Comparative study between ligature with thread or metallic clamping by means of laparoscopy with the purpose of experimental biliary obstruction in swines[1]

Estudo comparativo entre ligadura com fio ou clampeamento metálico por videolaparoscopia para obstrução biliar experimental em suínos

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ABSTRACT
PURPOSE: To induce a total extra-hepatic obstructive jaundice in swines, by ligation of the common bile duct by laparoscopic surgery.
METHODS: Eight swines of the Landrace race, 36-day-old, originated from the same matrix, distributed in two groups. Group A: was used titanium metal clip to the common bile duct ligation in three animals; group B: were ligated with 2-0 cotton thread in five animals.
RESULTS: The ligation of the biliary ducts was performed successfully in all animals, with easy identification of the common bile duct by laparoscopy. There weren't difficulties in the procedures, mainly due to the increased surgical field provided by the excellent quality of light and image of the appliance. The clinical signs of jaundice were evident in the animals in seven days. In group A, two animals showed bile duct perforation near the clip, probably due to ischemic necrosis, progressing to peritonitis and death. In group B, five animals showed obstructive jaundice without any amendment.
CONCLUSION: Under the conditions of this study, we therefore recommend the use of unabsorbed wires to experimental biliary obstruction, in order to avoid complications, such as ischemia and necrosis, followed by perforation of the wall of the bile ducts.
Key words: Laparoscopy. Cholestasis. Ischemia. Comparative Study. Swine.

RESUMO
OBJETIVO: Induzir um quadro de icterícia obstrutiva extrahepática total em suínos, através da ligadura no ducto colédoco por meio de cirurgia videolaparoscópica.
MÉTODOS: Oito suínos da raça Landrace, com 36 dias de idade, originários da mesma matriz, foram distribuídos em dois grupos. Grupo A: utilizou-se clipe metálico de titânio para ligadura do ducto colédoco em três animais; grupo B: foi feita a ligadura com fio de algodão 2-0 em cinco animais.
RESULTADOS: A ligadura da via biliar principal foi realizada com sucesso em todos os animais, com fácil identificação do colédoco por videolaparoscopia. Não houve dificuldades nos procedimentos, principalmente devido ao aumento do campo cirúrgico proporcionado pela qualidade de luz e imagem do aparelho. Os sinais clínicos indicativos de icterícia foram evidentes nos animais em sete dias. No grupo A, dois animais apresentaram perfuração coledociana junto ao clipe por provável isquemia e necrose, evoluindo com coleperitônio e óbito. No grupo B, os cinco animais apresentaram quadro de icterícia obstrutiva sem qualquer alteração.
CONCLUSÃO: Nas condições desse estudo, recomenda-se a utilização de fios inabsorvíveis para obstrução experimental das vias biliares, a fim de evitar complicações, como isquemia e necrose, seguida de perfuração da parede das vias biliares.
Descritores: Laparoscopia. Colestase. Isquemia. Estudo Comparativo. Suínos.

Introduction

Cholelithiasis is one of the most common disorders of the biliary tree, considered an international public health problem, its prevalence is around 10% in national researchs[1-4]. The main risk factors for the development of gallstones in humans are age, heredity, parity and obesity; occurring mainly in people over 55 years, and a greater incidence among women[5-8].

There are several conditions that, in humans and animals, can cause obstruction in the biliary tree, occurring cholestasis[9-13]. However, in recent years, there was an increase in the diagnosis of asymptomatic lithiasis due to upgrading diagnostic methods routinely performed in patients[14-17]. Because of its frequency, many researchs are conducted to expand the knowledge about the pathogenesis and improving treatment of this disease[14,15,18-24].

The treatment of biliary obstructions depends on the time between occurrence and diagnosis of the injury and, the type, extent and location of same in the bile ducts[14,25], being the surgical treatment the most recommended[2,26]. In this context, the development and evolution of laparoscopic surgery have stimulated the execution of operative techniques increasingly complex; even considered safe, the procedure requires extensive anatomical knowledge and training aimed at reducing the incidence of postoperative complications[25,27-32].

