

The Prevention of the Development of Peritonitis with Different Disinfectants to Compare the Efficacy of Gastric Lavage at the NOTES

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Summary

In the implementation of natural orifice transluminal endoscopic surgery (NOTES) there is a risk of infection arising from the orifices of the body and the endoscope used. This study investigates the need for and efficiency of gastric lavage in reducing the risk of peritoneal infection in transgastric intraperitoneal surgery. In this study, Wistar-Albino rats were divided into 4 groups of 8. Gastric lavage was performed with three, but it was not performed on group 4. Blood samples were taken on the first and 14th days postoperatively, and a leukocyte count was made and C-reactive protein was measured. On the 14th postoperative day, the rats were sacrificed; aerobic and anaerobic culture specimens were taken from the peritoneal cavity, and biopsy samples were taken. Pathological examination of biopsy lavage in those groups, all for less inflammatory reaction and foreign body giant cell formation mikroabses were observed. In the all groups Leukocyte count and CRP measurements according to measurement values are rising. Culture examination of samples of seven different rats, while producing, anaerobic culture was not isolated in. As a result of gastric lavage should be done to ensure that surgery more safely performed of transgastric peritoneoscopy. There is no difference between the disinfectant solutions used, and the use of normal saline for gastric lavage is sufficient

Keywords: Endoscopic surgery, Transluminal peritoneoscopy, Disinfectants, Chlorhexidine, Povidone-Iodine, Saline

NOTES'da Peritonit Gelişiminin Önlenmesi İçin Farklı Dezenfektanlarla Yapılan Gastrik Lavajın Etkinliğinin Karşılaştırılması

Özet

Doğal açıklıklar yoluyla endoskopik cerrahi (NOTES) uygulamalarında vücut boşlukları ve kullanılan endoskoptan kaynaklanan enfeksiyon riski vardır. Bu çalışmada transgastrik intraperitoneal cerrahi uygulamalarında peritoneal enfeksiyon riskini azaltmada gastrik lavajın etkinliği ve gerekliliği araştırıldı. Çalışmamızda Wistar-Albino cinsi rat'lar 8'erli 4 gruba ayrıldı. 3 gruba farklı dezenfektanlar ile gastrik lavaj yapıldı, 4. gruba ise lavaj yapılmadı. Postoperatif 1. ve 14. günlerde kan alınarak lökosit sayımı yapıldı, C- reactive protein ölçüldü. Postoperatif 14. gün rat'lar sakrifiye edildi. Peritoneal kaviteden aerob ve anaerob kültür numuneleri, mide duvarı ve perigastrik peritondan biyopsi örnekleri alındı. 14. günde hayvanlar sakrifiye edildiklerinde makroskopik apse formasyonu olan hiçbir hayvan yoktu. Biyopsilerin patolojik incelemesinde lavaj yapılanlarda daha az olmak üzere tüm gruplarda inflamatuvar reaksiyon, mikroapse formasyonları ve yabancı cisim dev hücreleri gözlemlendi. Tüm gruplarda 1. ölçüm değerlerine göre Lökosit ve CRP ölçümlerinde yükselme oldu. Kültür numunelerinin incelemesinde 7 farklı ratta üreme olurken, anaerobik kültürlerde üreme olmadı. Sonuç olarak, transgastrik peritoneoskopide cerrahi uygulamaların daha güvenle yapılmasını sağlamak için gastrik lavaj yapılmalıdır. Bu amaçla kullanılan dezenfektanlar arasında fark yoktur. Serum fizyolojik ile lavaj yapılması yeterlidir.

Anahtar sözcükler: Endoskopik cerrahi, Transluminal peritoneoskopi, Dezenfektanlar, Klorheksidin, İyot, Serum fizyolojik



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INTRODUCTION

It is thought that with the implementation of natural orifice transluminal endoscopic surgery (NOTES), which aims to perform less invasive operations without any incision of the abdominal wall and complications associated with them can be eliminated¹. Within a short time of Kallou² first introducing transgastric peritoneoscopy (TP), surgeons and gastroenterologists showed great interest in the subject. Using animal models, researchers have described methods for entering the intraperitoneal area via the stomach, colon, vagina and bladder. However, these unconventional routes of entry carry a risk of infection either from the flora of the cavernous organ through which the instruments pass, or from the instruments themselves.

