

Original Article

Use of laparoscopy for diagnosing experimentally induced acute pancreatitis in dogs

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Diagnosis of acute pancreatitis in dogs remains a significant challenge despite the development of advanced diagnostic methodologies. Visual inspection and pancreas biopsy using laparoscopy are generally considered to be procedures free of complications when conducted on healthy animals. However, the usefulness of laparoscopy for diagnosing acute pancreatitis has not been assessed. In the present study, the efficacy of laparoscopy for diagnosing acute pancreatitis in dogs was evaluated in animals with experimentally induced acute pancreatitis. Gross appearance of the pancreatic area was examined by laparoscopy to survey for the presence of edema, adhesions, effusion, pseudocysts, hemorrhage, and fat necrosis. Laparoscopic biopsy was performed and the histopathologic results were compared to those of pancreatic samples obtained during necropsy. The correlation between laparoscopy and histopathologic findings of the pancreas was evaluated. The presence of adhesions, effusion, and hemorrhage in the pancreatic area observed by laparoscopy significantly correlated with the histopathologic results ($p < 0.05$). There was no significant relationship between the histopathologic and laparoscopic biopsy findings. Results of this study suggested that laparoscopic evaluation of gross lesions has clinical significance although the laparoscopic biopsy technique has some limitations. This method combined with additional diagnostic tools can be effective for diagnosing acute pancreatitis in dogs.

Keywords: acute pancreatitis, biopsy, dog, laparoscopy

Introduction

Pancreatitis is a common gastrointestinal disorder in dogs.

In humans, cases of pancreatitis are usually classified as acute or chronic based on a combination of clinical and pathological criteria that may be loosely applied to cats and dogs [1]. Acute pancreatitis is usually associated with nonspecific clinical signs, physical examination findings, and complete blood count (CBC) as well as serum biochemistry characteristics. Ultrasonography, high serum canine trypsin-like immunoreactivity, or canine pancreatic lipase immunoreactivity data may provide a high degree of confidence for a presumptive diagnosis of acute pancreatitis. However, this determination can only be tentative without direct examination of the pancreatic tissues. A definitive diagnosis of pancreatitis can only be made after conducting a pancreatic biopsy through exploratory laparotomy or laparoscopy [1,2]. In dogs, pancreatic biopsies are usually performed through exploratory laparotomy. This can cause potential complications such as deteriorated inflammation and hemorrhage. Therefore, pancreatic biopsies are conducted for only a limited number of dogs in practice [6,14].

Laparoscopy is a minimally invasive technique that can be used to visually inspect and biopsy organs in the peritoneal cavity [7,9]. This technique is commonly performed for pancreatic evaluation in humans because it enables the visualization of disease-related changes in the pancreatic parenchyma and enhances biopsy performance by identifying areas of inflammation, atrophy, or lesions with minimal complications [4]. In a study evaluating laparoscopic biopsies of the pancreases in normal dogs, no postoperative complications or secondary pancreatitis symptoms were observed, and the use of laparoscopic examination for canine pancreas was proposed [4]. Because the value of laparoscopy

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for diagnosing acute pancreatitis has not been thoroughly examined in dogs, the goal of the present study was to assess the use of laparoscopy for gross examination and biopsy of pancreatic lesions in dogs with acute pancreatitis.

Materials and Methods

Animals

Clinically healthy adult beagles (five males and five females) that were between 2 and 4 years old and weighed between 8 and 14 kg (BeagleKorea, Korea) were used for this study. All dogs were fed commercial adult dry food (Natural Balance; Natural Balance Pet Food, USA) and had access to tap water *ad libitum*. The dogs were observed for at least 1 week prior to the start of the experiments. CBC and serum biochemistry parameters including alanine aminotransferase (ALT), alkaline phosphatase (ALKP), albumin, total bilirubin, total protein, blood urea nitrogen (BUN), creatinine, amylase, lipase, calcium, phosphorus, and cholesterol concentrations were all found to be within the reference range for each dog. All procedures were approved by the Institutional Animal Care and Use Committee of Seoul National University (Korea).

