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Evaluation of two liver biopsy and rectal sealing techniques in dog cadavers undergoing Natural Orifices Transluminal Endoscopic Surgery (NOTES) by transrectal access

Avaliação de duas técnicas de biopsia hepática e selamento retal em cadáveres de cães submetidos a cirurgia endoscópica por orifícios naturais transretal

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Highlights

The rectal rupture pressure of cyanoacrylate adhesive is the same as rectal suture.
Liver biopsy with endoscopic oval forceps collecting samples from the liver surface.
The diathermy polypectomy endoscopic forceps biopsy provides larger diameter samples.

Abstract

The aim of this study was to evaluate two liver biopsy techniques by transrectal Natural Orifice Transluminal Endoscopic Surgery (NOTES) and compare tensiometric parameters of rectal sealing using 2-octyl cyanoacrylate glue or conventional rectal sutures in a dog cadaver model. In sixteen dog cadavers two liver biopsy techniques were performed via transrectal NOTES using either polypectomy diathermy forceps or endoscopic oval biopsy forceps. The cadavers were divided into two groups: Glue Group (GG) where rectal sealing was performed with 2-octyl cyanoacrylate glue and Suture Group (SG) with the rectal defect sealed with simple continuous extracorporeal 3-0 polydioxanone sutures. The rupture pressure of the seals was measured on a rectal burst test. The diathermy polypectomy endoscopic forceps biopsy technique was significantly faster ($p<0.001$) and provided larger diameter samples. Rectal sealing was significantly faster ($p<0.001$) in the GG. There was no difference between the two groups with regard to rupture pressure (258.5 mmHg) with air insufflation. Using endoscopic oval biopsy forceps, biopsy samples can only be collected from the surface of the liver, whereas polypectomy forceps with a diathermy loop can be used to collect samples from the tip of the hepatic lobe. There was no difference in rectal rupture pressure in the burst test between the cadavers where

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sealing was performed with rectal sutures and those where cyanoacrylate adhesive was used.
Key words: Adhesive. Experimental study. Liver. Rectal access. Rectum. Surgery.

Resumo

O objetivo do presente estudo foi avaliar duas técnicas de biopsia hepática realizadas por cirurgia endoscópica transluminal por orifícios naturais (NOTES) transretal e parâmetros tensiométricos do selamento retal usando adesivo de 2-octil cianoacrilato ou sutura retal convencional em cadáveres de cães. Em 16 cadáveres de cães, foram realizadas duas técnicas de biopsias hepáticas usando pinça diatérmica de polipectomia e pinça de biopsia oval. Os cadáveres foram divididos em dois grupos: sendo que o Glue Group (GG) recebeu o adesivo de 2-octil cianoacrilato para selamento retal e o Suture Group (SG), recebeu sutura contínua simples extracorpórea com fio polidioxanona 3-0. A pressão de ruptura retal foi mensurada por meio do teste de pressão intra-retal. A técnica de biopsia hepática com pinça diatérmica de polipectomia foi mais rápida ($p<0.001$) e providenciou amostras maiores. O selamento retal foi mais rápido no GG ($p<0.001$). Não houve diferença entre os grupos com relação a pressão de ruptura retal com insuflação de ar (258.5 mmHg). A biopsia hepática com pinça oval é limitada a colheita de fragmentos superficiais do fígado, já a pinça de polipectomia permite a colheita de fragmentos nas extremidades. Os parâmetros tensiométricos do reto, avaliados por meio do teste da pressão de ruptura retal foram semelhantes tanto em cães que receberam sutura retal quanto nos que receberam apenas o adesivo de 2-octilcianocrilato em cadaveres de cães.
Palavras-chave: Adesivo. Estudo experimental. Fígado. Acesso retal. Cirurgia.

Introduction

The clinical importance of liver biopsy in the diagnosis and prognosis of hepatobiliary diseases, has motivated the search for new, less invasive techniques and better equipment, since bleeding, subcapsular hematoma, sample size and prolonged time to perform the procedure constitute important disadvantages of frequently used techniques (Neuberger & Cain, 2021).

