Application of high frequency bipolar electrocoagulation LigaSure™ in appendix vermiformis of rabbits with or without acute inflammatory process[1]

Aplicação do eletrocoagulador bipolar de alta frequência LigaSure™ no apêndice vermiforme na vigência ou não de processo inflamatório agudo

Laura Cristina de Souza[I], Maíra Rocha Ortega[I], Eduardo Achar[II], Dante Simionato Netto[III], Marcelo Augusto Fontenelle Ribeiro Junior[IV]

[I]Graduate student, scientific initiation project, Medical School, UNICID, Sao Paulo-SP, Brazil. Acquisition of data and technical procedures.
[II]Associate Professor, Experimental Surgery Division, Medical School, UNICID, Sao Paulo-SP, Brazil. Anesthesia, technical procedures and critical analysis.
[III]Assistant Professor, Morphofunctional Laboratory, UNICID, Sao Paulo-SP, Brazil. Histological analysis, acquisition and interpretation of histological data.
[IV]Associate Professor, Experimental Surgery Division, Medical School, UNICID. Full Professor, Head, Department of Surgery, General Surgery Division, UNISA, Sao Paulo-SP, Brazil. Tutor, responsible for conception, design, intellectual and scientific content of the study; critical analysis and final approval of manuscript.

ABSTRACT

PURPOSE: To evaluate the efficacy of the use of LigaSure™ in appendectomy, with or without acute inflammatory process, and to compare with simple ligature and conventional therapy.

METHODS: A total of 30 rabbits (*Oryctolagus cuniculus*) randomly allocated in two groups, group A and B, of 15 animals each were used. The group A without acute appendicitis and the group B with acute appendicitis were submitted to appendectomy. After, the groups were subdivided into three groups, each group containing five rabbits submitted to simple ligature, conventional therapy and application of LigaSure™. We assessed macroscopic and microscopy parameters of appendiceal stump and operative wound.

RESULTS: The group with acute appendicitis that LigaSure™ was applied had fibrosis in 100% of animals, as well as in the other operative techniques used. It suggested that application of LigaSure™ is efficient as other techniques used in healing of appendiceal stump.

CONCLUSIONS: The application of LigaSure™ induces the formation of fibrosis in the appendiceal stump. The technique proved efficacy to induce enough fibrous tissue to obstruct leakage of enteric content.

Key words: Appendicitis. Appendectomy. Animal Experimentation. Ligation. Electrocoagulation. Rabbits.

RESUMO

OBJETIVO: Avaliar a eficácia da utilização do LigaSure™ na apendicectomia, com ou sem a presença de processo inflamatório agudo, comparando com ligadura simples ou técnica tradicional.

MÉTODOS: Um total de 30 coelhos (*Oryctolagus cuniculus*) foi alocado em dois grupos, grupos A e B, cada um composto por 15 animais. O grupo A não apresentava apendicite aguda e o grupo B com apendicite aguda, sendo os animais submetidos à apendicitectomia. Cada grupo foi divido em três subgrupos, cada um com cinco animais onde foram então submetidos à ligadura simples, técnica tradicional ou utilização do LigaSure™. Foram avaliados parâmetros macroscópicos assim como microscópicos do coto apendicular assim como da ferida operatória.

RESULTADOS: No grupo que apresentava apendicite aguda onde foi utilizado o LigaSure™, foi observado fibrose em 100% dos animais, assim como nos outros dois grupos. Com isto mostrou-se que a aplicação do LigaSure™ é tão eficiente quanto as outras técnicas utilizadas na cicatrizaçãoo do coto apendicular.

CONCLUSÕES: A utilização do LigaSure™ induz a formação de fibrose na região do coto apencicular. A técnica foi eficaz quanto à formação de tecido fibrótico evitando o extravassamento de conteúdo entérico.

Descritores: Apendicite. Apendicectomia. Experimentação Animal. Ligadura. Eletrocoagulação. Coelhos.

