MORPHOMETRIC ANALYSIS OF VASCULAR CLEFTS IN CHILDREN WITH SYMPTOMS OF ACUTE APPENDICITIS AND NEGATIVE APPENDECTOMY

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Objective: Many cases of clinically suspected acute appendicitis show no microscopic signs of acute inflammation. Negative appendectomy rates differ greatly, partly due to various criteria used by different institutions to define acute appendicitis. In our practice, we have noticed that many of the negative appendectomy specimens contain prominent vascular clefts. The objective of this study was to determine the possible significance of vascular clefts, which has not been investigated yet. Our hypothesis was that vascular clefts are early, as yet unrecognized signs of acute appendicitis. ***Methods:*** We conducted a retrospective study by searching for patients who had negative appendectomy at the Zagreb Children's Hospital (2014-2019). There were 151 patients aged 1-18 years, 124 of which were included in the study group and 27 in the control group. Vascular clefts, if present, were measured microscopically. Statistical analysis was performed using Kolmogorov-Smirnov, Kruskal-Wallis, Mann Whitney, $\chi 2$ and Spearman's rank correlation tests. The level of statistical significance was set at $p<0.05$. ***Results:*** Out of the 124 patients included in the study group, 50.8% were female (n=63) and 49.2% were male (n=61). Mean age of the patients was 11.5 years and median 12 years. Negative appendectomy specimens showed prominent vascular clefts in 94 of 124 (75.8%) study group patients. Vascular cleft width varied between 140 and 1751 μm. Twelve (9.7%) specimens showed no signs of vascular clefts, and 18 specimens had partial vascular clefts that did not penetrate muscular wall of the appendix and consequently could not be measured. We also showed that there was a statistically significant difference between the number of appendices that contained fecaliths in their lumina in the study group as compared to the control group ($p<0.01$). ***Discussion:*** Negative appendectomies are still a problem in the 21st century medical practice. Although many cases of clinically suspected acute appendicitis microscopically show no signs of inflammation, in some cases symptoms may regress after appendectomy has been performed, even if there are no histopathologic signs of inflammation. In everyday practice, we noticed that in cases of acute suppurative or phlegmonous appendicitis, a dense inflammatory infiltrate is often seen passing through prominent vascular clefts, which we define as fissures of the muscular layer of the bowel (or in this case appendiceal) wall through which blood vessels and peripheral nerve branches pass on their way to and from the bowel. We tried to determine the possible significance of these vascular clefts. We collected 124 negative appendectomy specimens from the archives of our Department of Pathology and Cytology, all of which were removed from pediatric patients at the Zagreb Children's Hospital due to clinically suspected acute appendicitis. None of the 124 appendices met our criteria for acute appendicitis. We found that 94 of 124 (75.8%) negative appendectomy specimens showed vascular clefts. We also calculated the Zagreb Children's Hospital negative appendectomy rate during the 5-year period, which was 9%. ***Conclusion:*** Our results show that prominent vascular clefts in the muscular layer of the appendiceal wall are frequently found in negative appendectomy specimens. These clefts could be implicated in the pathophysiology of acute appendicitis and might be one of the first signs of acute appendicitis.