Natural Orifice Translumenal Endoscopic Surgery (NOTES) is an advanced form of minimally invasive surgery (Rattner & Kalloo, 2006) with the advantage of providing transluminal access to the abdominal cavity without an abdominal incision. A variety of access routes for NOTES have previously been described in the literature, including: transvaginal, transgastric, transurethral, transcolonic and transrectal (Lima et al., 2006; Alcaraz et al., 2009; Trindade et al., 2015).

The advantages of a transrectal approach include: better endoscope reach and improved visualization of the cranial abdominal organs and elimination of the need for scope retroflexion as in the transgastric route. The transrectal approach also offers a larger caliber lumen for instrument insertion and specimen retrieval in both sexes (Ryou & Thompson, 2008). However, surgical closure of the rectal wall is challenging. Rectal wall healing is poor compared to other organs due to reduced collagen content and more collagenase-producing bacteria and other factors such as contamination

by microorganisms, friction and difficulty in local immobilization may delay healing (Soares et al., 2010). Tight suturing can impair tissue perfusion and oxygenation, hindering rectal healing (Irkorucu et al., 2009). Gluing is a good alternative for rectal wall sealing without tissue strangulation.

2-octyl cyanoacrylate is one of the most commonly used, commercially-available, wound adhesives, with indications for approximation of surgical incisions or lacerations secondary to trauma in dry and low friction areas (Saxena & Willital, 1999; Perera & Tavarez, 2024). Contraindications for the use of cyanoacrylate adhesives are limited. Specifically, they cannot be used in areas of tension, such as joints, areas subjected to friction, and areas showing infection and/or contamination with exudate (Borie et al., 2019; Perera & Tavarez, 2024). In addition, they cannot be used in conjunctival procedures and patients with allergy to cyanoacrylate (Borie et al., 2019). Successful use has been reported in a tongue laceration in a child and for cleft lip repair (Kazzi & Silverberg, 2013), and for reinforcement of colorectal anastomosis in an experimental model in rats (Kazzi & Silverberg, 2013) and pigs (Boersema et al., 2017).

Rectal closure is essential for the safe use of transrectal NOTES and the evaluation of different endoscopic biopsy options is important in the development of new techniques for collecting liver fragments. Thus, the aim of this study was to evaluate and compare two liver biopsy techniques performed via transrectal NOTES and compare tensiometric parameters of rectal sealing using 2-octyl cyanoacrylate-based glue or conventional suture with polydioxanone in an experimental canine model.

Material and Methods

Sixteen fresh frozen, adult dog cadavers, with no chemical preservation were donated by the Special Secretariat for Animal Rights (SEDA). There were 10 male and 6 female mixed breed cadavers, with an average weight of 15.9 ± 1.9 kg. The dogs had no history of gastrointestinal disorders or recent surgical procedures in the abdominal cavity and were not obese.

The cadavers were kept frozen for up to 24 hours before the procedure and were thawed by immersion in 100L of water. Feces was manually removed and colorectal washing with water was performed. The two methods of liver biopsy collection through the same transrectal access by NOTES were carried out in each cadaver. The cadavers were randomly divided into two groups of eight, according to the rectal sealing technique applied. In the Glue Group (GG) the rectal incision was sealed using 2-octyl cyanoacrylate and in the Suture Group (SG) the defect was closed with simple continuous extracorporeal sutures using 3-0 polydioxanone.

In both groups, the cadavers were positioned in left lateral recumbency. The rectal mucosa was exteriorized by the insertion of four stay sutures with 4-0 nylon which were pulled through the anus (Figure 1A), a 3 cm transverse incision was made in the rectal wall with a scalpel, 5 cm proximal to the external anal sphincter (Figure 1B). Doyen-type forceps were inserted through the incision created in the rectum for blunt dissection of the adjacent pelvic tissues so that a flexible colonoscope (Pentax®), 14 mm in diameter and 150 cm in length, could be inserted through the rectal incision, into the

abdominal cavity (Figure 1C). The cadavers were then placed in the supine position. Pneumoperitoneum was established with endoscopic abdominal insufflation (with ambient air). The volume of ambient air used for abdominal distension in the dog cadavers was established such that there was sufficient separation of the abdominal muscle wall from the abdominal structures and viscera to generate an adequate intracavitary work space. The abdomen was inspected, and the number of anatomical structures visualized was recorded.

