Assessment of the Vasoactive Intestinal Polypeptide (VIP) in Morphologically Normal Appendices Removed from Patients with Clinical Diagnosis of Acute Appendicitis

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Abstract

**Background:** Correct diagnosis of acute appendicitis is one of the most challenging issues in surgery. About 15% to 40% of all appendectomies result in the removal of morphologically normal appendices.

**Objective:** To assess values of the vasoactive intestinal polypeptide (VIP) in the presence of normal and inflamed appendices removed due to clinical diagnosis of acute appendicitis.

**Patients and Methods:** The appendices removed from 76 patients were divided into three groups as follows: group 1: 50 morphologically normal appendices that were removed due to clinical diagnosis of acute appendicitis; group 2: 10 normal appendices prophylactically removed during other surgical procedures; group 3: 16 inflamed appendices with a morphological aspect of acute appendicitis. Histological and immunohistochemical studies defined the characteristics of each appendix of all groups.

**Results:** Groups 1 and 2 did not differ between themselves, but group 3 showed a greater degree of color reaction for VIP than groups 1 and 2.

**Conclusion:** Morphologically normal appendices removed due to a clinical diagnosis of acute appendicitis do not present the immunohistochemical changes for the VIP that is found in inflamed appendices.

**Keywords:** Appendix; Appendicitis; Appendicectomy; VIP; Diagnosis; Treatment

Introduction

Acute abdomen, of the right flank (RF) is probably one of the most challenging problems in Medicine since it opposes vast possibilities of surgical and non-surgical disorders [1]. Acute appendicitis is listed among its causes due its frequency and diagnostic difficulties [1-3].

The probabilities of diseases related to acute abdomen vary regarding age, ethnic aspects and family characteristics. In Brazil, the prevalence of acute appendicitis is 11 per 1,000 inhabitants, per year [4,5]. This inflammation may occur in any age group, but is predominant between 15 and 30 years of age, and in white and brown skin color individuals [6,7]. Males are more affected, 1.1 to 3 cases for each female case [4,8].

Although the cause of appendicitis is still unknown, the presence of microorganisms is found always, as it occurs in all digestive system diseases. Obstruction of the lumen of the appendix, either by thickening of the wall or by foreign bodies seems to be associated to its etiopathogenesis [5,7,9,10]. However, a cause effect of these findings was not established.

Acute appendicitis is diagnosed based on the clinical complaints, leukocytosis and alterations of appendiceal aspect at imaging studies [11]. Clinical presentation includes hyporexia, migrating pain from epigastrium to the right flank and vomitings. Leukocyte count increases above 11×10^3/mm^3 in more than 75% of patients. Radiological studies of the abdomen show the presence of the fecal loading image inside a dilated cecum, in over 90% of acute appendicitis. This sign is present in less than 20% of patients with other inflammatory diseases of the right abdomen [12-14]. Other appendiceal disorders are also found at ultrasound, CT scan and NMR [14].

About 15% to 40% of all appendectomies result in the removal of appendices apparently normal when submitted to conventional histological staining [15]. Maresh and Masson found neuroendocrine lesions in appendices without signs of acute inflammation, suggesting association between clinical symptoms of appendicitis and the presence of lesions on the submucosal layer [16]. According to Di Sebastianio et al. there is a neural proliferation in the appendix, associated with the increase of an immunological reaction for substance P (SP) and VIP, in patients with clinical diagnosis of acute appendicitis without inflammatory reaction. These authors verified increase on the staining of nerve fibers for protein gene product 9.5 (PGP 9.5) in the mucosa of non-inflamed appendices, as well as the presence of SP and VIP. SP and VIP may be the cause of the pain on the RF in the presence or not of an inflamed appendix [17].

In the basal mucosa, the neurogenic vasodilatation is mediated not only by SP, but also by the release of VIP. Kubota et al. showed that the...
distribution of the VIP and its expression are changed in the inflammatory intestinal diseases. According to Di Sebastian et al. the changes in the peptidergic innervations in acute appendicitis may be related to localized pain [18].

The objective of this study was to verify the presence of VIP and its values in patients with clinical symptoms of acute appendicitis, with and without inflamed appendix.

Patients and Methods

This study was approved by the Committee of Ethics in Research of the Federal University of Minas Gerais under protocol number 0429/06. Seventy six patients of both sexes were divided into the following groups:

- group 1: (n = 50), 25 men and 25 women with clinical symptoms of acute appendicitis. Imaging studies reinforced this diagnosis. All patients underwent appendectomies. Examination of these appendices, macro- and microscopically, did not show any morphological disorder and were considered as normal. After the operation, all clinical manifestations of the disease disappeared completely on all patients and did not return over the follow-up period.

- group 2: (n = 10), 8 men and 2 women, underwent prophylactic removal of normal appendices during procedures for other intra-abdominal condition.

- group 3: (n = 16); 6 men and 10 women, operated on for acute appendicitis whose appendices were macro- and microscopically inflamed. Their symptoms disappeared after the surgical treatment.