Animal model of acute pancreatitis

After fasting for 24 h, the dogs were anesthetized with 0.5 mg/kg diazepam (Merode; Dong Wha Pharmaceutical, Korea) and 6 mg/kg propofol (Provive 1%; Claris Lifesciences, India) delivered intravenously. Anesthesia was maintained with isoflurane (Isoflurane; Rhodia Organique Fine, Korea). The animals received 25 mg/kg cephadrine (Cephadrine; GUJU Pharma, Korea) and 2 mg/kg tramadol (Tramadol HCl; Shin Poong Pharm, Korea) intravenously along with a 0.9% saline (Isotonic Sodium Chloride inj.; Dai Han Pharm, Korea) infusion.

The abdomen was aseptically prepared, a midline incision was made, and the pancreas was carefully pulled out of the abdominal cavity. An incision approximately 4 cm in length was then made in the descending duodenum starting 2 cm distal to the proximal duodenal flexus. The main pancreatic papilla was exposed and a 22 G intravenous catheter (Becton, Dickinson and Company, USA) was inserted. For eight dogs, 0.5 mL/kg of autologous bile being taken directly by cholecystocentesis was injected into the pancreatic papilla catheter. For two dogs that served as the control group, 0.9% saline was injected instead of bile. The incision site in the duodenum was then closed using simple interrupted absorbable monofilament suture (PDS II Sutures; Ethicon, USA). All dogs recovered without developing complications but experienced mild pain or discomfort. Twelve hours after the operation, the induction of acute pancreatitis was evaluated based on clinical signs, blood work, and abdominal ultrasound

(SSD4000; Hitachi Aloka Medical America, USA).

Laparoscopic examination and biopsy

Laparoscopy was performed under general anaesthesia with propofol (Provive 1%; Claris Lifesciences) 24 h after the operation to induce acute pancreatitis. A stat incision was made 2 cm caudally from the umbilicus and a Veress needle (Karl Storz, Germany) was placed through the abdominal wall. The abdomen was distended with CO₂ through the Veress needle, and abdominal pressure was maintained at 15 mmHg using an automatic CO₂ insufflator (Endoflator; Karl Storz). After adequate abdominal distension was achieved, a small skin incision about 2 cm in size was made in the middle abdomen on the right side 5 cm from the umbilicus. A cannula (Karl Storz) was placed through the right middle abdominal wall. A 5-mm telescope with a 30-degree view angle (Karl Storz) was placed inside the right cannula. Under visual control, another cannula was inserted into the left middle abdomen 5 cm from the umbilicus and abdominal exploration was performed using a palpation probe (Karl Storz).

Currently, there is no well-established classification by which the pancreas can be evaluated during laparoscopic examination. Therefore, gross appearance of the pancreas in the current study was carefully assessed and the severity of acute pancreatitis was classified according to the following three scores based on the presence of edema, fibrous adhesions to adjacent organs, peritoneal effusion, pseudocyst formation, interstitial hemorrhage, and fat necrosis: 0, absence; 1, mild; and 2, severe. After the pancreas was examined, the palpation probe was removed.

Two biopsy samples were taken from the tail edge of the right pancreas limb away from the pancreatic ducts using 5-mm punch biopsy forceps (Karl Storz). Two additional biopsy samples were taken from the edge of the liver using 5-mm oval cup biopsy forceps (Karl Storz). The biopsy samples were immediately fixed with 10% buffered formalin for histopathologic examination. All instruments and the telescope were removed, and the pneumoperitoneum was decompressed by opening one of the cannula valves and permitting the CO₂ to escape. The cannulae were then removed, and the puncture sites and skin were sutured using non-absorbable monofilament (Nylon; DuPont, USA).