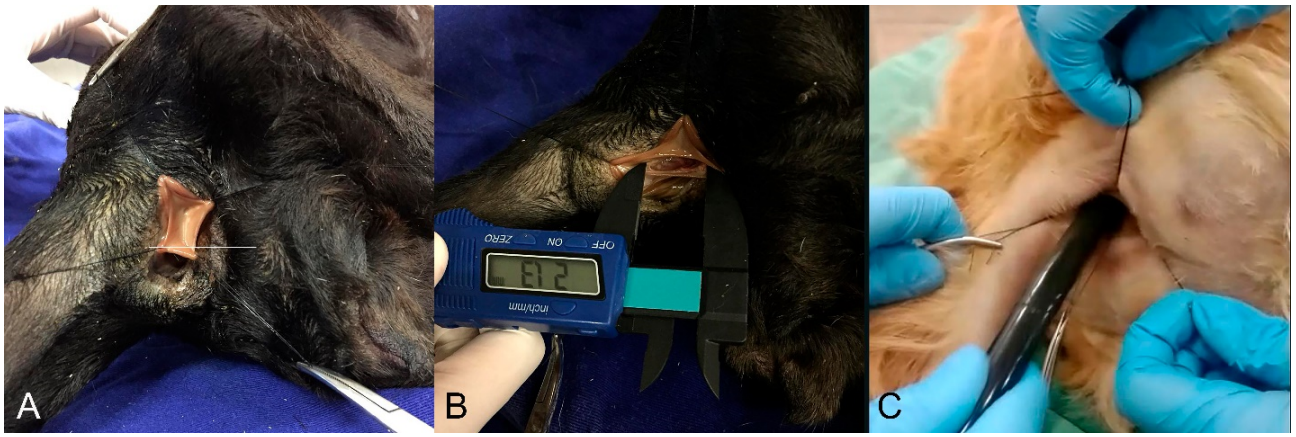

Figure 1. Transrectal access in dog cadavers undergoing two liver biopsy techniques by Natural Orifices Transluminal Endoscopic Surgery.
A) Exteriorization of the rectal mucosa. B) Rectal incision. C) Introduction of the endoscope through the incision created in the rectal mucosa into the abdominal cavity.

The liver was identified in all dog cadavers and the two liver biopsy techniques were performed. Firstly, one sample was collected from the edge of the left lateral lobe using oval endoscopic biopsy forceps (2.8 mm in diameter and 230 cm long), introduced through the working channel of the device. Next a second liver fragment was collected from the same hepatic lobe, using endoscopic polypectomy forceps with a diathermy loop (5.5 x 3.5 cm in diameter and 23 cm long). Diathermy at 36°C was used until the fragment was cut free and was then collected with oval endoscopic biopsy forceps. Liver biopsy samples, with both techniques, were always taken from the edge of liver. The size of the collected samples was recorded (Figure 2), any intra-abdominal abnormalities and complications related to the liver biopsy technique were also recorded. In the Glue Group (GG), the rectal incision was exposed externally by the previously placed repair stitches, the edges of the incision were positioned manually, dried and 2-octyl-cyanoacrylate glue (Dermabond®, Ethicon) was used to seal the defect. One capsule (0.5 ml) of Dermabond® was used to form a continuous glue bond,

including the entire incision and extending laterally for 0.5 cm, with the excess glue being removed. The maximum mechanical strength of the adhesive film was reached when the substance was no longer sticky, to the touch.