None of these patients presented any evidence of immune disturbance. The patients of groups 1 and 3 had only symptoms and signs of acute appendicitis; those of group 2 were operated on for abdominal diseases not related to the immunological system. No previous symptom suggesting acute appendicitis was reported by any patient of the three groups. The gap between the clinical presentation and the surgery ranged between six and 36 hours, depending on the necessity of complementary exams to make the diagnosis of acute appendicitis.

All appendices of the three groups were examined by two independent pathologists. Histological sections were performed on the immunohistochemical analysis with the method streptavidin-biotin. The immune markers were observed on the muscularis mucosa, on nerve plexuses and nerve fibers of the appendices. This study was performed according to the following protocol:

- stage 0: absence of immune marker activity;
- stage 1: immune marker activity present in 1/3 of the field; magnification x 400;
- stage 2: immune marker activity present within 1/3 and 2/3 of the field; magnification x 400;
- stage 3: immune marker activity present greater than 2/3 of the fields; magnification x 400.

Comparisons among the three groups used the Fisher’s exact test for small sample sizes, as well as the Kruskal Wallis parametric test for independent samples relative to the variable of interest. Significant differences were accepted at p<0.05.

Results

There was no difference among the three groups relative to distribution by sex, (p = 0.110). Figure 1 shows the three groups of patients on the level of immune staining for the vasactive intestinal polypeptide (VIP) (p<0.001 – Kruskal Wallis test).

One patient from group 1 was excluded due to laboratorial mistake on the immunohistochemical analysis. There was difference among the three groups for the degree of staining of the slides (p<0.001). On multiple comparisons among the groups it was observed that groups 1 and 2, whose appendices did not present inflammatory signs, did not differ, but group 3, with established inflammation, displayed a level of staining greater than groups 1 and 2. During the late postoperative period, none of patients of the three groups presented any symptom like those found in the patients of groups 1 and 3.

Discussion

The likelihood of characterizing acute appendicitis by aspects other than inflammation creates the necessity for extending the propedeutics to new neuroendocrine methods, such as the immunohistochemical analysis, looking for the identification of appendicular disorders even in the presence of morphologically normal aspects. The need for a more precise investigation of the appendix is indicated by the fact that all patients with clinical symptoms of acute appendicitis and that underwent the removal of apparently normal appendices healed completely and definitely, showing disappearance of all previously existent laboratorial and imaging indications of disease, immediately after surgery [1,4,15,19]. Only a disease of the appendix may explain the resolution of these manifestations without any other treatment.

The immunohistochemical analysis was performed only for VIP, for the three appendix groups studied. The appendices with established acute inflammation showed immune staining by VIP more intense than the histological normal appendices, either with RF pain or without clinical signs (Figure 1). Thus, the results of this study were opposite to those reported by Di Sebastian et al. which presented immune staining for VIP more intense in the group of morphologically normal appendices in patients with clinical symptoms of acute appendicitis [19].

A difference in the VIP immune staining was expected between morphologically normal appendices removed from patients with clinical symptoms of acute appendicitis and the normal appendices prophylactically removed. The presumed disease of the apparently
normal appendices with clinical appearance of appendicitis may be associated to appendiceal VIP increase, which was detected as immune staining (Figure 1).

Peptidergic nerves secrete neuropeptides other than VIP, which are pro-nociceptive and pro-inflammatory transmitters [19]. Considering that neurogenic process may not include inflammatory signs, Hofler et al. suggested the name "neurogenic appendicopathy" for appendices of morphologically normal aspect in patients with clinical symptoms of acute appendicitis [20]. This concept was revised since it was shown that there was an increase in the number of nerve fibers stained by antibodies against protein S-100, causative of RF pain. According Hofler et al. and Guller et al. either neurogenic appendicopathy or acute appendicitis present the same symptoms and signs, making impossible the distinction between these two conditions [19-21]. Sesia et al. revised this concept, including the neuropeptides VIP and SP as causative agents of this disorder [22].

In another study, VIP did not present greater expression in neurogenic appendicopathy in comparison with normal appendices without clinical manifestation [1], as it was seen in the present investigation. Thus, the findings of this study, as well as those reported in the literature, do not establish a definitive result, such as the role of the neuropeptides in the clinical manifestation of acute appendicitis.

In this study, there was no difference on VIP expression between morphologically normal appendices in patients with clinical symptoms of acute appendicitis and normal appendices prophylactically removed (Figure 1). This result is different from those reported by Partecke et al. who found VIP greatly stained in patients submitted to removal of normal appendices, more than on those with neurogenic appendicopathy [1]. As these authors performed only immunohistochemical analysis for VIP in patients already tested positively for protein S-100, expressed by cells of neural origin, their histological sections contained samples of appendices already positively tested for parasympathetic nerve fibers, where VIP is immune stained [23,24].

Based on the findings of this study, supported by the literature, the efficacy of the non-operative treatment for patients with clinical symptoms of appendicitis continues to be controversial. Even the necessity and the efficacy of the antibiotics are dubious in neurogenic appendicopathy that is not an inflammatory disease [24-27].

Conclusion

Patients with clinical symptoms, laboratorial and imaging findings of acute appendicitis submitted to appendectomy and whose appendices are morphologically normal did not show immunohistochemical changes on the vasoactive intestinal polypeptide (VIP), which is associated only with inflamed appendices.

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