

# Systemic Inflammatory Response After Natural Orifice Transluminal Surgery: Transvaginal Cholecystectomy in a Porcine Model

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## ABSTRACT

**Objective:** We analyzed circulating TNF- $\alpha$  and IL-6 to determine systemic inflammatory responses associated with transvaginal cholecystectomy in a porcine model.

**Methods:** Six female pigs were used for a survival study after transvaginal cholecystectomy (NOTES group) using endoscopic submucosal dissection (ESD) instruments and a single-channel endoscope. Blood was drawn preoperatively and 24 hours and 48 hours postoperatively. Four pigs were used as controls. In addition, laparoscopic cholecystectomy was performed in 2 pigs for laparoscopic control.

**Results:** In all 6 pigs in the NOTES group, no major intraoperative complications occurred. No significant differences were found between control, laparoscopic, and NOTES groups in terms of preoperative IL-6 level ( $P=0.897$ ) and at 24 hours ( $P=0.790$ ), and 48 hours postoperatively ( $P=0.945$ ). Similarly, there was no significant difference in mean preoperative ( $P=0.349$ ) and mean day 2 postoperative TNF- $\alpha$  levels ( $P=0.11$ ). But a significant increase in day 1 postoperative TNF- $\alpha$  levels in the laparoscopic group compared with that in the control and NOTES groups was observed ( $P=0.049$ ). One limitation of our study is that the sample size was relatively small.

**Conclusion:** NOTES is safe in animal models in terms of anatomical and cellular level changes with minimal systemic inflammatory host responses elicited. Further study needs to be carried out in humans before NOTES can be recommended for routine use.

**Key Words:** NOTES, Natural orifice transluminal endoscopic surgery, Transvaginal surgery, Cholecystectomy, Cytokines, Acute inflammatory response.

## INTRODUCTION

Natural orifice transluminal endoscopic surgery (NOTES) has developed rapidly in recent years, and transvaginal cholecystectomy has been successfully performed in humans.<sup>1</sup> Researchers are actively evaluating different peritoneal accesses<sup>2-4</sup> and development of novel instruments and innovative procedures.<sup>5</sup> There have been few reports, however, on the immunological effects of NOTES. In the development of laparoscopic surgery, tremendous research has been conducted to address immunological and inflammatory responses following laparoscopy. Laparoscopic procedures are associated with less host inflammatory response compared with that in open surgery. This has been well demonstrated in colorectal resection<sup>6</sup> and liver resection.<sup>7</sup> In addition, a more favorable cellular and humoral immunological response has been observed in laparoscopic colorectal resection in terms of a lower level of circulating IL-6 and TNF- $\alpha$ .<sup>8</sup> Theoretically, with the lower degree of trauma induced by NOTES, a lower perioperative proinflammatory response would be expected. In the current study, we aimed at analyzing the systemic inflammatory responses associated with transvaginal cholecystectomy in a porcine model.

## METHODS

This study, using transvaginal cholecystectomy in the porcine model, was carried out in the Animal Laboratory, Department of Surgery, University of Hong Kong. The pigs, weighing 30 kg to 35 kg, were cared for by a registered veterinarian working in our laboratory before and after the procedures. Before the experiment, the pigs were housed in a large animal care facility and were maintained in an environment of  $20\pm 1^\circ\text{C}$ . They were quarantined and allowed to acclimatize in the animal facility for a minimum of 1 week before being used. All animals were given a standard diet and water ad libitum until the time of the experiment. The study was approved by the Committee on the Use of Live Animals in Teaching and Research of the University of Hong Kong. All experiments conducted in pigs followed the Animal Welfare Guidelines for the care and use of laboratory animals.

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## Experimental Design

### Phase I

Six female pigs were used for the survival study after transvaginal cholecystectomy performed with endoscopic submucosal dissection (ESD) instruments and a single-channel endoscope. The pigs were kept alive for 2 weeks and then sacrificed. Blood was drawn preoperatively and within 24 hours to 48 hours postoperatively.

### Phase II

Four female pigs served as the control group. Preparation was the same as in the pigs used for transvaginal cholecystectomy except that no operation was performed and anesthesia was reversed 180 minutes after induction. Blood was drawn within 24 hours to 48 hours afterwards.

### Phase III

Laparoscopic cholecystectomy was performed in 2 female pigs as a laparoscopic control. Blood was drawn within 24 hours to 48 hours postoperatively.