Introduction

Acute appendicitis is the most frequent abdominal inflammatory disease. It mainly affects young adults of reproductive age[1]. It is the most common and emergency cause of inpatients pre-diagnosed with acute abdominal pain in emergency rooms. For this reason, the appendectomy became one of the more common surgical emergencies done in general hospitals services[2]

This disease usually has a satisfactory evolution if the appropriated operative treatment is done. Although, cases of difficult or later diagnosis might have some acute complications[3]

So far, however, has been little discussion on the etiology and epidemiology of acute appendicitis. Two most common theoretical are: the bacterial dissemination, which leads to inflammatory process of cecal appendix[4] and the obstruction of appendical lumen, which cause stasis, hypersecretion and growth of already presented bacterial[5].

Surgical techniques to date have used simple ligature of appendix verminformis and conventional technique, which consists in simple ligature of invagination of appendical stump as described by Ochner[6].

The LigaSure™ is a feed-back-controled bipolar electrocoagulation system designed to seals effectively vessels up to 7mm in diameter with minimal thermal spread. This device applies a precise amount of mechanical pressure and radiofrequency energy to tissue, causing fusion of the opposing layers by creating a seal of denatured collagen, which can be transected[7]. The superiority of LigaSure™ is that the tissue fusion is created by the denaturation of proteins, thus forming a true seal rather than creation of a proximal thrombus.

Because collagen is abundantly present in intestinal wall, we assumed that the device would have been capable of creating intestinal walls seals, and would have had enabled secure appendectomy[7].

The aim of this paper is to evaluate the efficacy of the use of LigaSure™ vessel sealing system in appendectomy with or without acute inflammatory process.

Methods

A total of 30 New Zealand white rabbits (*Oryctolagus cuniculus*), from a certified animal breeder, weighing between 3.000g to 3.500g were used. This is the animal model par excellence for experimental appendectomy[8], both males and females. This study was submitted and approved by Ethics Committee on animal use.

The animals were randomly allocated into two groups (Group A and B) of 15 animals. Group A submitted to appendectomy without acute appendicitis and Group B with acute appendicitis.

In the period of the study animals were housed in cages at room temperature, were fed with rabbit chow, and had free access to water. All operations were done in the surgical laboratory of the medical school of UNICID.

The animals were pre-anesthetized with acepromazine (1mg/Kg), xylazine (0.5mg/Kg) and ketamine (0.5mg/kg) administered via intramuscular injection and after 10 minutes they received volatium anesthetic with isoflurane anesthesia, which was maintained during the surgery.

Access to the abdominal cavity was obtained through a 5cm incision median laparotomy in peritoneal region. Appendix verminformis originated from the cecum was found and exteriorized. In all cases meso-appendix and appendiceal base were ligated and appendectomy was done subsequently. To induce appendicitis in the group B we performed the procedure described by Dennis[9].

The groups were further subdivided into three groups, each group containing five rabbits submitted to: 1- simple ligature, a ligature of appendix base approximately 1cm from the cecal appendix, transfixed with 4-0 prolene suture; 2- conventional technique as described by Ochsner[6], which is, a ligation of the appendiceal stump, and then purse-string invagination of the cecal base; 3- appendectomy using LigaSure™ device placed approximately 1 cm from transition appendix, and then appendiceal artery ligation.

After appendectomy, the cecal stump was replaced into the peritoneal cavity in the right lateral position. The abdominal wall was closed in two layers. Polypropylene 4-0 was used to close peritoneum and muscle with a knotless suture anchor, and the skin was closed with mononylon 4-0 continuous simple suture.

The animals were allowed to live for 14 days, and then they were sacrificed. The abdomen was opened through a pediculated U-shaped incision from the chest. We evaluated the following macroscopic parameters: adhesion within cavity line sutured, adhesion between loops of small intestine and intracavitary abscess.

Subsequently the cecum region where appendectomy was previously removed. It was washed with tap water and embedded in formaldehyde of 10%. The pathological material for analysis was prepared in the laboratory of structural biology and histology of Paulista School of Medicine, Federal University of Sao Paulo (UNIFESP). After appropriated fixation and longitudinal sections

of the pieces, they were stained with hematoxylin and eosin and masson's trichrome stain to microscopy analysis.