Ten dogs were euthanized with a KCl solution (Potassium Chloride inj.; Dai Han Pharm) under general anesthesia induced by propofol 12 h after laparoscopy. Immediately after each dog was sacrificed, the pancreas was removed and fixed in 10% buffered formalin for histopathologic examination. The pancreas was divided into four sections (A1 to A4) with A1 beginning at the right tail of the pancreas and A4 ending at the left tail of the pancreas.

Pancreatic samples obtained during laparoscopic biopsy and necropsy were dehydrated, embedded in paraffin, and then stained with hematoxylin and eosin (Sigma-Aldrich,

USA). An experienced pathologist who was unaware of the sample identity examined the pancreas sections and categorized the acute pancreatitis severity based on the percentage of necrotic lesions in the pancreas using the following scale: 0, no necrotic lesions; 1, < 15% of necrotic lesions; 2, 15 to 30% of necrotic lesions; and 3, > 30% of necrotic lesions.

Correlations between results of the laparoscopic examination and histopathology performed after necropsy were assessed by calculating Spearman's rank correlation coefficient (r_s) using GraphPad 5.0 (GraphPad Software, USA) and Origin 6.1 (OriginLab, USA).

Results

Gross appearance of the pancreas with experimentally induced acute pancreatitis observed through laparoscopy is presented in Table 1 and Fig 1. In five out of eight dogs with acute pancreatitis, the pancreas had adhered to the duodenum, mesentery, great omentum, and/or pylorus region of the stomach. Mild adhesions could be removed using a palpation probe while the severe adhesion could not be detached. Abdominal effusion, which was indicated by the presence of low-viscosity serosanguineous fluid, was easily identified by laparoscopy. In three out of eight dogs with acute pancreatitis, the abdominal organs, especially those in the inflamed pancreatic region, were difficult to evaluate because of the effusion. One of the control dogs also developed mild effusion in the abdominal cavity. However, clear serous fluid different from the fluid observed in the animals with acute pancreatitis was observed. No dehiscence was found at the suture site in the descending duodenum, which was also examined by laparoscopy. Peripancreatic fat necrosis and

saponification that appeared as focal white granules were easily observed around the pancreas during examination (Fig. 1). Fat necrosis occurred concurrently with adhesion development. Edema and hemorrhage in the pancreas were relatively difficult to view in the entire parenchyma due to the presence of adhesions along with extensive effusion, and were generally found only in the focal area of the pancreas. No pancreatic pseudocysts were identified in any dogs with acute pancreatitis or in the control group.

Quality of the pancreatic biopsy samples was adequate for examination with the exception of one in which

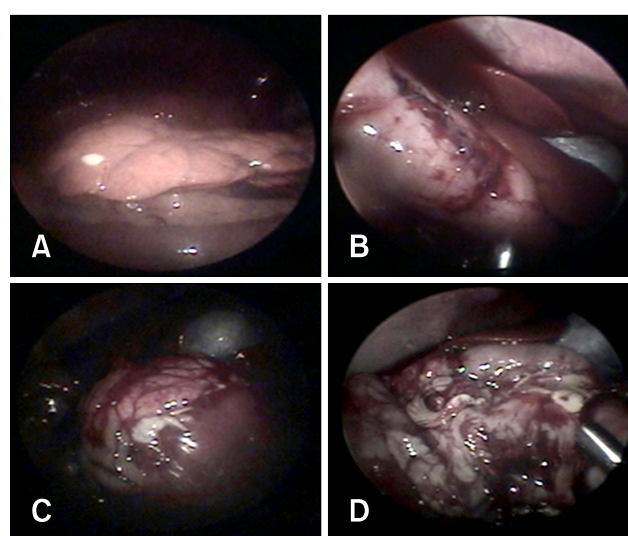


Fig. 1. Laparoscopic views of the pancreas of dogs in which acute pancreatitis was experimentally induced. (A) Edema, (B) peritoneal effusion, (C) pancreatic hemorrhage, and (D) fat necrosis.