The Natural Orifice Surgery Consortium for Assessment and Research in the USA (NOSCAR) announced clinical experiments in research and development and potential problems in March 2006¹. This dealt with ways of preventing iatrogenic infection caused by entry into the peritoneal cavity. In the implementation of NOTES, there is a risk of infectious complications caused by leakage of the contents of the transited organs such as the oral cavity, the colon or the vagina into the peritoneal cavity.

When TP is performed, incomplete closure of the opening in the stomach can cause the spread of stomach contents into the peritoneal cavity and septic complications, and antibiotic prophylaxis and lavage are recommended to prevent such infectious complications³. However, sufficient research has not yet been done to determine whether lavage is necessary, or if so which disinfectants are more effective, or what side effects they may have^{4,5}.

This study was performed with the aim of determining the effects of gastric lavage for the purpose of reducing the risk of peritoneal infection when the transgastric route is chosen to access the peritoneal cavity by natural routes.

MATERIAL and METHODS

The study was approved by the Local Ethics Committee on Animal Experiments of Ankara University, decision No. 2009-51-254, and performed in the animal laboratory of the Medical Faculty of the same university in accordance with that decision.

Animals

Thirtytwo female Wistar-Albino rats (208-248 g) were obtained from the university's animal rearing unit. The study was carried out in conformity with the Helsinki Declaration (the 2008 revision of the 1964 resolution), directive No. 25464 of 16.5.2004 of the Turkish Ministry of Agriculture and Rural Affairs, and the law on the protection of experimental animals No. 5199 of 01.07.2004. The

animals were placed in wire cages with floors covered in wood-shavings suitable for them to feed, rest, and defecate. The temperature of the room where the cage was averaged 22°C, and the day was divided into 12 h of daylight and 12 h of darkness. Water and feed was provided with libitum up to 3-4 h before the experiment. The animals were weighed and group and serial numbers were assigned.

Protocol

The rats were divided into 4 groups of 8 for the study. Gastric lavage was performed on Group 1 with a 0.5% solution of chlorhexidine, on Group 2 with 0.5% Povidone-iodine, and Group 3 with 0.9% saline. Gastric lavage was not performed on Group 4, the control group. The rats were left hungry for 3-4 h, and 2 h before anaesthesia 10 mg (40 mg/kg) of cephazoline sodium was administered intramuscularly as a prophylactic. Gastric lavage was performed with 1.5 cc of solution using a 6f diameter plastic catheter.

Preoperative Care and Anaesthesia

The animals were taken into the laboratory on the day of the experiment. 30 min before the implementation 5 mg/kg of Xylazine HCl (Alfazyne 2%, B.V. 3440 AB, P.O. Box 78, Woerden Holland) was administered IM as an anaesthetic, and 70 mg/kg of Ketamine HCl (Eczacıbaşı Sağlık Ürünleri San. Tic. A.Ş. 39780, Tekirdağ, Turkey) was given IM as a neuromuscular blocking agent. In order to determine the depth of anaesthesia, spontaneous movements, reduction of breathing rate and shallowness of breathing, and response to touching on the plantar surface of the feet were checked. After sufficient anaesthesia was achieved, the animals' tails were hung down for 2 min to secure venous dilation, and blood was taken from the ventral vein of the tail or the lateral saphenous vein in order to count leukocytes and CRP.

Surgery

After sufficient anaesthesia had been achieved, the upper left quadrant of the abdomen was shaved, and the operation area was cleaned and disinfected with 7.5% Povidone-iodine. One hour after peritoneal lavage, a mini laparotomy was carried out in sterile conditions. A local injection was made into the incision line of Bupivacaine HCl 5 mg 0.1 ml (Marcaine 0.5% flacon 20 ml, AstraZeneca, Brixham, UK). The skin, muscle and peritoneum layers were penetrated with a sterile scalpel. The liver was displaced upwards using sterile instruments and the stomach was exposed by means of forceps. The stomach was pierced with a No 16 needle containing 2 ml of 0.9% saline, and stomach contents were drawn into the syringe by negative pressure. These stomach contents were then sprayed into the peritoneum in simulation of NOTES. The needle puncture hole in the stomach was then closed by suturing

with 4/0 polypropylene thread. Total operation time was 10-15 min.