Key words: appendix, appendicitis, vascular cleft, negative appendectomy rate

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INTRODUCTION

Appendectomy is one of the most common emergency surgical procedures (1). However, in many cases of clinically suspected acute appendicitis, resected appendectomy specimens show no signs of inflammation. Negative appendectomy rate (NAR) is a quality metric used in the management of acute appendicitis, which determines the frequency of nontherapeutic appendectomies (1,2). NAR varies broadly in the literature from 1% to 40% (1). It is important to emphasize that NAR is also determined by definition of negative appendectomy (3). There are different criteria to define appendicitis and negative appendectomy (3). The most common definition of negative appendectomy is the absence of inflammation or pathology in the appendix, although some use stricter criteria such as absence of intramural neutrophils in the appendix (3). High NAR has been justified by some because there is a belief that the morbidity associated with negative appendectomies is not severe enough compared to the risk of developing appendiceal perforation (2). NAR has been in decline over the past decade, which can be attributed to better diagnostics, and some authors ascribe it to the increased use of ultrasound and computed tomography (CT) scans (2). However, what if the appendices that appear normal on histologic slides actually show early signs of appendicitis of which we are not yet aware? One study found evidence for an inflammatory pathologic condition at the molecular level in a histologically normal appearing appendix (4). Some suggest neurogenic appendicitis to be a possible causative mechanism of pain, especially in children. Cases such as these could theoretically explain the improvement of clinical symptoms after negative appendectomy (5). We conducted a retrospective study on negative appendectomy specimens focusing on the presence or absence of vascular clefts on histologic hematoxylin and eosin (H&E) stained slides. Vascular clefts are fissures of the muscular layer of the bowel wall through which blood vessels and peripheral nerve branches pass on their way to and from the bowel (Figure 1).

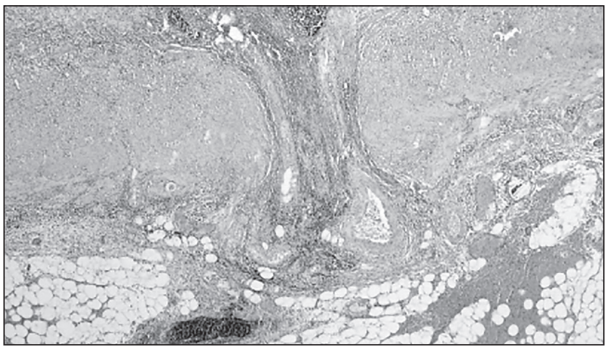

Fig. 1. *Vascular cleft with inflammatory infiltrate penetrating the muscular layer of the appendix.*

OBJECTIVE

The objective of this study was to determine the prevalence of prominent vascular clefts in negative appendectomy specimens, measure their width, determine whether there are fecaliths present in appendiceal lumina, and compare the findings to the specimens from the control group. Our hypothesis was that vascular clefts are early, as yet unrecognized signs of acute appendicitis.

MATERIAL AND METHODS

A retrospective review was conducted on patients having undergone appendectomy at the Zagreb Children's Hospital between January 1, 2014 and March 31, 2019. Patients were identified by searching through pathologic records of Department of Pathology and Cytology, Sestre milosrdnice University Hospital Centre. All resected appendices were serially sectioned and submitted to histologic analysis in their entirety. They were cut by microtome in multiple 3-5 μm thick sections. At our institution, acute appendicitis is histologically defined by the presence of neutrophils in the muscular layer of the appendix, and all other cases were considered to be negative appendectomies and were included in the study. Microscopic slides were retrieved and re-examined to determine the presence or absence of vascular clefts. We measured the width of vascular clefts at their point of entry through the muscular wall microscopically using the Olympus camera (Figure 2). If more than one cleft were found in the specimen, the widest cleft was measured and subjected to statistical analysis. The study included 151 patients aged 1-18 years. Out of these 151 patients, 124 were assigned to the study group. These patients showed clinical signs of acute appendicitis and were treated operatively as such; however, histology did not confirm the diagnosis of acute appendicitis. The remaining 27 patients were assigned to the control group. Their appendices were removed during abdominal surgery for non-related reasons as a precautionary measure. Statistical analysis was performed using Kolmogorov-Smirnov, Kruskal-Wallis, Mann Whitney, χ^2 and Spearman's rank correlation tests. The level of statistical significance was set at $p<0.05$. The analysis was made using GraphPad Prism version 8.0.0.