Table 1. Gross appearance of the pancreatic area examined by laparoscopy and scores indicating the presence of edema, adhesions, effusion, pseudocysts, hemorrhage, and fat necrosis in 10 beagles

Gross appearance	Dog									
	Control		Animals in which acute pancreatitis was experimentally induced by injecting autologous gall bladder bile acid							
	A	B	1	2	3	4	5	6	7	8
Edema	0*	0	1	2	2	0	2	1	2	2
Adhesions	0	0	0	1	1	0	0	2	2	1
Effusion	0	1	0	2	2	0	1	1	2	1
Pseudocysts	0	0	0	0	0	0	0	0	0	0
Haemorrhage	0	0	0	2	2	0	1	1	2	1
Fat necrosis	0	0	0	2	2	0	0	1	0	0

*Gross appearance of the pancreas was scored on a scale of 0 to 2 with 0, normal appearance of the pancreatic regions; 1, mild change of pancreatic regions; and 2, severe changes of the pancreatic regions.

peripancreatic fat inflammation was observed. The biopsy sites were closely monitored for bleeding throughout the study. It was eventually confirmed that no significant complications developed following any of the procedures.

All dogs recovered very quickly within 0.5 to 1 h of laparoscopy. The animals showed signs of discomfort and pain during the recovery period. However, no significant changes in clinical symptoms were observed before or after the procedures.

Gross appearance of the pancreas viewed during necropsy was characterized by generalized fat necrosis, adhesion development, and hemorrhage (Fig. 2). These changes were found to be more severe around the pancreatic body whereas symptoms in the right tail area of the pancreas were less severe or absent. Both the pancreatic and liver biopsy sites were assessed. There was little evidence of procedures-associated complications. No gross pathologic changes were observed although mild extra-pancreatic hemorrhage was noticed in two cases. The severity of acute pancreatitis was classified on a scale of grade 1 to 3 based on the presence of necrotic lesions in the pancreas identified during histopathologic examination after necropsy (Fig. 3 and Table 2).

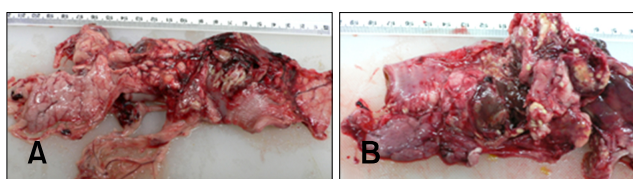


Fig. 2. Gross appearance of the pancreas of a dog in which acute pancreatitis was experimentally induced. (A) Overall view of a pancreas with acute pancreatitis and (B) a magnified view of the necrotized pancreas.

The laparoscopic scores were compared to the histopathologic grades based on the severity of pancreatic necrosis found in samples obtained during necropsy. There was a significant correlation between the laparoscopic scores (indicating the degree of adhesion development, effusion, and hemorrhage) and histopathologic scores ($p < 0.05$). No remarkable necrosis or inflammation was found in the laparoscopic biopsy samples during histopathologic examination compared to the necropsy samples. Biopsy samples of the liver acquired during laparoscopy showed no evidence of pathologic changes.

