th>Duration of operation</th>
<th>Penetration site</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT</td>
<td>21 minutes</td>
<td>Upper part of the lesser omental sac</td>
</tr>
<tr>
<td></td>
<td>24 minutes</td>
<td>Not in the lesser omental sac</td>
</tr>
<tr>
<td></td>
<td>20 minutes</td>
<td>Posterior lower part of the lesser omental sac</td>
</tr>
<tr>
<td>ML</td>
<td>15 minutes</td>
<td>Middle and upper part of the lesser omental sac</td>
</tr>
<tr>
<td></td>
<td>12 minutes</td>
<td>Middle part of the lesser omental sac</td>
</tr>
<tr>
<td></td>
<td>18 minutes</td>
<td>Middle part of the lesser omental sac</td>
</tr>
</tbody>
</table>

Differences between endoscopic and standard anatomical images: EESTS was performed, using the submucosal tunneling technique, as follows: 1) Three pigs were fixed in the raised right shoulder position; 2) Blue methylene solution (1:2) was used for intraoperative localization; 3) A full-thickness incision was made in a progressive longitudinal manner; 4) Endoscopic and standard anatomical images were recorded and differentiated.

Statistical analysis

Statistical analyses were performed using SPSS statistical software version 22.0 (SPSS, Inc.; Chicago, IL, USA). Student’s t-test was used for continuous variables and the results are presented as mean ± standard deviation (SD). P≤0.05 indicates statistical significance.

Results

Preoperative localization

In the ML-group, 3 endoscopes were entered in the abdominal cavity through the same route from the posterior wall of gastric cardia to the lesser omental sac. In the CTL-group, 3 endo-
scopes were entered in the abdominal cavity through different routes. One of these was not entered into the lesser omental sac (Table 1), however. Duration of operation in the ML-group (21.67 ± 2.08 minutes) was shorter than that in the CTL-group (15.00 ± 3.00 minutes), with P = 0.034 (Table 1).

Surgical positioning

Factors including entrance of the tunnel, duration of operation (P = 0.242), straightness of the tunnel, endoscopic vision, and operation difficulty were investigated, finding that the raised right shoulder position was optimal (Figure 5; Table 2).

Incision approaches for the muscularis propria

Factors including incision length of gastric parietal muscle wall, flexibility of the endoscope, straightness of the tunnel, and practicability of further operations were assessed and differentiated. Results revealed that the progressive
longitudinal full-thickness incision was optimal (Table 3).

**Differences between endoscopy and standard anatomical images**

The endoscope was entered in the abdominal cavity in a straight direction through a series of surgical procedures, such as inverted T-shaped incision, progressive longitudinal full-thickness incision, and blunt dissection of the lesser omental sac (Figure 6A-C). After dissecting the porcine corpses, the endoscope was penetrated through the abdominal cavity from the posterior gastric wall to the lesser omental sac (Figure 6D). The abdominal aorta was observed in the status of the endoscope, as shown in Figure 6E. The left hepatic lobe and inferior vena cava were observed by twisting the body of the endoscope to the right (clockwise) or left (counter-clockwise) direction (Figure 6F). The posterior gastric wall and spleen were observed by continuously turning the endoscope upward (Figure 6G). The pancreas was observed by inserting the endoscope along the path of the spleen, as illustrated in Figure 6H. Endoscopic
vision was obscured when the body of the endoscope was twisted to the right or left, which led to great difficulties in observation and operation. After dissecting the porcine corpses, standard anatomical images and locations of the endoscope were found, as shown in Figure 6I.

**Discussion**

EESTS is a technique that utilizes a flexible endoscope through the gastric wall and to organs surrounding the stomach in the abdominal cavity. It cannot be performed by strict aseptic manipulation, however, and is closed completely by endoscopic clips, leading to a high ratio of infection and other complications. Therefore, finding an effective approach to protect the integrity of the intestinal mucosa has become increasingly significant [16, 17]. The submucosal tunneling technique creates a submucosal tunnel between mucous layer and intrinsic myometrium, allowing the endoscope to be enter into the extraluminal cavities and finally closed completely [18-20]. In the present study, the EESTS strategy was proposed using the submucosal tunneling technique. In additional, it was revealed that the proposed approach could maintain the integrity of the overlying mucosa, minimizing contamination from intra-luminal contents and bacteria.