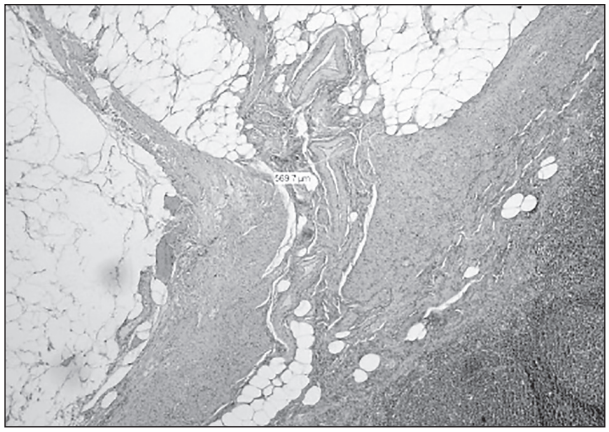

Fig. 2. *The width of the vascular cleft was measured at its point of entry through the muscular wall using the Olympus camera.*

RESULTS

A total of 1376 appendectomies due to the symptoms of acute appendicitis were performed at the Zagreb Children's Hospital during the 5-year period (from January 1, 2014 to March 31, 2019). Out of these 1376 appendectomy specimens, 124 showed no signs of inflammation, which means that the negative appendectomy rate (NAR) for the study period at the aforementioned institution was 9%. Out of the 124 patients included in the study group, 50.8% (n=63) were female and 49.2% (n=61) were male. Mean age of patients in the study group was 11.5 years and median 12 years. Ninety-four of 124 (75.8%) negative appendectomy specimens showed prominent vascular clefts. Vascular cleft width varied between 140 and 1751 μm. Twelve (9.7%) specimens showed no signs of vascular clefts, and 18 (14.5%) specimens had partial vascular clefts that did not penetrate muscular wall of the appendix on the sections analyzed and therefore were not measured. Out of the 27 patients included in the control group, 44.4% (n=12) were female and 55.6% (n=15) were male. Mean age of patients in the control group was 8 years and median 9 years. Nineteen of 27 (70.4%) control group specimens showed prominent vascular clefts. Vascular cleft width varied between 168 and 864 μm. Eight (29.6%) specimens had partial vascular clefts that did not penetrate muscular wall on the sections analyzed. The mean cleft size was larger in the study group than in the control group (363.72 *vs*. 301.75 μm), however, statistical analysis did not yield significant p values (p=0.36). We think this was the result of a relatively small control group. In the study group, 63.7% (n=79) of appendices contained fecaliths in their lumina as compared with 25% in the control group (p<0.01). The presence of fecaliths did not affect cleft size in the study group (337.41 μm with fecalith present *vs*. 410.10 μm with no fecalith, p=0.20).

DISCUSSION

The pathogenesis of appendicitis is still poorly understood (6,7). Commonly accepted theories are based on pathophysiologic changes caused by luminal obstruction, such as continued mucosal secretion and inflammatory exudation increasing intraluminal pressure and consequential obstruction of lymphatic drainage, edema and mucosal ulceration, venous obstruction, and finally ischemic necrosis (8-10). Even though appendicitis can be caused by direct luminal obstruction induced by fecaliths, lymphoid hyperplasia, impacted stool or rarely by an appendiceal neoplasm, according to recent theories these do not seem to be regular occurrences (5,6). The full spectrum of causes of appendicitis is not known yet, and some theories focus on genetic and environmental factors and infections (5). The lifetime incidence of acute appendicitis is 8.6% for men and 6.7% for women, but the incidence of appendectomy carried out for different reasons is 12% for men and 25% for women (9). The incidence of acute appendicitis roughly follows the development of lymphoid tissue, with the highest incidence in late teens and twenties (7,11,12). An accurate preoperative diagnosis of acute appendicitis is difficult and clinical assessment remains crucial in the management of suspected acute appendicitis (11,13). The typical presentation of acute appendicitis includes periumbilical pain, nausea, vomiting, loss of appetite, low fever, and malaise. Within 6-18 hours, the pain localizes in the right lower quadrant with associated guarding and rebound tenderness (11). About 20%-33% of patients present with atypical findings (11). Acute appendicitis requires early surgical intervention since appendiceal rupture causes significant rise in morbidity and mortality. Trying to reduce the risk of perforation by lowering the threshold for surgical intervention has led to a high NAR (11,13). NARs are higher in women of childbearing age (11). The reported complication rate following negative appendectomies is 6.1% (11). Negative appendectomies impose high cost to the patient and the healthcare system. CT scans and diagnostic laparoscopy should be only used in selected cases (11). The gross pathology and histology of acute appendicitis are quite variable. The outer appearance may not correspond to the degree of inflammation found on histologic slides (13). Acute suppurative or phlegmonous appendicitis is defined as neutrophilic inflammation of the appendiceal wall. Diagnostic criteria for acute appendicitis are controversial, as some believe that mucosal neutrophilic infiltration with superficial ulceration can be considered as an early criterion for acute appendicitis, whereas others require the presence of neutrophilic infiltration of muscularis propria (13). Since fecaliths and enteric infections can also cause superficial ulceration and mucosal inflammation (13), and as some studies suggest that mucosal inflamma-

