ORIGINAL ARTICLE

Role of dissemination of microorganisms during laparoscopic appendectomy in abscess formation

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ABSTRACT

BACKGROUND: The aim of this study was to investigate the potential contributory role of laparoscopic appendectomy in the occurrence of postoperative intra-abdominal infections.

METHODS: A prospective single-center study including 48 patients who underwent laparoscopic appendectomy was conducted between August 2010 and September 2011. Two peritoneal samples were obtained from each patient in the pre- and post-appendectomy period. Aerobic and anaerobic microbiological cultures were obtained from the samples. The data were analyzed with statistical methods.

RESULTS: The mean age of the 48 patients (29 male, 19 female) was 10.9 years. Among the pre-appendectomy aerobic cultures, microorganisms were isolated in 18 of the patients (38%), with *Escherichia coli* being the most common. In post-appendectomy aerobic cultures, various bacteria were isolated in 7 patients (14.6%), with the numbers of bacteria statistically significantly reduced ($p<0.05$). Anaerobic microorganisms were isolated in 12 patients (25%) and 4 patients (8.3%) in pre- and post-appendectomy cultures, respectively, with *Bacteroides fragilis* the most common organism; there was a significant reduction in the bacterial count ($p<0.05$). Each patient was regarded as their own control.

CONCLUSION: Our results suggest that laparoscopic appendectomy does not cause an increase in intra-abdominal infections, and particularly not infections associated with anaerobic bacteria.

Key words: Aerobic and anaerobic infections; *Bacteroides fragilis*; *Escherichia coli*; intra-abdominal abscess; laparoscopic appendectomy.

INTRODUCTION

Acute appendectomy is one the most common surgical procedures in children and requires urgent and appropriate treatment; otherwise, it may progress to complicated intra-abdominal infections.[1,2] Minimally invasive surgery has gained acceptance for application in acute and complicated appendicitis, as it offers reduced scarring of the abdomen, earlier recovery, and a shorter hospital stay.[3,4] Studies have suggested that an infected appendix can contaminate adjacent tissues during surgery because the intra-abdominal pressure of carbon dioxide (CO_2) provides a suitable condition for the spread of infection, particularly by anaerobic microorganisms.[5]

In this study, we aimed to investigate the effects of laparoscopic appendectomy on the spread of intra-abdominal infections.

MATERIALS AND METHODS

This prospective study included patients aged 2 to 18 years who underwent laparoscopic appendectomy between August 2010 and September 2011 in our Department of Pediatric Surgery. Patients were admitted to the study after parental consent and ethical committee approval were obtained. Demographic variables, clinical presentations, radiological findings, pathological diagnosis, preoperative laboratory parameters (white blood cell count, C-reactive protein [CRP] levels), length of hospital stay, and time since onset of complaints

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were recorded on a standard data sheet. Postoperative abscess formation, wound infections, and antibiotic resistance were recorded. Afebrile patients with normal bowel movement were discharged.

Patients with open appendectomies, Meckel diverticulitis, and appendectomies that had not been completed laparoscopically were excluded. Suspected appendicitis was evaluated pathologically, and patients with negative pathology were excluded.

Surgical Technique

Laparoscopic appendectomy, utilizing the three-trocar technique, was performed as the standard method. A 10 mm trocar was inserted into the abdomen via the open technique through an intraumbilical incision. After creating a pneumoperitoneum with CO_2 at a pressure between 8 and 10 mmHg, two 5 mm trocars were introduced at the left lower abdomen and at the midline above the symphysis pubis under direct vision of a 5 mm 30° laparoscope.[6] A grasper was used to identify the appendix and to dissect retroperitoneal adhesions. When the tip of the appendix was freed, the mesoappendix was divided by hook cautery. An intracorporeal appendectomy was performed via two-loop ligation, with a division between the loops. The appendix was removed with a grasper held from the closed side, without a bag, through the (umbilical) trocar. After removal of the appendix, a second peritoneal sample was obtained.

Bacteriological Analyses

Peritoneal samples were obtained twice for isolation of microorganisms. The first peritoneal samples were collected at the beginning of the operation, and the second were obtained following the appendectomy. They were transported to the laboratory in Portagerm bottles recommended for the transport of liquid specimens (BioMerieux, France). All the samples were inoculated on sheep-blood agar, chocolate agar, and MacConkey agar and incubated at 35-37°C for 24-48 hours. Significant growth of microorganisms in culture was identified using conventional and semiautomatic methods, namely BBL Crystal Identification Systems (Becton Dickinson, USA).