Assess any changes in liver and biliary tree due to biliary obstruction is of great importance, because it aids in clinical diagnosis of the patient, improving the quality and efficiency of treatment. For this, is required the development of experimental works, both to deepen knowledge of anatomy or in search for an ideal model for research as, also, for training and capacity of the surgeon to intervene surgically in the biliary tree[18,20,21,25,27,28,33].

In order to seek a physiological and anatomical reconstruction more suitable for extensive lesions of the biliary tree, was proposed a study of biliary reconstruction by interposing a conduit derived from a segment of small intestine, similar to the one that Monti and collaborators have proposed for urinary diversion with continence mechanisms[34,35]. However, was necessary a experimental model enabling standardize a procedure that produces a pattern of obstructive jaundice by the extrahepatic biliary ligation, allowing for the previous acknowledgment of the surgical site, indicating the use of specific techniques to, a second moment, propose the reconstruction of the extrahepatic biliary tract. In this context, this research aimed to induce a total obstructive jaundice in swines, through the common bile duct ligation by laparoscopic surgery using 2-0 cotton thread or titanium metal clip, which compared the efficacy of the materials used and, also, description of the alterations observed in clinical evaluations, ultrasound examinations of the gallbladder and pathological analysis of the animals.

Methods

This project was evaluated by the Ethics Committee in Research of the Faculty Dr. Maeda Francisco and was approved under protocol n. 19/2008.

Experimental delineation

This research was developed in the Faculty Dr. Maeda Francisco (FAFRAM/FE), located in Ituverava-SP, Brazil, was used the structure of the swine farm and the laboratories and facilities of the Veterinary Hospital. Were utilized eight swines (*Sus scrofa*), Landrace race, with 36-day-old and average weight of 9,17 (±1,69) kg, originating from the same matrix. They were followed from birth with individual clinical evaluations twice a week. The data collected and tests performed were stored in individual records.

The animals were divided randomly into two groups. Group A, with three animals subjected to bile duct obstruction by placing a metallic clip Titanium 9,0 mm long and 1,0 mm wide and, Group B, five animals that received ligature with 2-0 cotton thread.

Ultrasound analysis

Ultrasound examination pre-obstruction was made on the day preceding the procedure obstructive and the examination post-obstruction, the same day of the correction of the biliary route; by food and water fasting and after ten minutes of sedation with acepromazine (0,2mg/Kg/IM). The images were recorded on video and printed paper. Were evaluated, for comparison purposes, the transverse and longitudinal diameters of the gallbladder.

Anesthesia and surgical procedures

The pre-operative procedures were common to all animals. The animals remained under water and food fasting for six hours. Arriving at the hospital received a cold shower bath for body cleansing, were sedated with acepromazine (0,2mg/Kg/IM) and, subsequently, underwent trichotomy and antisepsis of ventral abdominal region. After ten minutes of sedation, were taken to the operating room and accommodated on supine position on the operating table. The medial saphenous vein was catheterized for infusion of glucose-saline solution and administration of dissociative anesthetic based on the combination of tiletamine and zolazepam (5,0mg/Kg/IV), also being administered fentanyl (0,025mg/Kg/IV) and atropine sulfate (0,5mg/Kg/IV). Anesthesia was maintained with doses of one-third to half the original dose of the dissociative anesthetic. During surgery the animals were supplemented with oxygen by face mask.

Confirmed the anesthesia, laparoscopic procedure was initiated with a supra-umbilical midline incision of 1,0 cm to insert the Verres needle and perform the pneumoperitoneum, the intracavitary pressure was adjusted to 11,0 mmHg. Then four trocaters were placed: (1) two of 5,0 mm, located in a region caudal to the right costal margin and another at the level of right iliac fossa, (2) and two of 10,0 mm, one located 1,0 cm supra-umbilical and another caudal to the left costal margin (Figure 1).