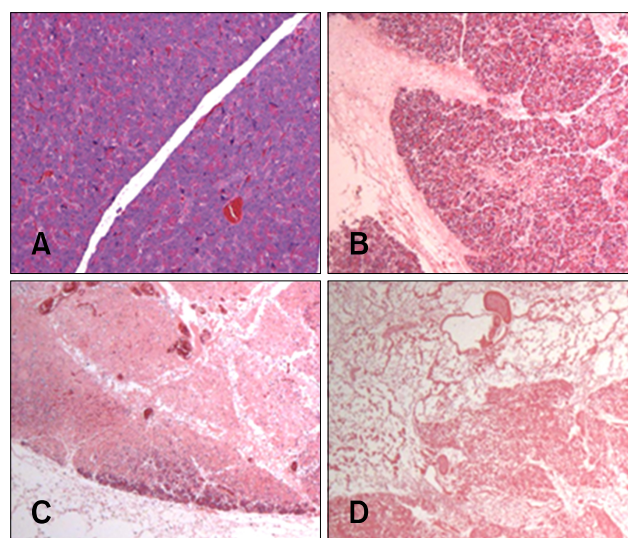


Fig. 3. Histopathologic examination (400× magnification) of hematoxylin and eosin-stained sections of the pancreases from dogs in which acute pancreatitis was experimentally induced. (A) Grade 0, normal; no necrotic lesions. (B) Grade 1, mild; < 15% of necrotic lesions. (C) Grade 2, moderate; 15 ~ 30% of necrotic lesions. (D) Grade 3, severe; ≥ 30% of necrotic lesion.

Table 2. Results of the histopathologic examination of the pancreases obtained after necropsy of 10 beagles

Pancreas section*	Dog									
	Control		Animals in which acute pancreatitis was experimentally induced by injecting autologous gall bladder bile acid							
	A	B	1	2	3	4	5	6	7	8
A1	0**	0	1	2	1	1	2	3	2	2
A2	0	0	1	2	2	1	3	3	3	2
A3	0	0	1	2	2	1	1	3	3	1
A4	0	1	1	3	3	1	1	3	3	2
Grade (mean ± SD)	0 ± 0	0 ± 0	1.0 ± 0	2.25 ± 0.5	2.0 ± 0.82	1.0 ± 0	1.75 ± 0.96	3.0 ± 0	2.75 ± 0.5	1.75 ± 0.5

*The pancreas of each dog was divided into four sections with A1 beginning at the right tail of the pancreas and A4 ending at the left tail of the pancreas. **Severity of acute pancreatitis was histopathologically classified as grade 0 to 3 based on the percentage of necrotic lesions in the pancreas using the following scale: grade 0, no necrotic lesions; grade 1, < 15% of necrotic lesions; grade 2, 15 ~ 30% of necrotic lesions; and grade 3, ≥ 30% of necrotic lesion.

Discussion

Acute pancreatitis is a disorder caused by numerous factors including drugs and infectious agents as well as metabolic, nutritional, and immune dysfunction [10]. Premature activation of enzymes initiated by the complement cascade, trypsinogen activation, and the release of superoxide radicals subsequent to ischemia are also known to cause acute pancreatitis [13]. Numerous experimental techniques including hyperstimulation by administration of caerulein, carbamylcholine, or scorpion venom; obstruction of the pancreatic duct, traductal injection of bile, fatty acid, or enzymes; pancreatic ischemia, and certain dietary regimens can induce pancreatitis in dogs [11]. Among these procedures, injection of autologous gallbladder bile into the main pancreatic duct has been shown to promote severe acute pancreatitis within 12 h and is comparable to bile reflux pancreatitis *in vivo* [5,12]. Therefore, autologous bile injection was used to induce acute pancreatitis in the present study.

Laparoscopy has been used as an effective method for evaluating gross lesions and collecting biopsy samples from the pancreas in humans [15]. Pancreatic biopsy conducted using laparoscopy has been performed in healthy dogs [4] as well as ones with pancreatitis [18], and was reported to be generally free of complications. However, the value of this procedure for identifying cases of acute pancreatitis along with the associated risk has not been thoroughly examined [4]. Results of the present investigation indicated that adhesion development, effusion, and hemorrhage, which are relatively easy to identify and less subjective characteristics, were correlated with the degree of pancreatic necrosis determined by histopathology. Another investigation previously showed that laparoscopic appearance corresponded to histopathology findings for seven out of nine dogs (78%) with pancreatic disease [18].