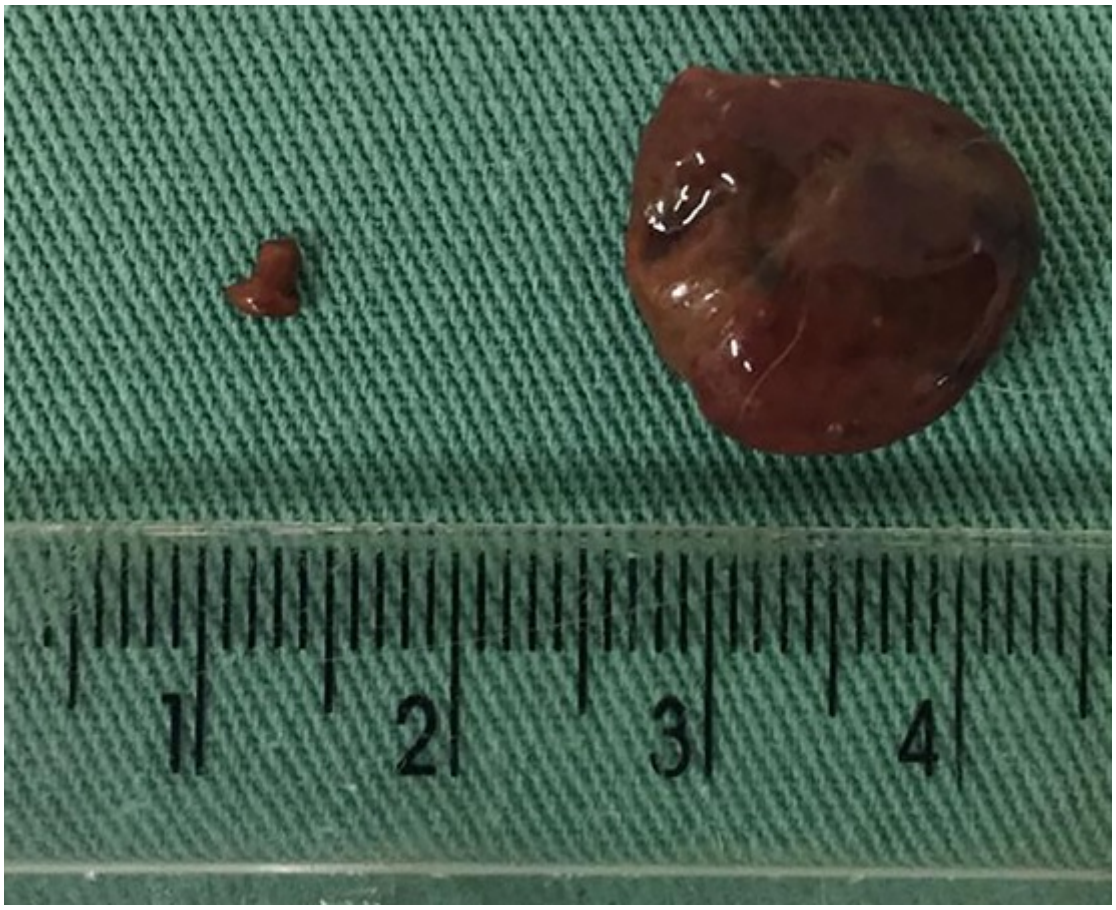

Figure 2. Tissue samples obtained from liver biopsies performed with different endoscopic forceps using transrectal NOTES in dog cadavers. The larger fragment was collected with polypectomy endoscopic forceps with a diathermy loop (right side) and the smaller fragment (left side) with endoscopic oval biopsy forceps.

In the Suture Group (SG), a continuous 1-layer anastomosis was created using synthetic sutures (3-0 polidioxanone, PDS, Ethicon®, J&J, USA).

At the end of each surgical procedure a section of rectum, 1.5 cm proximal and distal to the enterotomy was resected. The ends of the rectal section were ligated proximally using 2 square knots of monofilament nylon. The in vitro burst test was performed by introduction a urinary catheter inserted into the distal end of the isolated segment and

secured with surgical suture to create a watertight seal. The catheter was connected to a Y device with one branch connected to 20ml syringe (used to inject air) and the other to a manometer (Medex Inc™ – USA) to measure intrarectal pressure (mm Hg) (Figure 3A). The rectum was immersed in a glass container of water, at a depth of 5 cm to 10 cm, and pressure within the rectal segment was progressively increased by air injection. Rupture of the seal was identified by the escape of air bubbles (Figure 3B).