During the procedure, were identified, isolated and dissected peri-hilar structures and performed laparoscopic common bile duct ligation as distal as possible (Figure 2), with intention to produce a total extra-hepatic obstructive jaundice. After the ligation procedure, hemostasis was reviewed, the cavity washed with saline preheated at a temperature ±37.5°C, dissolved the pneumoperitoneum, followed by laparorrhaphy with 2-0 cotton thread and 3-0 mononylon at skin.

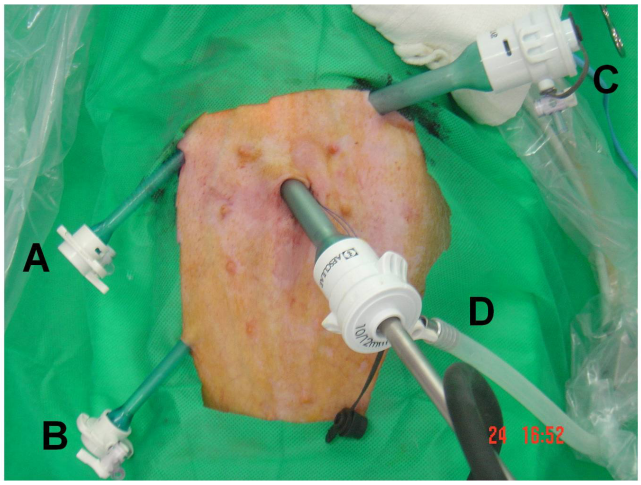

FIGURE 1 - Sites of placement of trocars in the abdomen of pigs subjected to procedure of the common bile duct ligation by laparoscopy. **A** - flow area to the right costal margin; **B** - level of the right iliac fossa; **C** - caudal to the left costal margin; **D** - pre-umbilical

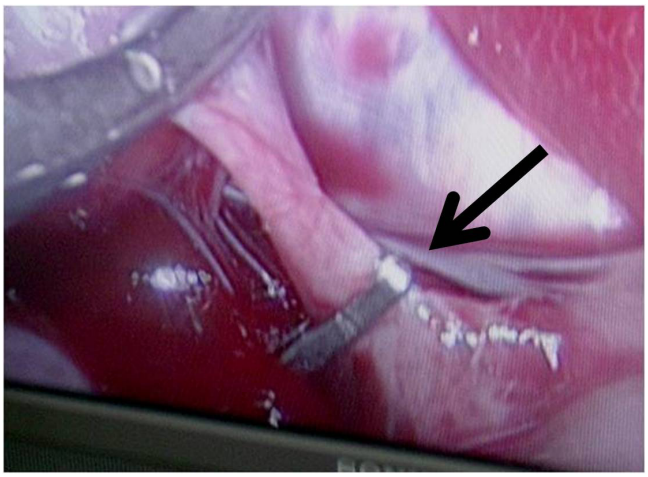

FIGURE 2 - Picture showing the site chosen for the common bile duct ligation aiming biliary obstruction in swine, as well as showing ligation performed with titanium metal clip (arrow)

Seven days after the obstructive procedure was performed laparotomy, by supra-umbilical incision at approximately 8.0 cm, for inspection of the abdominal cavity (we used the same anesthetic protocol used for the procedure obstructive) and evaluating the site of ligation choledoco, as well as correction of the biliary pathway. Laparorrhaphy was made with 2-0 cotton thread and 3-0 mononylon at skin.

Postoperative observations

In the postoperative periods (obstruction and unobstruction) the animals were kept under constant care, to monitor the healing of surgical wounds and administering anti-inflammatory (dexamethasone, dose of 0,025mg/Kg/IM/day), analgesic (flunixin meglumine, dose of 2,2mg/Kg/IM/day) and preventive antibiotic (enrofloxacin, dose of 2,5mg/Kg/IM/day). The average weight of animals at the end of the experiment was 9,42 (±2,19) kg.

During the execution of the experiment two animals died and underwent autopsy examination.

Results

The main bile duct ligation was performed successfully in all animals, with easy identification of the bile duct by laparoscopic technique.