Definitions

Acute appendicitis was defined as inflammation or suppurative inflammation of the appendix without perforation and without gangrenous or abscess formation. Complicated appendicitis was defined as gangrenous or perforated appendicitis with or without an abscess or peritonitis. The diagnosis was confirmed with intraoperative macroscopic findings and/or pathology.[7]

Antibiotic Therapy

All patients received a single dose of amoxicillin clavulanic acid preoperatively in the operating room. If the appendicitis was acute, antibiotic therapy was continued with amoxicillin clavulanic acid. If there were complications, combination therapy with gentamicin and metronidazole was added. The antibiotic therapy was modified according to culture results.[7-9]

Statistical Analyses

The Statistical Package for the Social Sciences (version 16.0 software) was used to analyze the results. The results were expressed as mean±standard deviation (SD) for continuous variables and as a percentage for qualitative variables. The distribution of the variables was analyzed with the Kruskal-Wallis test. Differences were assessed using the paired t test or the Mann-Whitney U-test, as appropriate. Nominal variables were assessed by the Pearson chi-square test and by Fisher's exact chi-square test. Wilcoxon's signed-rank test was used for related samples. Statistical significance was considered as $p<0.05$.

RESULTS

Forty-eight patients (29 male, 19 female) aged 2-18 years, with a mean age of 10.9 years, were included in the study. Twenty cases had acute appendicitis, 12 had suppurative appendicitis, 9 had local perforated appendicitis, and 7 had perforated appendicitis and generalized peritonitis. The mean length of the hospital stay was 3.2 (range, 1-10) days. The mean CRP level was 54.8 mg/dl (range, 0.1-284), and the mean leukocyte count was 15.130/mm^3 (range, 5.100-29.000). With the use of preoperative ultrasound, the mean noncompressible appendix diameter was found to be 8.3 mm (range, 6-13). Intra-abdominal fluid without the presence of an abscess was found in 15 (17.4%) of the patients, and an abscess was present in 1 patient (2%) (Table 1).

Table 1. Demographic findings of the patients

	Mean values	Range
Age (years)	10.9	2-18
Leukocytes (/mm^3)	15000	5100-29000
C-reactive protein (mg/dl)	54.8	0.1-284
Diameter of appendicitis on USG (mm)	8.3	6-13
Length of hospital stay (days)	3.2	1-10

Aerobic microorganisms were isolated in 18 of the 48 patients (38%) in the first cultures. *Escherichia coli* was the most common microorganism, in 12 of the 18 pre-appendectomy aerobic cultures, with an incidence of 66.6%. In 2 patients, both *E. coli* and *Pseudomonas aeruginosa* were present in the first cultures. *Klebsiella oxytoca*, beta-hemolytic streptococci, and methicillin-resistant *Staphylococcus aureus* (MRSA) were also isolated from the pre-appendectomy aerobic cultures. Post-appendectomy aerobic cultures were positive in 7 patients. *E. coli* was again the most common microorganism in the second cultures, being positive in 5 patients. Both *E. coli* and *P. aeruginosa* were also identified in 2 patients.

E. coli was resistant to ampicillin-sulbactam in 10 patients (83%) and to gentamicin in 3 patients (25%). Ampicillin-sulbactam- and gentamicin-resistant *E. coli* in these 3 patients was sensitive to ceftriaxone (Table 2).

Anaerobic microorganisms were isolated in the pre-appendectomy cultures of 12 (25%) of the 48 patients. *Bacteroides fragilis* was the most commonly identified microorganism in 10 (83.3%) of the patients. In 4 of these patients, *Peptostreptococcus* accompanied *Bacteroides*, which was the second most common (50%) anaerobic microorganism. The second culture results were positive in only 4 patients. In the post-appendectomy anaerobic cultures, *Bacteroides* was also the most common microorganism. The decrease in the bacterial count in the post-appendectomy anaerobic cultures was also significant ($p<0.05$). In 2 patients, *Peptostreptococcus* accompanied *Bacteroides*. The number of patients in which a positive culture was reported for each bacterium is listed in Table 3. In our study, each patient acted as their own control in lieu of choosing a control group consisting of open appendectomies.