Pancreatitis can lead to the production of septic or non-septic exudates, and is often associated with various degrees of peripancreatic fluid accumulation. Pancreatic inflammation along with inflammatory mediators increase endothelial permeability and promote the recruitment of neutrophils as well as other phagocytic cells. This may lead to the accumulation of peripancreatic fluid and exudates [3]. In the current study, abdominal effusion was easily identified by laparoscopy and hindered exploration of the abdominal contents including the pancreas. Although pancreatic parenchyma edema was identified in several dogs, this was not significantly correlated with the degree of pancreatic necrosis, suggesting that identification of edema was relatively subjective and this condition was more localized than adhesion development or hemorrhage.

Pancreatic pseudocysts are a complication of pancreatitis and formed by a collection of sterile pancreatic juices enclosed by a well-defined wall composed of granulation or

fibrous tissue [1]. These structures can be easily distinguished from abdominal effusion by ultrasonography and cystic fluid analysis [16]. Pancreatic pseudocysts can develop secondary to acute pancreatitis but are not lesions essential for diagnosing the disease. In the present investigation, pancreatic pseudocysts were not found during laparoscopy or necropsy in any of the dogs with acute pancreatitis.

Peripancreatic fat necrosis and acinar cell necrosis are currently recognized as important indicators of morbidity and mortality in cats [17]. However, the importance of these conditions has not been widely studied in dogs. In the present investigation, peripancreatic fat necrosis observed during laparoscopy was not significantly correlated with the severity of acute pancreatitis determined by histopathology. However, future studies should be conducted to specifically evaluate the association between fat necrosis and morbidity and/or mortality in dogs with acute pancreatitis.

Histopathology of laparoscopic biopsy samples from the canines with acute pancreatitis had minimal histopathological changes. This finding indicated that laparoscopic biopsy has a low value for diagnosing acute pancreatitis although the procedure is relatively safe. This limitation may be due to several factors. First, pancreatic inflammation tends to occur in discrete areas within the pancreas rather than being diffusely distributed throughout the whole organ [8,19]. In the present study, biopsy was performed in only the right pancreatic limb. Even the biopsy region was selected based on the gross evaluation but the histopathologic result would not be in exactly accord with findings of gross lesion. Samples from the left pancreatic limb were not obtained because of difficulties with the anatomic approach. This represented a critical limitation of laparoscopic biopsy for thoroughly assessing pancreatic inflammation. Second, a single biopsy sample is insufficient for excluding pancreatitis when making a diagnosis. Furthermore, there is no preferred site for pancreatic biopsy collection unless gross lesions are present [8]. Finally, animal positioning during laparoscopy influences the examination field. In the current study, a right lateral mid-abdominal approach was used for laparoscopy. Only the proximal portion of the left pancreatic lobe is generally visualized [7]. The right limb of the pancreas, duodenum, extra-hepatic biliary system, and liver were easily viewed, but complete exploration of the left pancreatic limb was unsuccessful in all dogs. Additionally, it was difficult to examine the entire pancreas due to adherence to the surrounding organs in several dogs.

Laparoscopic examination can be used as a supplemental method for diagnosing acute pancreatitis based on adhesion formation, abdominal effusion, and hemorrhage. Moreover, laparoscopic biopsy can be performed to collect samples from areas containing gross pancreatic lesions for histopathologic examination with minimal complications. However, risks associated with anesthesia should be considered, especially in compromised patients, and could

be a main limitation of laparoscopic examination. Although laparoscopic sampling has several limitations because a laparoscopic-based diagnosis may be based not only on biopsy results but also gross examination findings, the clinical significant and applicability of laparoscopy to identify acute pancreatitis cases in small animal practice is presumptively valuable.

Acknowledgments

This study was supported by the BK PLUS program for Creative Veterinary Science Research, and Research Institute for Veterinary Science, College of Veterinary Medicine, Seoul National University, Korea.

Conflict of Interest

There is no conflict of interest.

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