After the procedure of biliary obstruction, the most common clinical signs presented by the animals were strong yellow colored urine, icteric skin and mucous membranes in the sharp or discrete levels (Figure 3); faeces lighter shade than that observed before the ligation and hepatomegaly on palpation. By comparing the diameters ultrasound transverse and longitudinal gallbladder obtained in pre and post-obstructive postoperative, one can show considerable increase in the thickness of it (Figure 4).

With respect to cotton yarn and clip titanium, materials used for the ligated bile duct animals, was observed that the five ligations of the biliary tree performed with cotton thread presented clinical symptoms of obstructive jaundice without any degenerative changes of the common bile duct. Of the three ligation with metal clip, two died; one occurring two days after the obstructive procedure and the other four days after the procedure, during necropsy was observed with the drilling common bile duct clip and biliar peritonitis, the third animal presented with obstructive jaundice without any degenerative changes of the common bile duct.

During exploratory laparotomy, there was markedly increased liver volume in six animals, with moderately pale colored and markedly yellowish, firmer consistency, and increase in size and consistency of the gallbladder and extrahepatic bile ducts. In two necropsy, beyond these same changes hepatobiliary, areas of fibrinous inflammation were observed with adherence to intestinal segments in the Glisson's capsule, due to biliar peritonitis.

FIGURE 3 - Swine subjected to experimental biliary obstruction by ligation of the common bile duct, where there is jaundiced skin and intensely yellow color of urine