### Anesthesia

All pigs were fasted overnight before the procedure and were given 0.5 mg/kg ketamine and 1.2 mg atropine sulphate by intramuscular injection as a premedication before anesthesia. An ear vein was used for injection of 0.5 mg/kg of Dormicum, 0.1 mg/kg of Pavulon, and 0.01 mg/kg fentanyl for induction. The trachea was intubated with a cuffed endotracheal tube using an illuminated laryngoscope with an extra-long blade. Anesthesia was then maintained with 0.5% to 1.5% isoflurane. Oxygen and nitric oxide was administered in a ratio of 1:3. Periodic injection of Pavulon and fentanyl was used to ensure adequate anesthesia and muscle relaxation. On completion of the operation, 1 mg of neostigmine and 1.2 mg of atropine were used for anesthesia reversal, and the pigs were extubated. Ampicillin in a dose of 500 mg was injected intravenously just before the procedure and continued for 5 days every 8 hours. Buprenorphine 0.05 mg/kg to 0.2 mg/kg was given every 12 hours for 3 days after the operation for pain control. The pigs were sacrificed with 200 mg/kg of pentobarbital sodium 2 weeks after the experiment.

### Preoperative Preparation

All operating instruments were sterilized according to the standard autoclave protocol, whereas the endoscope was

disinfected by immersing it for 20 minutes in 2.4% glutaraldehyde (Cidex, Johnson and Johnson, New Brunswick, NJ) and then rinsed with sterile water. The peritoneal access and cholecystectomy were performed with a single-channel endoscope (GIF-Q240X, Olympus Optical Co, Ltd, Tokyo, Japan). The anesthetized female pig was put in the lithotomy position with back support. The skin was prepared with povidone-iodine solution, and the vagina was irrigated with the same solution. Every effort was made to ensure strict asepsis during the whole procedure. A urinary catheter was also inserted under endoscopic guidance.

### Peritoneal Access

A Veress needle was inserted for creation of carbon dioxide pneumoperitoneum and maintenance of intraperitoneal pressure at 12 mm Hg. The pig was then put in a Trendelenburg position. The urogenital sinus was cannulated with the endoscope, and the vagina cavity was entered. After the cervix was identified, 2 approaches were used for accessing the peritoneal cavity: (1) the anterior vaginal wall was dissected with a hook knife (Olympus Optical Co, Ltd, Tokyo, Japan), and air was insufflated during colpotomy to maintain an optimal endoscopic view; the entrance to the peritoneal cavity was recognized by a sudden increase in monitored intraperitoneal pressure, and the hook knife then served as a guidewire for insertion of the endoscope into the peritoneal cavity; (2) The endoscope was passed beyond the cervix to enter the retroperitoneal plane, and the peritoneum was incised using a hook-knife to gain access to the peritoneal cavity.

### Cholecystectomy

One 5-mm trocar was inserted under direct endoscopic vision for retraction of the gallbladder. The endoscope was then manipulated so that minimal looping would be created and an end-on view of the cystic duct could be achieved. The gallbladder was retracted cranially and laterally. The covering peritoneum was incised and dissected using a combination of hook knife and hot biopsy forceps. After the cystic duct and artery were isolated, both were clipped together with endoscopic HemoClip (Olympus Optical Co, Ltd, Tokyo, Japan) and divided with endoscopic scissors (Olympus). The gallbladder was then dissected from its liver bed by using a hook knife or IT (Insulated Tip) knife, or both, (Olympus Optical Co, Ltd, Tokyo, Japan) with PD-60 and ENDOPLASMA (Olympus Optical Co, Ltd, Tokyo, Japan) where appropriate. Use of the IT knife was advantageous as it could protect the liver

from diathermy injury and minimize the chance of accidental gallbladder perforation.

### Specimen Retrieval

After clearing accumulated intraperitoneal fluid by suction via the endoscope, the gallbladder was grasped with either a pair of biopsy forceps or an endoscopic snare under direct vision. It was then retrieved via the vagina. The vaginotomy was left open after the procedure.

### Cytokines Measurements

Porcine serum was prepared by centrifuging the clotted blood for 10 minutes at 3000 rpm, and the serum was then stored in aliquots at  $-80^{\circ}\text{C}$ . After collecting all specimens required for experimentation, TNF- $\alpha$  and IL-6 were quantitatively measured by the ELISA method [R&D Systems (Quantikine), Minneapolis, MN, USA]. The colored product formed at the end of assays was measured according to the manufacturer's instructions, and the concentrations of TNF- $\alpha$  and IL-6 were subsequently determined from their standard curves. Interassay precision was ensured by testing 2 samples for each serum specimen collected.

### Statistical Analysis

The level of TNF- $\alpha$  and IL-6 is expressed as mean. The differences between operated on and control groups were determined by using the one-way ANOVA test. All statistical analysis was calculated using SPSS for Windows version 11.5 (Chicago, IL) (Tables 1 and 2).