tion of the appendix is not related to the symptoms of appendicitis (14), we considered the presence of neutrophils in muscularis propria to be the proper histologic criterion for the diagnosis of acute appendicitis. Negative appendectomy specimens in some cases have shown nonspecific changes such as cytokine elevation or neurogenous hyperplasia, which some believe may be the cause of clinical symptoms (13). However, to our knowledge, no one has yet investigated clinical significance of vascular clefts and their possible role in the pathogenesis of acute appendicitis. We searched the PubMed database using keywords such as "vascular clefts" and "appendix" or "appendicitis" in search for studies focused on or mentioning vascular clefts. We found an article describing widened vascular clefts as sites of weakness in the muscular layer of the appendiceal wall with a potential of developing appendiceal diverticula (14). No other mention of vascular clefts of appendiceal wall has been found.

In our study, we demonstrated that most (75.8%) of the negative appendectomy specimens contained prominent vascular clefts. Even though the mean cleft size was larger in the study group than in the control group, statistical analysis did not yield significant p values. This might be the result of a relatively small control group, which was a shortcoming of the study based on pediatric population. It is quite difficult to obtain healthy controls for comparison since all the specimens allocated to the so-called control group had been removed during surgery due to acute abdominal symptoms. This was the major limitation of our study. However, it may be possible to determine clinical significance of the presence and/or width of vascular clefts by comparing them to the severity of clinical symptoms or inflammation parameters (white blood cell count, C-reactive protein, etc.). We also showed that there was a statistically significant difference between the number of appendices that contained fecaliths in their lumina in the study group as compared with the control group ($p<0.01$). This finding is in line with the traditionally accepted theories about the pathophysiologic changes caused by luminal obstruction, which in this case might be connected to the development of vascular clefts and symptoms of appendicitis, even though the presence of fecaliths did not affect cleft size in the study group. The NAR calculated for the Zagreb Children's Hospital during the 5-year period (from January 1, 2014 to March 31, 2019) was 9%, which did not differ significantly from the NARs calculated at other institutions.

CONCLUSION

Our study demonstrated that prominent vascular clefts were present in most (75.8%) of the negative appendectomy specimens. Due to difficulties in obtaining control group specimens, we could not prove a statistically significant difference in vascular clefts between the study group and control group, but there was a statistically significant difference between the number of appendices containing fecaliths in the study group as compared with the control group. The possibility remains that vascular clefts might be one of the early signs of acute appendicitis. Further investigation is needed.