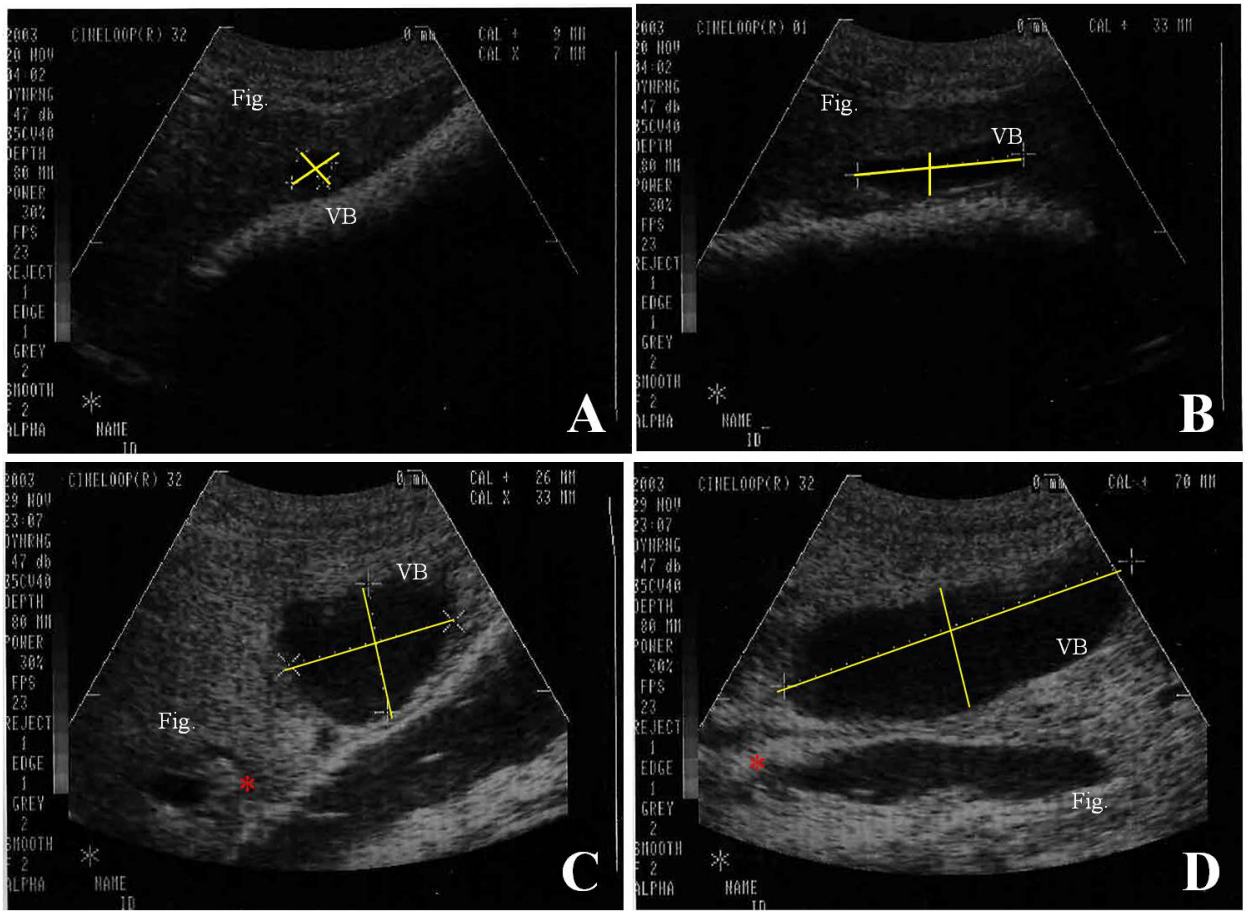

FIGURE 4 - Ultrasound images of gallbladder (lines) in swine subjected experimental biliary obstruction. A - transversal diameter pre-obstruction; B - longitudinal diameter pre-obstruction; C - transversal diameter post-obstruction; D - longitudinal diameter post-obstruction. Observe the increase in organ size (lines) when comparing A with C and B with D. Is observed, also, increased echogenicity of hepatic parenchyma in C and D (*). FAFRAM/FE - 2010

Discussion

We used the swine (*Sus scrofa domesticus*) as an experimental animal because it is resistant, easy to handle and with a good possibility of standardization, beyond are considered a suitable animal model for diseases linked to lifestyle human[33,36,37]. When the physiology of humans is compared to the dog, rat, mice and other species used in testing these similarities are much more distant, however the swine model is the one with anatomical and functional characteristics closer to human, and its use in scientific research is an old practice[38-41]. Thus, these animals are frequently used in experimental studies associated to various morphological and functional changes that affect several organs and systems, including the liver and bile ducts, from simple histologic examination to more complex surgical procedures, providing a experimental model for developing or improving techniques and subsequent application in humans[33,37,38,42].

In this context, the main bile duct ligation was performed successfully in all animals in this experiment, with easy identification of laparoscopic common bile duct, which is the most used and considered safe in treating disorders of the biliary tract, especially due to the low rate of complications and advantages such as less postoperative pain, shorter hospital stay and better cosmetic results, despite being invasive[24,30,31,43].

Obstructive procedures occurred without difficulty, mainly due to the visibility of the structures proper proportion through the screen provided by the device. This fact, which is associated with the maximization of the image of the organs in video monitors, allows you to define the exact site of action, providing safe interventions to vital structures, even in complicated cases[28,29,44,45].

Experimental induction of extrahepatic cholestasis is a process that does not always succeed, requiring a high degree of technical quality, patience, concentration and continuous training[46,47]. But, seven days after the procedure for biliary obstruction, the animals showed strongly yellow urine, skin and mucous membranes icteric, ranging from mild to severe; stools lighter shade than that observed before ligation, so as to hepatomegaly palpation. Signs compatibles with what is described on cholestasis in humans[1,48,49], dogs[9,18], mices[20] and rats[22,23]. However, in less time than that observed in other animal species researchs[20,22].

The development of jaundice justifies the color changes of skin and mucous membranes observed, because bilirubin is deposited in tissues such as in high concentrations[49,50]. In the case of urine, strong staining occurs due to increased concentration of bilirubin in the bloodstream, that pass through the glomeruli and is excreted, this is a characteristic change of cholestasis[19]. In the case of faeces, a reduction or absence of excretion of bilirubin in the intestinal lumen causes changes in faeces color making them clearer (reduction) or whitish (absence)[50].