Figure 3. The rectal burst test to evaluate rectal sealing.
A) Introduction of a urinary catheter into the distal portion (*) of the isolated segment which is secured with surgical sutures to create a watertight seal. The catheter is connected to a manometer with a 20ml syringe (black arrow) to measure intrarectal pressure (mm Hg). B) The rectal segment is immersed in a glass container of water, at a depth of 5 cm to 10 cm, and pressure within the rectal segment progressively increased by air injection from the syringe.

The evaluation of the operative time included the total operative time (TOT), from the incision of the rectum to the closure of the rectal defect in each group; biopsy time (BT), from the beginning of the insertion of the biopsy forceps to the removal of the specimen, and the time of rectal sealing only (TRS), from the removal of the flexible

endoscope from the abdominal cavity to closure of the rectal defect.

For the transrectal NOTES technique TOT, access incision size, liver visualization and collection of appropriate biopsy material, technical difficulties, and the occurrence of iatrogenic events were recorded.

Quantitative variables were described as mean and standard deviation or median and range of variation. Qualitative variables were described by absolute and relative frequencies. The Wilcoxon test was used to compare sample sizes and total time of liver biopsy using endoscopic forceps, and oval biopsy forceps with polypectomy with a diathermy loop. The t-student test was used to compare the means between the groups (GG and SG). The Mann-Whitney test was used in case of asymmetry. When comparing proportions, Fisher's exact test was applied. The significance level was set at 5% ($p<0.05$) and the analyzes were performed using the SPSS version 21.0 program.

Results and Discussion

The median TOT for the transrectal NOTES technique in each group was 26 minutes (9-68 min.). Two cadavers had a diaphragmatic hernia, making access to the left hepatic lobe difficult due to its thoracic location but, although the operating time was increased in these cases, the total operative time was not increased.

Transrectal access to the abdominal cavity was possible in all cadavers, with no differences in incision size ($p=0.602$) between the GG (2.25 cm ± 0.44) and the SG (2.15 cm ± 0.22).

During creation of pneumoperitoneum, iatrogenic insufflation of the omentum occurred in one cadaver in SG. This made it difficult to establish pneumoperitoneum and, consequently, difficult to visualize and locate the abdominal wall for spatial orientation.

In all cases the peritoneal surface could be visualized, and the liver inspected. In dorsal recumbency, it was possible to evaluate the right and left lateral hepatic lobes, gallbladder, stomach, intestinal loops, omentum, falciform ligament and diaphragm surface in all cadavers. Through endoscopic retroversion, it was possible to visualize the bladder in only two cadavers from the SG (25%). Diaphragmatic hernia was diagnosed in two animals in the GG (25%) with the left hepatic lobe located in the thoracic cavity. In these cases, it was possible to inspect the thorax and evaluate the left pulmonary lobe.

The dorsal recumbency and the absence of auxiliary instruments for organic manipulation of the intestinal loops and the falciform ligament, made it difficult to assess the spleen and kidneys, with identification of the spleen in only two cadavers from the GG (25%) and two cadavers in the SG (25%), and the kidneys in only one animal in the GG (12.5%).

The median total time of liver biopsy with endoscopic oval biopsy forceps was significantly shorter (md=1.0 min) than with diathermy polypectomy forceps (md=5.5 min).

The biopsy sample obtained using diathermy forceps was significantly larger ($p<0.001$), with a median of 204 mm^3 (139-332 mm^3), than that obtained using the oval biopsy clamp technique, with a median 8.5 mm^3 (4-12 mm^3).

A number of complications and technical difficulties were associated with liver biopsy with diathermy forceps including: initial loss of sample (subsequently recovered) in the abdominal cavity in two animals in the SG (25%); difficulty in correctly positioning the diathermy loop at the tip of the liver tissue, and the care needed to avoid sample damage or inadvertent cauterization of adjacent organs.

The incision size (p=0.602) and size of the resected rectum (p=0.191) did not differ between groups. The rectal incision in the GG cadavers was 2.25±0.44 cm for endoscopic oval biopsy forceps and 2.60±0.81 cm for diathermy polypectomy forceps, while in the SG group these were 2.15±0.22 cm and 3.11±0.65 cm, respectively.