## RESULTS

Twelve female pigs were used for the present study in which 4 were assigned to the control group without intervention after general anesthesia with the same protocol previously mentioned (C). Two pigs underwent laparoscopic cholecystectomy (L), and 6 pigs underwent NOTES transvaginal cholecystectomy with a single-channel endoscope and ESD instruments (N). In all 6 pigs in the treatment group, satisfactory control of the cystic complex was achieved, and no major intraoperative complications occurred. Cholecystectomy was performed uneventfully in the laparoscopic control group without any morbidity. The mean operating time for the NOTES group was 123 minutes (range, 92 to 175). The mean preoperative IL-6 was 59.54 pg/mL for the control group (C), 63.5 pg/mL for the laparoscopic group (L), and 50.47 for the NOTES group (N) ( $P=0.897$ ). Day 1 postoperative IL-6 levels were 67.31 pg/mL (C), 68.68 pg/mL (L), and 57.37 pg/mL (N),

respectively ( $P=0.790$ ). Postoperative day 2 IL-6 levels were 59.67 pg/mL (C), 54.07 pg/mL (L), and 57.54 pg/mL (N) ( $P=0.945$ ). No significant difference was found in the 3 groups at different times. Similarly, no significant difference was noted in mean preoperative TNF- $\alpha$ : 79.94 pg/mL for the control group (C), 82.68 pg/mL for the laparoscopic group (L), and 53.99 for the NOTES group (N) ( $P=0.349$ ). Mean postoperative day 2 TNF- $\alpha$  levels were 69.19 pg/mL (C), 105.12 pg/mL (L), and 63.29 pg/mL (N) ( $P=0.11$ ). But there was a significant increase in postoperative day 1 TNF- $\alpha$  level in the laparoscopic group compared with the control and NOTES groups: 63.53 pg/mL (C), 93.83 pg/mL (L), and 50.59 pg/mL (N) ( $P=0.049$ ).

## DISCUSSION

For the past 2 decades, laparoscopic surgery has been shown to be associated with less postoperative pain and faster postoperative recovery. This is usually attributed to the small incision, less manipulation, and avoidance of exposure of internal viscera during laparoscopic surgery. Together with the improved operative outcomes, the minimally invasive approach is merited with triggering fewer inflammatory responses in the patient.<sup>9</sup> Inflammatory cytokines, as an indirect measurement of host acute inflammatory response, are usually used in the laboratory and clinically to assess the magnitude of the acute-phase reaction. Significantly lower levels of TNF- $\alpha$ , IL-1, and IL-6 and other inflammatory markers were released in the laparoscopic surgery group compared with markers released in the open surgery group.<sup>10–20</sup> With the recent increased interest in NOTES, the impact of transvaginal cholecystectomy on host response is being studied.

In our experiment, we compared the postoperative inflammatory response between laparoscopic, NOTES, and control groups in a porcine model. Circulating cytokines, being the most sensitive indicator for host trauma, objectively reflect host immunological response. The differences in host immune responses between open and laparoscopic cholecystectomy have been well validated in the literature, and therefore we did not include an open cholecystectomy control group. We demonstrated no significant difference in terms of TNF- $\alpha$  and IL-6 levels 48 hours after surgery. However, a significant increase in TNF- $\alpha$  levels was detected in the laparoscopic group compared with control and NOTES subjects on postoperative day 1. But the rise is not significant when comparing laparoscopic and NOTES groups alone ( $P=0.059$ ). It is difficult

**Table 1.**  
Mean IL-6 Level (pg/mL)

	Preoperative	Day 1	Day 2
Control	59.54	67.31	59.67
Laparoscopic	63.5	68.68	54.07
NOTES*	50.47	57.37	57.54

\*NOTES = natural orifice transluminal surgery.

**Table 2.**  
Mean TNF- $\alpha$  Level (pg/mL)

	Preoperative	Day 1	Day 2
Control	79.94	63.53	69.19
Laparoscopic	82.68	93.83*	105.12
NOTES†	53.99	50.59	63.29

\*P = 0.049.

†NOTES = natural orifice transluminal surgery.

to predict whether it was due to a transient inflammatory host response to the skin incision, surgical procedures, or purely due to chance alone. Because only 2 subjects were included in the laparoscopic group, it is difficult to draw a conclusion about the significance of the greater cytokine response in laparoscopic surgery compared with that in NOTES. However, with smaller and fewer abdominal incisions, less inflammatory response should be triggered compared with that in laparoscopic or open surgery. Although the sample size is small for the whole study, it is logical to conclude that NOTES is safe in terms of the microcellular level in pigs. This finding needs to be further validated in randomized control trials in humans.

Besides surgical access, another potential hazard in NOTES nowadays is the control of pneumoperitoneum. It is not uncommon to overgenerate pressure while manipulating endoscopes within the peritoneal cavity. Consistent or repeatedly high intraabdominal pressure causes abdominal compartment syndromes, with the potential danger of hemodynamic disturbances, impairing end-organ perfusions, or even pulmonary embolism. However, it is not an easy task to detect all these subtle changes in the porcine model unless invasive monitoring is adopted perioperatively. Therefore, pigs survival does not necessarily equal clinical safety. Our study further supports the safety in NOTES in humans.

## CONCLUSION

NOTES is safe in animal models on the anatomical and cellular levels with minimal systemic inflammatory host responses elicited. Further study needs to be carried out in humans before NOTES can become part of routine practice.

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