Histological parameters were evaluated according to following criteria:

	Definition
Acute inflammatory process	Presence of congestion, edema and Polymorphonuclear leukocyte infiltration
Chronic inflammatory process	Presence of mononuclear cells infiltration (lymphocytes, plasmocytes and histiocytes)
Granulation tissue	Presence of fibroblasts and neovascularization in a loose extracellular matrix
Fibrosis	Identification and concentration of collagenous fibers

Statistical analyses were done using the Chi-square test[10] to compare the three techniques related to frequencies of macro and microscopy analyses of the results. The statistical significance was set at 0.05 or 5%. The Fisher's exact test[10] was also used to compare groups with and without appendicitis regarding to frequency of fibrosis.

Results

Macroscopic aspects

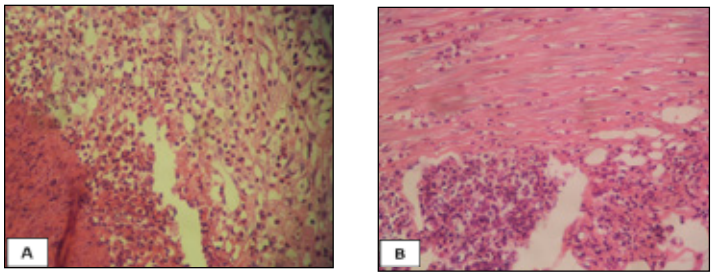

FIGURE 1 - Histological sections of the wall in the vermiform appendix showing exuberant neutrophilic infiltrate, edema and necrosis associated with mural specimen without inducing appendicitis, operated using the LigaSure™ (**A**), compared to specimens without inducing appendicitis operated using simple ligation (**B**) (HE, 400X).

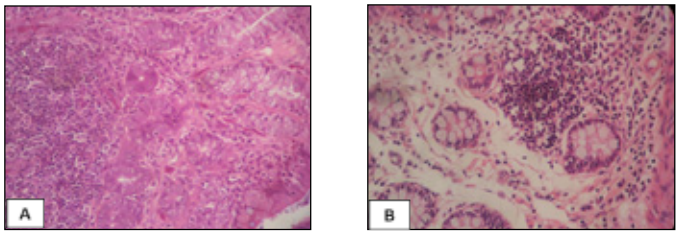

FIGURE 2 - Histological sections of the appendicular mucosa showing exuberant lymphocytic infiltrate including reactive follicular hyperplasia in the specimen with induction of appendicitis, treated with simple ligation (**A**), compared to mucosa with mild lymphocytic inflammatory infiltrate, sometimes with lymphoid arrangements in specimen with induction of appendicitis treated with LigaSure™ (**B**) (HE, 400X).

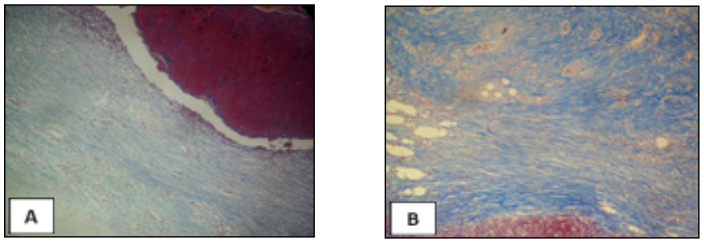

FIGURE 3 - Histological sections of the appendiceal wall, stained with Masson's trichrome (100X), with mild fibrosis in the specimen without induced appendicitis treated with simple ligation (**A**), compared with the specimen **B**, with moderate to marked degree of fibrosis in animal induction of appendicitis treated with LigaSure™.

TABLE 1 – Rabbits from groups A and B in the three operative techniques for the adhesion within cavity line sutured.

	Group A with appendicitis				***Group B without appendicitis***			
Technique	*Yes*	*No*	*Total*	*Yes %*	*Yes*	*No*	*Total*	*Yes %*
Simple ligature	3	2	5	60.0	2	3	5	40.0
Conventional technique	1	4	5	20.0	3	2	5	60.0
LigaSure™	0	5	5	0.0	1	4	5	20.0
TOTAL	4	11	15	26.7	6	9	15	40.0
	Not analyzed				Not analyzed			

TABLE 2 - Rabbits from groups A and B in the three operative techniques for the adhesion between loops of small intestine.