The rectal sealing time in the GG was shorter (1±1 min) (p<0.001) than in the SG (5±11 min). However, this did not significantly affect the total surgical time (p=0.505).

There was no difference between the two groups in the burst pressure (258.5 mmHg) with air insufflation. Rupture of the seal was identified by the escape of air bubbles at the same pressure.

The endoscopic biopsy technique using oval biopsy forceps proved to be quick and simple. However, the other diathermy biopsy technique proved to be of medium complexity, with the main difficulty being the loss of samples in the retroperitoneal cavity in two cadavers. In addition, it was difficult to position the diathermy loop at the tip of the hepatic lobe and great care was needed in the use of diathermy so as not to compromise the sample and to avoid inadvertent cauterization of adjacent organs. These concerns mean that specific training is essential for this technique.

Specific site identification for collection of liver biopsy specimens in liver disease is extremely important as disease may be localized within areas of the liver (Rothuizen & Twedt, 2009). Thus, since the endoscopic diathermy polypectomy forceps are limited to collecting samples from the tip of the hepatic lobe this technique cannot be used when changes are localized to the deeper regions of the liver (Bravo et al., 2001).

Closure of colonic and rectal defects can be achieved with clips/staples or sutures. Prospective randomized trials have not demonstrated any differences between stapled and hand-sewn anastomosis in terms of eakage rates. Either continuous or interrupted sutures can be used to perform an intestinal anastomosis (Chen, 2012). No randomized trials have addressed the question of whether interrupted sutures have a significant advantage over continuous ones; however, retrospective reviews have not demonstrated any advantage of one method over the other (Koruda & Rolandelli, 1990) and there was no evidence that two-layer sutures yielded a lower rate of postoperative leakage than single-layer (Chen, 2012). In our study, we used continuous suture in a single-layer because the size of the defect was small, and the study design was based on studies that used NOTES by the transrectal access (Akça et al., 2015; Trindade et al., 2015; Senft et al., 2016).

The use of 2-octyl cyanoacrylate for incision sealing was rapid and straightforward to perform. There was no leakage during the application of the glue and additional applications of adhesive were not required.

The 2-octyl cyanoacrylate is a synthetic adhesive that polymerizes quickly, sealing the contact tissues in less than one minute (Shapiro et al., 2001). This adhesion speed, together with the ease of application, resulted in a shorter sealing time in the GG than in the SG. In this study, the rectal burst test was chosen as the measure for the evaluation of the anastomosis because it is considered a physiological test that reproduces the force vectors normally transmitted over the wall of the digestive tract when pressure is applied through the internal circumference of the organ (Heibel et al., 2006). This burst test has previously been used as a surrogate marker of anastomosis healing in animal models (Hjortrup et al., 1989; Mantzoros et al., 2004) and was considered the most appropriate test to the experimental model used.

Both rectal sealing techniques had the same rupture pressure (258.5 mmHg). Different values were found by Hjjortrup et al. (1989) where the median bursting strength in the rectum seven days after the operation was 280 mm Hg for the combined anastomosis and fibrin adhesive and 260 mmHg for the sutured anastomosis in the control group. In our study, the GG was repaired with cyanoacrylate alone rather than in combination with sutures.

It was not possible to measure the pneumoperitoneum pressure used for abdominal distension due to the limitation that the flexible endoscopy device. Since increased sufflation pressure could improve visualization of the structures, as well as the performance of the technique, both techniques were performed and compared in the same cadaver once an adequate workspace had been generated.

Limitations of this study included the use of cadavers, which may not represent physiologic conditions *in vivo* for example: absence of rectal and hepatic bleeding and postoperative complications such as abdominal contamination due to transrectal access, pain, time of permanence of the rectal adhesive and quality of healing. Hence, future clinical studies on live patients are needed.

Conclusion

Liver biopsy with endoscopic oval biopsy forceps is limited to the collection of tissue located on the surface of the organ, whereas polypectomy forceps with a diathermy loop may be preferred in cases where it is necessary to collect lesions from the tip of the hepatic lobe. There was no difference in rectal rupture pressure in the burst test between the cadavers where rectal sealing was performed with sutures and those where cyanoacrylate adhesive was used.

Declaration of conflict of interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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