Postoperatively, exudative fluid developed in 5 patients, and the mean duration to the diagnosis of abscess formation was 5 days (3-7 days). The diagnosis of an abscess was confirmed by ultrasonography. In large, accessible abscesses more than 3 cm in diameter, percutaneous drainage with ultrasound was performed. *E. coli* was isolated from abscess cultures in 2 patients, and the remaining 2 only showed leukocytosis. *E. coli* was the most common microorganism in the pre-appendectomy cultures. All the abscesses developed after perforated appendicitis, except one (Table 4). Wound infection developed in 5 of the patients, but it resolved with conservative treatment.

DISCUSSION

Laparoscopic appendectomy is performed for acute and complicated appendicitis in children. Following the removal of the appendix, infections may occur, except in cases of limited intraluminal acute appendicitis. Complicated appendicitis can

Table 2. Susceptibility/resistance of *E. coli*, the most frequently identified microorganism, to commonly used antibiotics

E. coli **(n=12)**	**Susceptible**	**Susceptible (%)**	**Resistant**	**Resistant (%)**
Ampicillin-sulbactam	2	16.6	10	83.3
Gentamicin	9	75	3	25
Ceftriaxone	12	100	–	0

Table 3. Number of patients in which positive culture was reported is listed for each bacterium

Aerobic bacteria			**Anaerobic bacteria**		
	Preapp	**Postapp**		**Preapp**	**Postapp**
E. coli	10	5	*B. fragilis*	4	2
E. coli+					
P. aeruginosa	2	2	*B. fragilis+Peptostreptococcus*	4	2
Klebsiella oxytoca	2	0	Bacteroides spp.	2	0
Beta-hemolytic streptococci	2	0	*Peptostreptococcus*	2	0
MRSA	2	0			
Total	18	7		12	4

Preapp: Pre-appendectomy; Postapp: Post-appendectomy; MRSA: Methicillin-resistant *Staphylococcus aureus*.

For aerobic and anaerobic microorganisms isolated in pre- and post-appendectomy cultures, there were significant decreases in the number of positive cultures for aerobic ($p<0.01$) and anaerobic ($p<0.05$) microorganisms between pre- and post-appendectomy cultures.

Table 4. Ultrasonography (USG) and culture results of patients who developed an abscess

Patient appendicitis	Type of USG	Preoperative USG	Postoperative culture results	Preoperative
1	Perforated + generalized peritonitis	Appendix not visualized Minimal fluid	30x30 mm abscess	*K. oxytoca*
2	Localized perforated	Appendix 8 mm, uncompressed Fluid absent	30x30 mm abscess	*E. coli*
3	Perforated + generalized peritonitis Minimal fluid	Appendix 13 mm, uncompressed	Three abscesses 24x15 mm	*E. coli* *Peptostreptococcus*
4	Acute	Appendix 6 mm, uncompressed, Minimal fluid	31x62x69 mm abscess	*E. coli*
5	Localized Perforated	Appendix not visualized, Minimal fluid	Two abscesses 50x18, 8x6 mm	*E. coli*

lead to the development of abdominal abscesses and generalized peritonitis.[4,8] During laparoscopic surgery, the anaerobic environment and the manipulation of the appendix may result in infections in the abdomen. In open appendectomies, the abdomen is exposed to normal air containing 20% oxygen, which is toxic to anaerobic microorganisms. Serour et al.[10] reported that intra-abdominal CO_2 in the pneumoperitoneum may increase the risk of intraperitoneal infections in laparoscopic appendectomies. Although some studies have shown no difference between open and laparoscopic appendectomies in terms of infection, we hypothesized that CO_2 insufflation may facilitate the spread of bacterial colonization. CO_2 insufflations at a continuous pressure (10-12 cm H_2O), entrance of the ports, movement of the appendix during the preparation of the mesoappendix, and removal of the appendix via intraoperative or transumbilical excision provide a suitable environment for the spread of microorganisms, particularly anaerobes. The intraluminal microorganisms can be flushed out into the intraperitoneal area. Contaminated pieces of fecaliths can also spread throughout the abdomen during aspiration. The contamination can increase complications.[11] Some studies have found that there was no significant bacterial translocation or dissemination of microorganisms in the peritoneum after appendectomy.[12] In our study, we did not observe any increase in the numbers of microorganisms in the post-appendectomy cultures of peritoneal fluid after manipulations associated with appendectomy, aspiration of abscesses, and the removal of the appendix.

Bacteroides is the most common Gram-negative anaerobic bacteria encountered in appendicitis, and it can play a significant role in complications in appendicitis. In pre-appendectomy cultures, we isolated these bacteria in 25% of patients, a number compatible with that reported in the literature. However, *B. fragilis* was present in only four patients in the second batch of cultures, thereby showing a significant decrease.