Recovery and Postoperative Observation

The animals were taken back to their cages and temperature and breathing were monitored. On the first and 14th postoperative days, blood was taken from either the lateral or the ventral vein of the tail; a leukocyte count was performed and C-reactive protein (CRP) was measured by the turbidimetric method. Weight, feeding habits, clinical signs of sepsis and bowel movements were monitored daily. On the 14th postoperative day the rats were sacrificed and the intraperitoneal area was examined. The presence of purulent material was accepted as an abscess. Aerobic and anaerobic samples were taken from the peritoneal cavity. Care was taken when taking culture samples to prevent contamination. The aerobic samples were transferred by Stuart transport medium and aerobic samples by Cary-Blair medium and delivered within 30 min to the microbiology laboratory. Aerobic samples were sprinkled on to 5% sheep's blood agar and incubated at 37°C for 4 h, and then evaluated. The anaerobic samples were sprinkled on to blood agar and evaluated after 72 h incubation at 37°C in anaerobic conditions. Gram staining was carried out on the reproductive colonies. The colonies were named taking into account their colony characteristics and Gram staining characters by choosing suitable cards in a VITEK 2 (bioMerieux, France) automated system.

The specimens from the gastric incision site and peritoneal surface were assessed by gross examination and submitted in 10% Formalin solution for microscopic histopathological examination. The specimens were later embedded in paraffin, sectioned on a manual microtome, and stained with haematoxylin solution and eosin. All slides were reviewed by the same pathologist.

Statistical Analysis

The Kruskal Wallis test was used to compare the 4 groups with regard to the results of the first and second measurements of leukocytes and CRP, and the Wilcoxon signed-rank test was used to test for differences between the first and second measurements of each group^{6,7}.

RESULTS

There was no difference between the groups regarding the first and second leukocyte measurement (P = 0.175 and P = 0.643 respectively). In comparisons within the groups, there was an increase in the second measurement as compared with the first measurement except in the groups where gastric lavage was performed using 0.9% saline (Table 1).

There was a difference between groups in the first and second CRP measurements (P= 0.022 and P = 0.004

respectively). In the double comparisons, there was a difference between the 0.9% saline group and the control group at the first measurement, and at the second measurement there were differences between the iodine group and the chlorhexidine group, and the iodine group and the 0.9% saline group (P = 0.022 and P = 0.005 respectively). CRP values of all groups were higher in the second measurement than in the first, but except for the iodine group this did not reach statistical significance (Table 2).

When the animals were sacrificed on the 14th day, none showed any macroscopic abscess formation. However, it was observed in all groups that the hepatogastric ligament and the perigastric fatty tissue had closed over the sutured hole in the stomach, and that micro abscesses had formed. Propagation occurred in seven aerobic cultures, but no obligate anaerobic bacteria were propagated in the cultures. Culture results are shown in Table 3.

Microscopic tissue micropathological examination revealed that the gastrotomy sites of all groups had chronic inflammatory changes and foreign-body giant-cell reactions (Fig. 1). In addition, micropathological examination of the rats on which gastric lavage had been performed with chlorhexidine and 0.9% saline showed

Table 1. Comparison of leukocyte counts between groups *

Tablo 1. Grupların lökosit sayılarına göre karşılaştırılması

| Name of Disinfectants | 1st Count | 2nd Count | P Value |
|-----------------------|-------------|--------------|---------|
| Chlorhexidine | 5.762±3.863 | 10.463±3.729 | 0.021 |
| Povidone iodine | 1.925±1.955 | 7.863±3.572 | 0.036 |
| Normal saline | 4.000±2.964 | 8.825±4.114 | 0.093 |
| Group of control | 3.050±3.402 | 9.650±4.196 | 0.025 |

* Values were measured in 10³/μL

Table 2. Comparison of CRP measurements between groups *

Tablo 2. Grupların CRP ölçüm değerlerine göre karşılaştırılması

| Name of Disinfectants | 1st Count | 2nd Count | P Value |
|-----------------------|-------------|--------------|---------|
| Chlorhexidine | 51.50±21.57 | 68.13±33.48 | 0.150 |
| Povidone iodine | 56.50±12.68 | 101.38±19.33 | 0.012 |
| Normal saline | 46.88±19.11 | 60.63±17.70 | 0.069 |
| Group of control | 74.38±13.13 | 79.63±12.14 | 0.249 |