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SAŽETAK

MORFOMETRIJSKA ANALIZA VASKULARNIH KLEFTOVA U DJECE SA SIMPTOMIMA AKUTNOG APENDICITISA I NEGATIVNOM APENDEKTOMIJOM

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Cilj: Mnogi slučajevi klinički dijagnosticiranih akutnih apendicitisa ne pokazuju znakove akutne upale. Stopa negativnih apendektomija znatno varira, dijelom i zbog različitih kriterija koje razne institucije primjenjuju u definiciji akutnog apendicitisa. U našoj svakodnevnoj praksi primijetili smo da mnogi od uzoraka negativnih apendektomija sadrže izražene vaskularne kleftove. Cilj ove studije bio je odrediti moguće značenje vaskularnih kleftova koje nitko dosad nije istražio. Naša hipoteza je bila da su vaskularni kleftovi rani, dosad neprepoznati znakovi akutnog apendicitisa. ***Metode:*** Proveli smo retrospektivnu studiju tražeći bolesnike s negativnom apendektomijom u Klinici za dječje bolesti Zagreb (2014.-2019.). Pronašli smo 151 bolesnika u dobi 1-18 godina, od kojih smo 124 uključili u istraživanu, a 27 u kontrolnu skupinu. Vaskularne kleftove smo mjerili mikroskopski. Učinjena je statistička analiza pomoću Kolmogorov-Smirnovljeva, Kruskal-Wallisova, Mann Whitneyeva, $\chi 2$ i Spearmanova rank korelacijskog testa. Razina statističke značajnosti utvrđena je na $p<0,05$. ***Rezultati:*** Od 124 bolesnika u istraživanoj skupini 50,8 % je bilo ženskih (n=63) i 49,2 % muških (n=61). Srednja dob bolesnika bila je 11,5 godina, a medijan 12 godina. Devedeset i četiri od 124 (75,8 %) uzorka negativnih apendektomija pokazivalo je izražene vaskularne kleftove. Širina vaskularnih kleftova je varirala između 140 i 1751 µm. Dvanaest uzoraka (9,7 %) nije pokazivalo znakove vaskularnih kleftova, a 18 uzoraka je imalo djelomične vaskularne kleftove koji nisu penetrirali kroz čitavu debljinu stijenke apendiksa i stoga se nisu mogli izmjeriti. Također smo pokazali statistički značajnu razliku u broju apendiksa koji su sadržavali fekolite u lumenu između istraživane skupine i kontrolne skupine ($p<0,01$). ***Rasprava:*** Negativne apendektomije su i dalje problem u medicinskoj praksi 21. stoljeća. Iako mnogi slučajevi kliničke sumnje na akutni appendicitis mikroskopski ne pokazuju znakove upale, u nekim slučajevima simptomi se mogu povući nakon provedene apendektomije, iako nema patohistoloških znakova upale. U svakodnevnoj praksi primijetili smo da u slučajevima akutnog supurativnog i flegmonoznog apendicitisa gusti upalni infiltrat često prolazi kroz izražene vaskularne kleftove, koje definiramo kao procjepe mišićnog sloja stijenke crijeva (u ovom slučaju apendiksa) kroz koji krvne žile i periferni ogranci živaca prolaze u crijevo. Pokušali smo odrediti značenje ovih vaskularnih kleftova. Sakupili smo 124 uzorka iz arhive našeg Zavoda za patologiju i citologiju, od kojih su svi odstranjeni iz pedijatrijskih bolesnika zbog kliničke sumnje na akutni apendicitis. Nijedan od 124 apendiksa nije ispunjavao naše kriterije za akutni apendicitis. Utvrdili smo da su vaskularni kleftovi bili prisutni u 94 od 124 (75,8 %) uzorka negativnih apendektomija. Također smo izračunali stopu negativne apendektomije za Kliniku za dječje bolesti Zagreb u petogodišnjem razdoblju koja iznosi 9 %. ***Zaključak:*** Naši rezultati pokazuju da su izraženi vaskularni kleftovi u mišićnom sloju stijenke apendiksa često prisutni u uzorcima negativnih apendektomija. Ti kleftovi bi mogli biti uključeni u patofiziologiju akutnog apendicitisa i mogli bi biti među prvim znakovima akutnog apendicitisa.

Ključne riječi: crvuljak, upala crvuljka, vaskularni kleft, stopa negativne apendektomije