	Group A with appendicitis				***Group B without appendicitis***			
Technique	*Yes*	*No*	*Total*	*Yes %*	*Yes*	*No*	*Total*	*Yes %*
Simple ligature	5	0	5	100.0	5	0	5	100.0
Conventional technique	5	0	5	100.0	5	0	5	100.0
LigaSure™	5	0	5	100.0	5	0	5	100.0
TOTAL	15	0	15	100.0	15	0	15	100.0
	Unnecessary analysis				Unnecessary analysis			

TABLE 3 - Rabbits from groups A and B in the three operative techniques for intracavitary abscess.

	Group A with appendicitis				***Group B without appendicitis***			
Technique	*Yes*	*No*	*Total*	*Yes %*	*Yes*	*No*	*Total*	*Yes %*
Simple ligature	1	4	5	20.0	1	4	5	20.0
Conventional technique	2	3	5	40.0	2	3	5	40.0
LigaSure™	0	5	5	0.0	4	1	5	80.0
TOTAL	3	12	15	20.0	7	8	15	46.7
	Not analyzed				Not analyzed			

TABLE 4 - Rabbits from groups A and B in the three operative techniques for acute inflammatory process (Figure 1).

	Group A with appendicitis				***Group B without appendicitis***			
Technique	*Yes*	*No*	*Total*	*Yes %*	*Yes*	*No*	*Total*	*Yes %*
Simple ligature	0	5	5	0.0	4	1	5	80.0
Conventional technique	0	5	5	0.0	1	4	5	20.0
LigaSure™	3	2	5	60.0	2	3	5	40.0
TOTAL	3	12	15	20.0	7	8	15	46.7
	Not analyzed				Not analyzed			

TABLE 5 - Rabbits from groups A and B in the three operative techniques for chronic inflammatory process (Figure 2).

	Group A with appendicitis				***Group B without appendicitis***			
Technique	*Yes*	*No*	*Total*	*Yes %*	*Yes*	*No*	*Total*	*Yes %*
Simple ligature	5	0	5	100.0	5	0	5	100.0
Conventional technique	5	0	5	100.0	5	0	5	100.0
LigaSure™	5	0	5	100.0	5	0	5	100.0
TOTAL	15	0	15	100.0	15	0	15	100.0
	Unnecessary analysis				Unnecessary analysis			

TABLE 6 - Rabbits from groups A and B in the three operative techniques for fibrosis (Figure 3).

	Group A with appendicitis				***Group B without appendicitis***			
Technique	*Yes*	*No*	*Total*	*Yes %*	*Yes*	*No*	*Total*	*Yes %*
Simple ligature	3	2	5	60.0	5	0	5	100.0
Conventional technique	3	2	5	60.0	5	0	5	100.0
LigaSure™	1	4	5	20.0	5	0	5	100.0
TOTAL	7	8	15	46.7	15	0	15	100.0
	Not analyzed				Unnecessary analysis			

TABLE 7 - Rabbits from groups A and B in the three operative using LigaSure™ for fibrosis.

	Fibrosis			
Groups	*Yes*	*No*	*Total*	*Yes %*
Group A – without appendicitis	1	4	5	20.0
Group B – with appendicitis	5	0	5	100.0
TOTAL	6	4	10	60.0

Fisher's exact test
P=0.0238

Animals submitted to operatory technique with LigaSure™ had higher percentage of fribrosis than those with appendicitis.

Discussion

Several animals have been used in experimental studies of acute appendicitis. Despite some of them, like chimpanzees, that have cecal appendix resembling to human, are difficult to obtain in laboratory routine[11]. Others, like dogs[12-15], do not present a true cecal appendix.

Among animals used in experiments, rabbit was chosen to investigations of acute appendicitis[9,16]. This animal have an appendix vermiformis with anatomical structure resembling to human, including the production of mucus, that enables appearance of inflammation after obstruction of the appendix lumen[9].

We choose the simple ligature and conventional technique because these two operative techniques have been frequently used to treat appendiceal stump[17,18]. Currently, however, there is no clear consensus regarding what technique is optimal from clinical[19,20] and experimental[21,22] point of view.

The use of LigaSure™ in the treament of appendiceal stump is justified because it has a capacity to denaturation of proteins and subsequently fusion of the tissue[7], which might represents secure to appendectomy.