The abdominal aorta is located at the lesser curvature of the posterior wall of the gastric cardia. If NOTES is performed through the cardia, it may result in severe complications, including diaphragm injury, mediastinal emphysema, or pneumothorax. Thus, the lesser curvature of the posterior wall of the gastric fundus was selected as the endoscopic access route to the abdominal cavity. However, a flexible endoscope could not maintain a straight state after perforating due to the large angle between the gastric fundus and esophagus. Hence, entering an endoscope into the abdominal cavity through the same endoscopic access route would be a technical challenge. In the present study, blue methylene solution (1:2) was injected into the submucosal layer in the site of the right posterior wall of the cardia, appearing to be an effective method for surgical localization. In the ML-group, the endoscope could be entered in the abdominal cavity through the same endoscopic access route. Results indicated that the duration of operation in the ML-group was shorter than that in the CTL-group. Accordingly, this was carefully investigated. First, in the ML-group, localization of the methylene blue could be used to navigate the endoscope to enter in the tunnel, creating a straight tunnel. When a blue mark was observed, a full-thickness incision could be made at this site, allowing the endoscope to enter the abdominal cavity. In the CTL-group, the observation of the direction of the tunnel by repeatedly withdrawing back the endoscope from tunnel when creating a tunnel was very time-consuming. Second, the methylene blue solution was injected in the same site for localization, allowing for easy observation in the gastric fundus near the cardia. This resulted in the entrance of the endoscope into the abdominal cavity through the same endoscopic access route. In conclusion, methylene blue solution (1:2) for surgical localization provides an appropriate basis for further investigation.

However, another problem presented, causing tunnel establishment to be difficult. The posterior wall of the esophagus was located at the three o’clock position. To solve this problem, surgical positioning was further investigated by differentiating the left-lateral, supine, and raised right shoulder positions. Surgery in the L-group was the most difficult, because the channel of the used endoscope was located at the eight o’clock position, causing the head of the used endoscope accessory to be at the six o’clock position. Simultaneously, the posterior wall of the esophagus located at the three o’clock position was not an optimal site for this surgery. The endoscope should be turned left by 90° to maintain the posterior wall of esophagus on the six o’clock position during the procedure. This resulted in difficult operations. Creating a tunnel and no straight tunnel was time consuming, making the surgery more difficult. This position presents an advantage, including appropriate endoscopic vision. In the S-group, the posterior wall of the esophagus was at the six o’clock position and had poor endoscopic vision, due to fluid accumulation in the tunnel entrance. In the RR-group, the right upper limb and shoulder of the pigs were raised to 15-20°. The posterior wall of the esophagus was at the five to six o’clock position. This group had the easiest operation procedure, the shortest duration of operation, and optimal endo-
scopic vision. In conclusion, the raised right shoulder position was the most appropriate surgical positioning.

In the present study, the best endoscopic access route to the abdominal cavity, when using the submucosal tunneling technique, was successfully determined by EESTS. Moreover, it was revealed that the endoscope would become inflexible when passing through the transverse incision to the abdominal cavity. In this case, if the endoscope was continuously entered in the abdominal cavity, the endoscope would turn in any direction, making subsequent surgeries extremely difficult. Therefore, another strategy was developed to explore an optimal incision approach for the muscularis propria. Three groups were introduced: transverse full-thickness incision, longitudinal full-thickness incision, and progressive longitudinal full-thickness incision groups. In the T-group, bad flexibility of the endoscope was due to the limited incision length of 1.5 cm and the block of the muscularis in the oral side, which caused that the endoscope to enter in the abdominal cavity at a certain angle. In the L-group, the flexibility of the endoscope was related to the limited incision length. Moreover, the limited incision length of 1.5 cm that belonged to the L-group had the same effect as that in the T-group. However, an incision length of 2 cm would provide a wide entrance. In this case, if the endoscope was entered through the abdominal cavity, the endoscope would turn in any direction, leading to another problem (greater injury). This would be detrimental to postoperative recovery, the sphincter of cardia dysfunction after the long incision of myotomy, and gastroesophageal reflux disease. Transverse and longitudinal full-thickness incisions were both in one-off total myotomy approaches, which could easily injure large vessels in the spatium intermusculare and extra lumen. However, a progressive longitudinal full-thickness incision could overcome these problems by performing the incision from shallow to deep. The incision by layer made hemostasis (electrocoagulation) easy in the operation, maintaining a clear endoscopic vision. Furthermore, an incision with a length of 2 cm provided enough space to enter the endoscope into the abdominal cavity in a straight state, as well as keeping the flexibility of the endoscope. In conclusion, a progressive longitudinal full-thickness incision presents several advantages.

Subsequently, the surgery was performed with the help of the methylene blue solution (1:2) for localization in the raised right shoulder position and the use of a progressive longitudinal full-thickness incision. This caused the endoscope to enter in the abdominal cavity through a route starting from the posterior wall of the gastric cardia to the middle part of the lesser omental sac. Endoscopic and standard anatomical images were differentiated to define landmarks during the operation.

**Conclusion**

To reduce the abdominal complications of NOTES, a novel strategy was proposed, EESTS. This strategy used the submucosal tunneling technique. This approach could protect overlying mucosa to the maximum extent. In the present study, some preliminary explorations were performed, finding that the raised right shoulder position for surgery, methylene blue solution (1:2) for preoperative localization, and progressive longitudinal full-thickness incisions were optimal. Afterward, differences between endoscopic and standard anatomical images were differentiated.

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**Disclosure of conflict of interest**

None.

**Abbreviations**

NOTES, Natural orifice transluminal endoscopic surgery; EGJ, Esophagogastric junction; POEM, Peroral endoscopic myotomy.

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