In questionable cases or require additional supporting information, or, even, for differential diagnosis, the interpretation of clinical signs should always be correlated with laboratory tests, ultrasound exams or other methods of diagnostic imaging[2,13,47,51,52].

Ultrasound is a practical technique, fast and inexpensive, being considered as a screening assay in the initial evaluation of patients with suspected hepatobiliary tract disorders[7,8,12,13,53], mainly due to its high sensitivity and specificity[1,16]. Through this examination, can visualize hepatomegaly in cases of cholestasis associated with obstruction, with the characteristic signs of increased volume of the organ and the presence of smooth margins, and there may be diffuse and increased echogenicity, and inadequate definition of the portal vessels; in biliary tract, is more common visualization increase of size in the gallbladder and dilation of ducts[12,16,17]. Data corroborate the ultrasound examination performed in the post-obstruction of this research, which we found a considerable increase in the diameter of the gallbladder.

With respect to cotton yarn and clip titanium there was not found publication about the use of these materials aimed to biliary obstruction in swines. However, some studies have been conducted to determine the safety of ligation of anatomical structures with titanium clips, such as Kerbl *et al*.[54], which revealed that the obstruction induced with titanium clips was safer than the ligatures made with silk or metal clasps. Nelson *et al*.[55] and Beltran *et al*.[56], using swines as models, considered safe clips provided by the obstructions in the mesenteric vessels and cystic ducts, respectively, and these authors did not describe complications, such as rupture, in their experiments.

Soares *et al*.[57], when performing ligation with one, two or three titanium clips, or with cotton thread 0 (zero) in dogs, reported that there is no difference between the ligature made with cotton thread and made two or three titanium clips, showing similar resistance when subjected to the same pressure. However, Branco Filho *et al*.[58], reported that of 45 human nephrectomies performed using ligation of the renal vessels with two titanium clips and cotton thread, there was need for further surgery in six hours for ligating the renal artery in one case, whose titanium clips were released. Maia *et al*.[23], using only thread in the common bile duct ligation, in rats, results could obstructive, without describing complications during the experiment. Same situation described by Crema *et al*.[18] in biliary obstruction experiment with dogs, and Dutra *et al*.[59], also rats.

These data tally with what was found in this experiment, in which five ligation of the biliary tree with a cotton thread presented clinical symptoms of obstructive jaundice without any degenerative changes of the common bile duct. However, the three ligation with metal clip, two drilling common bile duct presented with the clip, developed with biliar peritonitis and consequently died after three and five days post-obstruction. This fact may have resulted from the procedure for biliary obstruction, which by compressing the common bile duct caused cholestasis with consequent distention of the ducts of the biliary ducts and gallbladder, and the same thinning of the wall, leading to compression of vessels responsible for irrigation with consequent progressive necrosis of hypoxic cell wall, causing rupture and leakage of bile into the peritoneal cavity and biliar peritonitis, taking the animal to death[47,49,60-62].

Rupture of the gallbladder is usually associated with lesions of other structures such as mechanical irritation caused by stones in the wall of the organ, intravesicular pressure increase attributed to obstruction or severe inflammation and necrotizing ischemia, intravesicular pressure increase attributed to obstruction or severe necrotizing inflammation and ischemia, leading to biliar peritonitis and, consequently, evolution to death and increased mortality of traumatized[9,11]. Being that, among other causes, ischemia represents 5 at 10% of cases of acute cholecystitis. However, perforation of the gallbladder, biliar peritonitis, perivesicular abscess and biliary fistula are the main complications of acute cholecystitis[63,64].

Conclusions

Under the conditions of the experiment was conducted, we concluded that swines represent a suitable experimental model of obstructive jaundice, being the interval of seven days enough for the animals develop the characteristic clinical signs of jaundice, beyond cholestasis noticeable at the ultrasound examination. However, the use of nonabsorbable wires proved to be more effective than titanium metallic clips in experimental procedures aimed at the common bile duct ligation in swines.

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