Because of the small size of the sample assessed, we did not reject the hypotheses of similarity among these three techniques used in the group without appendicitis (P=0.0920) for the presence of adhesion within cavity line sutured. However, our results suggested a higher adhesion frequency within cavity line suture when simple ligature was applied.

Regarding to adhesion between intestinal loops, Kross[23] observed a more density and quantity when conventional technique was used, and compared with simple ligature.

In the present study, adhesions were observed in all animals in the two operative techniques used, and in those that acute inflammatory process was induced. Within adhesions we also observed evidences of intracavitary abscesses, however it did not show statistical significance.

Kross[23] towards histological findings to inflammatory infiltration observed that, acute inflammatory reaction was more intense in conventional technique than in simple ligature. In another study done by Cabral[24] the acute inflammatory process was more intense when conventional technique was used.

In our study, however, the group with appendicits had acute inflammatory process. The animals from the subgroup of LigaSure™ (60%) whereas those with appendicitis (80%) submitted to simple ligature and those submitted to appendectomy by LigaSure™ (40%), and yet the animals submitted to conventional technique (20%) had acute inflammatory process, which differ from findings reported in the literature.

We observed the chronic inflammatory process in 100% of animals from the both groups in the two techniques used.

To analysis of fibrous from the collagen fiber count, Kross[23] observed that fibrous was less intense in the group submitted to simple ligature compared to those submitted to conventional technique. In this study, there were no differences in fibrous techniques between simple ligature and conventional technique with the presence or not of acute appendicitis.

In a comparative study with rats, Elemen[25] using LigaSure™ and other techniques for appendectomy assessed the fibrosis process by measuring of hydroxyproline, which is a abundant amino acid found in the collagen fibers. They also observed higher levels of hydroxyproline when LigaSure™ was applied. They associated this result with the device capacity to promote denaturation of proteins, fusion of tissue layers and to create a seal of collagen, which enabled a better healing.

In our study when LigaSure™ was applied, in the group without appendicitis, the collagen fibers production was not

induced in comparison with conventional therapy, and simple ligature. On the other hand, in the group with acute appendicitis the use of LigaSure™ induced fribrosis in 100% of animals as well as the other two operative techniques applied. It suggested that application of LigaSure™ is efficient as other studied techniques in healing the appendiceal stump.

Recently several studies compared the application of LigaSure™ with the use of endodissector and endoclip to divide the mesoappendix. Although the use of eletrocautery is the most common and less expensive procedure to divide the mesoappendix, many authors feel that it is an unsafe method during laparoscopic approach. Ponsky[26] has demonstrated that in a serie of 442 cases the use of LigaSure™ and other devices like harmonic scalpel and endostapler seems to be unwarranted, and the use of eletrocautery for the mesoappendix and endoloops for the appendix was safe, effective and cost-efficient.

Sucullu[27] demonstrated that the use of LigaSure™ during appendectomy significantly reduced the operative time (P=0.036) when compared to a conventional approach (endodissector and endoclip) to the mesoappendix. There were no significant differences in complication rates, use of analgesics and hospital stay between the groups.

Aydogan[28] presented similar results when compared the electrothermal vessel-sealing system versus endoclip in laparoscopic appendectomy. Ligasure was used in 127 patients and endoclips were used in 153 cases. No statistically significant differences regarding hospital length of stay, complication rates were observed. The authors concluded that the vessel-sealing system facilitates the dissection of mesoappendix and shortens the operative time in the laparoscopic approach. Yang [29] also pointed that the application of LigaSure simplify the procedure and reduce the operative time showing no complications related to the use of the system.

A limitation of our study was the lack of findings with statistical significance, perhaps because of the small size of the sample in each subgroup.

Conclusions

The application of LigaSure™ induces the formation of fibrosis in the appendiceal stump. Therefore, it suggests that this is an effective technique to create enough fibrous tissue to obstruct leakage of enteric content. Further studies in this field with large sample size are mandatory to validate such findings, and finally evaluate this technique in humans.

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Correspondence:
Marcelo Augusto Fontenelle Ribeiro Júnior
Rua Professor Enéas de Siqueira Neto, 340 Campus I-UNISA
04829-300 São Paulo – SP Brasil
Tel.: (55 11)2141-8565 / 6843-9911
mfribeiro@unisa.br

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