* Values were measured in 10³/μL

Table 3. Comparison of culture results between groups

Tablo 3. Grupların kültür sonuçlarına göre karşılaştırılması

| Groups | Propagating Bacteria |
|------------------|-----------------------------------|
| Chlorhexidine | <i>Neisseria spp.</i> |
| | <i>Escherichia coli</i> |
| Povidone Iodine | <i>Staphylococcus chromogenes</i> |
| | <i>Streptococcus spp.</i> |
| Normal Saline | <i>Staphylococcus chromogenes</i> |
| | <i>Aerococcus viridans</i> |
| Group of Control | <i>Lactobacillus spp.</i> |

micro abscess formation (Fig. 2). Except for an inflammatory reaction in the iodine gastric lavage group and the control group, no special pathology was observed.

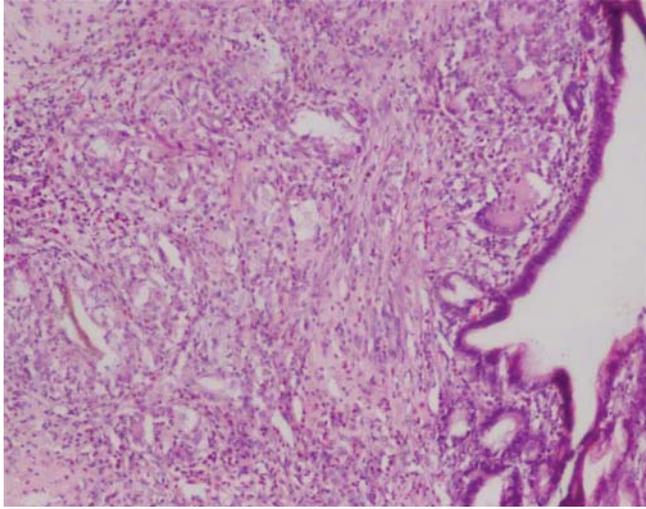


Fig 1. Foreign-Body Giant Cells from one of the rats on which gastric lavage was performed with 0.9% saline

Şekil 1. 0.9% sodyum klorür ile gastric lavaj yapılan sıçanlardan birinde yabancı cisim dev hücrelerinin görünümü

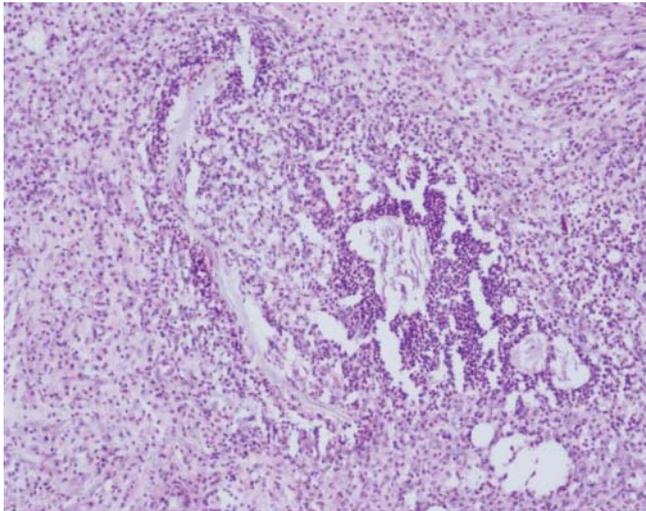


Fig 2. A View of micro abscesses on one of the rats on which lavage was performed with 0.9% saline

Şekil 2. 0.9% sodyum klorür ile gastric lavaj yapılan sıçanlardan birinde küçük apse odaklarının görünümü