Low numbers of aerobic bacteria were also found in the second cultures relative to the first (pre-appendectomy) cultures ($p<0.05$). *E. coli* has been reported to be the most commonly isolated organism in appendicitis in the literature.[13] Although laparoscopic procedures were associated with decreased numbers of *E. coli* in the second cultures, complications occurred due to antibiotic resistance.

Aided by ultrasonography, abscesses that were larger than 3 cm and easily accessible were drained percutaneously. Laparoscopic drainage is an effective procedure for intra-abdominal abscesses when percutaneous drainage is not possible.[11,13] *E. coli* was the most commonly identified organism isolated from the pre-appendectomy cultures (4/5, 80%) and from the abscesses (2/5, 40%). The bacterium was also resistant to ampicillin-sulbactam (100%) and gentamicin (50%). In the cases that were resistant to ampicillin-sulbactam and gentamicin, the antibiotic therapy was altered to ceftriaxone and tazobactam. The treatment was successful with these drugs. Patients who had abscesses smaller than 3 cm were treated with antibiotics. We think that the abscesses were the result of ampicillin-sulbactam-resistant *E. coli*. Following the replacement with ceftriaxone and tazobactam, all the abscesses resolved.[8]

The numbers of anaerobic organisms were reported to be decreased following laparoscopic appendectomy, but they were responsible for complications, such as a longer hospital stay and, particularly, abscess formation.[4,11] Although the numbers of *E. coli* were decreased in the second cultures following laparoscopic procedures, *E. coli* was responsible for complications due to antibiotic resistance and virulence. *E. coli* was isolated from the abscesses that developed postoperatively.

The limitations of this study are the relatively small number of cases and the difficulty in the production of anaerobic microorganisms in laboratory cultures.

In conclusion, laparoscopy is gaining acceptance in complicated cases of gangrenous or perforated appendicitis in children.[4,6] Suspicions have been raised about a potential increase in the rates of infection, particularly with anaerobic bacteria, due to the anaerobic atmosphere of the pneumoperitoneum, contamination with the contents of the appendix during the preparation of the mesoappendix, and the removal of the appendix via the right paracolic area of the abdomen.[14] Our study showed that laparoscopic appendectomy did not increase intra-abdominal rates of infection, and particularly not with anaerobic bacteria. Intra-abdominal abscesses following laparoscopic appendectomies can develop due to bacterial virulence and the resistance of intraluminal organisms.

Conflict of interest: None declared.

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KLİNİK ÇALIŞMA - *ÖZET*

Laparoskopik apendektomi sırasında apse oluşumunda mikroorganizmaların rolü

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AMAÇ: Bu çalışmada, laparoskopik apandektomi operasyonunun karıniçi enfeksiyon oluşumundaki rolü değerlendirildi.
GEREÇ VE YÖNTEM: Ağustos 2010-Eylül 2011 arasında laparoskopik apandektomi yapılan 48 çocuk hasta çalışmaya alındı. Çalışmamız tek merkezli ve prospektif olarak yapıldı. Her hastadan ameliyat sırasında apandektomi öncesi ve sonrası, aerob ve anaerob olmak üzere dörder adet kültür örneği alındı. Hastaların demografik bilgileri toplandı. Sonuçlar istatistiksel olarak karşılaştırıldı.
BULGULAR: Hastaların ortalama yaşı 10.9±3.3 idi, 29'u erkek, 19'u kızdı. Apendektomi öncesi alınan 48 aerob kültürün 18'inde üreme oldu (%38), ***E.coli*** en sık üreyen mikroorganizma idi. Apendektomi sonrası aerob kültürlerin 7'sinde (%15.9) üreme oldu, bakteri sayısı anlamlı olarak azalmıştı ($p<0.05$). Anaerobik kültürlerde ise 48 hastanın 12'sinde (25%) apandektomi öncesi, 4'ünde (8.3%) ise apendektomi sonrası üreme oldu. ***B. fragilis*** ensık izole edilen organizma idi. Azalma istatistiksel olarak anlamlı idi ($p<0.05$).
TARTIŞMA: Bizim sonuçlarımız laparoskopik apendektominin karıniçi enfeksiyon riskini, özelikle de aneorop enfeksiyonların riskini artırmadığı yönünde idi.
Anahtar sözcükler: Aerob ve anaerob enfeksiyon; Bacteroides fragilis; Escherichia coli; karıniçi apse; laparoskopik apendektomi.

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