DISCUSSION

There are various sources of infection in transluminal endoscopic surgery. The sources of infection in NOTES are the endoscope and accessories, the passage through which the endoscope passes, and the target organ of the surgery. Infections arising from microorganisms from the natural entry passages can be partly or wholly prevented by lavage with non-absorbable antimicrobial solutions. In the first study by Kalloo et al.², infection developed with the formation of intra-abdominal micro abscesses

in two pigs on which antibiotic gastric lavage was not performed. In a follow-up study, gastric lavage was performed and infection did not develop⁸. In other studies, it has been reported that infection developed in spite of similar prophylaxis. In a study by Merrifield et al.⁹, two out of 5 animals on which transgastric cholecystectomy had been performed died of purulent peritonitis. Buck et al.¹⁰ showed that bacterial colonization was reduced in 37 pigs on which gastric lavage had been performed with 500 ml of sterile saline. Nevertheless, these studies reached the conclusion that the risk of intra-abdominal infection was higher than with open or laparoscopic surgery, even with prophylactic antibiotics and lavage. Steele et al.¹¹ reported that in animals in which liver biopsies had been taken by TP following gastric lavage, no infectious complications occurred. Narula et al.¹² found that there was no peritoneal contamination from species isolated in gastroscopic contents in 10 patients on whom diagnostic TP was performed but gastric lavage was not performed, and that there was not enough pathogenic transfer to cause a clinically significant reaction, and reached the conclusion that stomach lavage with non-absorbable antibiotics or disinfectants was unnecessary. In our study, bacteria were propagated from cultures taken from 7 rats, but macroscopic abscess formation of clinical importance was not observed. Although perigastric micro abscesses were observed in all groups in our study, these were not enough to show clinical importance. In this respect, there was no difference between the groups where lavage was and was not performed.

The disinfection of instruments used in TP will prevent infectious agents from outside from affecting the interior of the abdomen. As these instruments are used on a large number of patients they must be thoroughly disinfected so as to prevent infections passing from one patient to another. With this aim, and particularly to kill such viruses as HIV, instruments must be soaked for long periods in 2% glutaraldehyde or 5% chlorhexidine solutions, or even disinfected with ethylene oxide. In a study by Pai et al.¹³, only one animal died from sepsis after peritoneoscopy with a well-disinfected gastroscope. In that study it was suggested that the spread of developing adhesion, infections was limited by the transluminal applications. Magno et al.¹⁴ reported that they did not observe intra-peritoneal septic complications in animals on which they used sterile instruments and performed gastric lavage before peritoneoscopy, but that in the groups where they did not do this, they observed micro abscesses on the line of the incision. Sterile plastic catheters of diameter 6F were used for gastric lavage in the study, and all rats received prophylactic 10 mg Cephalozine sodium IM 2 h before anaesthesia.

The closure of transluminal openings by secure methods is of vital importance in the prevention of

infection¹⁵. If the closure of the organ entered is inadequate, bacteria spreading into the abdomen from the incision line may cause infection. In gastrotomy a tampon, patch or clip may be used for this purpose^{16,17}. Kalloo et al.⁸ used an endoscopic clip and tampon technique and animals recovered without problems, opening up new horizons in this field. Bergman et al.¹⁸ reported closing gastrotomy openings in dogs with bioabsorbable mesh. After 2 weeks, there was no leakage in any of the dogs. In a histological study a normal stomach with early recovery was reported. However, it must be remembered that this study was carried out on dogs, which are more resistant to infection than humans. There are various other approaches and suggestions relating to closure. Some authors report pulling the omentum in towards the stomach and clipping it similar to a Graham patch. There are criticisms of this technique that only the mucosa and not the seromuscular layer are brought into contact. According to the principles of surgery, the seromuscular layer must be brought together for a full recovery. Merrifield et al.⁹ carried out TP on 5 animals, 2 of which died of purulent peritonitis because the clips used to close the gastric opening were insufficient. Pauli et al.¹⁹ observed clinically insignificant micro and macro-abscesses in the gastric wall of 2 of 5 pigs on which they performed an entry to the intraperitoneal area by forming a sub-mucosal tunnel in the gastric wall. It was stated that intraperitoneal abscesses did not form in any of these animals, and that they all recovered. Writers have reached the conclusion that bacterial contamination occurred during TP and that there was no later contamination. Perretta et al.²⁰ reported that one pig died of sepsis out of 6 in which they had closed a gastrotomy opening with a nitinol patch which they had developed themselves. In our study, the gastric opening made with a No 16 needle was closed with 4/0 polypropylene sutures. When the animals were sacrificed, the recovery of the scar in the sutured part of the stomach wall was seen to be normal. Pathological examination of biopsies taken from this area showed inflammatory and foreign body reactions in all groups. In the chlorhexidine and normal saline groups, the formation of micro abscesses was observed in addition to these findings.

Bacteria isolated in our study were opportunistic microorganisms such as *Lactobacillus spp.* or gram negative enteric bacilli found in the gastrointestinal flora such as *E. coli* and *Neisseria spp.* These are opportunistic pathogens of low virulence found in the flora of the gastric lumen or other parts of the gastrointestinal system. That is, their presence, given the existence of the immune system, does not mean that they could cause infection. Ryou et al.²¹ took 2.0x2.0 cm full-thickness specimens from the stomachs and colons of 6 adult female pigs which had been euthanized and after treating them for 10 min with 5 different solutions cultured them on blood agar. It is reported that after this only those which had received

IV Cephalozine or gastric lavage showed a reduction in the number of bacteria in the stomach, and those which had received both lavage and Cephalozine prophylaxis showed a reduction to zero. In our study, we were unable to give the number of bacteria per ml (CFU) because the specimens which were taken for culture were not of a standard quantity.

It may be considered that gastric lavage is unnecessary; because gastric acidity means that the pH of the stomach is not suitable for the proliferation of bacteria. A proportion of authors who are of this opinion support the idea that the use of these methods to prevent infection during TP is not always necessary. It has been proved by various studies that when a proton pump inhibitor (PPI) is given and the gastric pH rises, a more suitable environment is created for the proliferation of bacteria. Ramamoorthy et al.²² aspirated stomach contents and injected them into the intraperitoneal area in a controlled experiment on 30 Sprague-Dawley rats. The rats were sacrificed after 2 weeks and cultures were taken, and it was found that in the group which had received PPI there was 60% proliferation, while in the group which had not received PPI there was 20% proliferation. In studies on humans it was observed that cases who had received PPI showed an increased transfer of bacteria from the stomach to the intraperitoneal area after TP. Narula et al.¹² compared cases who did and did not use PPI from among 50 TP patients, and found that although more bacteria were isolated from the intraperitoneal area of individuals who used PPI, this number was not sufficient to be the cause of a clinically significant infection.

It is possible to assess the development of infection indirectly by inflammation mediators. A change in these mediators can indicate at an early stage an increase in the number of microorganisms, which does not show clinical signs but which brings the immune system into action and constitutes a risk of infection^{23,24}. CRP and leukocytes are among those mediators. In our study, the CRP measurement and the leukocyte count were higher on the 14th day after TP than on the first day. These increases are probably related to localized intragastric infections.

There is a risk of peritonitis in open enteroscopy such as choledochoscopy and gastrotomy in the course of emergency or elective operations. This kind of entry is called a clean-contaminated wound in surgery and the development of postoperative infection can be prevented by means of antibiotic prophylaxis²⁵. Microorganisms isolated in elective surgical entries where the gastrointestinal system is opened are of endogenous origin. NOTES is achieved through controlled clean-contaminated wounds. The 10-20% infection risk in animals after trans-luminal endoscopy can be reduced by prophylactic antibiotics or gastric lavage^{2,9}. In our study, no macroscopic abscesses or generalized peritonitis was encountered in

sacrificed rats after laparotomy. Perigastric micro abscesses were not widespread enough to be clinically significant.

The use of a syringe to aspirate stomach contents rather than carrying out gastric perforation and the use of a small animal like a rat were disadvantages of this study. Advantages were the performance of the study on a sufficient number of animals and the provision of better control. While the methods used in the study were not exactly those of NOTES, stomach contents were spread in the perigastric area and the opening in the stomach was sewn up as a primary as far as possible in accordance with the principles of NOTES.

Another aspect is the importance of whether or not micro abscesses were of an extent to cause clinical signs. This must be observed in the recovery period from normal surgery carried out on a significant number of animals and humans. Judgment or suggestions on this topic cannot be made until a sufficient number of comparative clinical studies have been carried out.

Finally, NOTES is a less invasive surgical intervention than classical laparoscopy. Instruments will enable more reliable prevention of infection arising in transluminal surgery from the organ used for transluminal passage and the target organ on which surgery is performed. Pre-operative gastric lavage performed for preventing the spread of infection when the transgastric route is used for NOTES is effective. However, until this topic is clarified with further studies in animals and humans, gastric lavage, prophylactic antibiotics and disinfected endoscopes must be used to prevent the development of infection after TP.

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