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Type I/type III collagen ratio associated with diverticulitis of the colon in young patients

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ABSTRACT

Background: The incidence of diverticulitis in young patients is rising, whereas the type I/III collagen ratio of the colon decreases with age. Perhaps a lower type I/III collagen ratio in younger patients may predispose these patients to the development of the disease.
Method: The purpose of this study was to evaluate the collagen content and type I/III collagen ratio in patients with diverticulitis *versus* a control group. Patients who underwent a colon resection were identified. Three groups of patients were created for analysis: those with diverticulitis aged <50 y, >50 y, and a control group. Tissue samples were stained with Sirius red/fast green and photographed. Photos analysis was performed to quantify the amount of type I collagen and type III collagen. The type I/III collagen ratio was calculated for each patient and compared.
Results: The quantity of type I collagen and type III collagen was higher in patients with diverticulitis aged >50 y ($P = 0.04$ and $P < 0.0001$, respectively); however, the collagen ratio was greatest in those patients with diverticulitis aged <50 y ($P = 0.01$). Further analysis demonstrated a significant higher type I/III ratio in all patients aged less than 50 y compared with all patients aged over 50 y ($P = 0.04$).
Conclusions: Our study demonstrated that diverticulitis in the younger patient was not associated with a lower type I/III collagen ratio. It appears that the decrease in collagen ratio of the colon with age is associated with an increase in type III collagen deposition.
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Introduction

Diverticulosis is a common problem, especially in western society. In the United States, the prevalence of diverticulosis is 5% by the age of forty, increasing to 65% by the age of 80 y, with 25% of these patients developing diverticulitis at some point.[1] Although the incidence of diverticulosis increases with age, the mean age of patients hospitalized with diverticulitis is actually declining from 71.2 y in 1995 to 68.1 y in 2004.[1] In addition, an increasing number of patients younger than 50 y old are being hospitalized with diverticulitis, accounting for 18%-34% of diverticular related admissions.[2] Although diverticulitis in younger patients is no longer considered a more virulent disease as Hinchey classifications, hospital duration, successful nonoperative management, and the need for emergent surgery are similar regardless of age.[3,4] A large

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meta-analysis did demonstrate that younger patients have a higher recurrence rate.[5] Clearly diverticular disease in younger patients continues to pose significant questions.

The development of diverticulosis and subsequent diverticulitis is believed to be multifactorial. Increased intraluminal pressure, thickening of the colonic muscular layer, a diet low in fiber, and genetic factors have all been hypothesized to predispose patients to the development of colonic diverticula.[6] These remain unproven, however. In addition, patients with connective tissue disorders, such as, Marfan Syndrome or Ehlers-Danlos, have a tendency to develop pan diverticulosis.[7] The development of diverticulosis in these patients is believed to be due to a defect in collagen synthesis.[7]

Although there are at least 14 different types of collagen, type I and type III collagen are the two predominant types implicated in the development of diverticulosis in patients with connective tissue disorders.[7,8] Type I collagen is composed of rigid fibrils and is the predominant type found in the body.[9] Type III collagen, typically present during the early phase of wound healing, is thinner and generally regarded as immature and weak.[10] The typical ration of type I to type III collagen is four to one.[11] Collagen defects, particularly a higher rate of elastic Type III collagen, have been shown to be a factor in other diseases, such as hernia formation and aortic dissection.[12,13] Synthesis of type III collagen has also been shown to be increased in diverticulosis,[6] and although previous studies have demonstrated a decrease in type I/III collagen ratio associated with diverticulosis, these studies have been limited.[7]

In addition to proliferation of type III collage, collagen crosslinking is also increased in diverticulosis.[8] This collagen crosslinking increases with age, causes the tissue to become more rigid, resulting in the characteristic diverticular outpouching with increased intraluminal pressure.[8] In addition, matrix metalloproteinase-1 expression is reduced in patients with diverticular disease.[7] A down regulation of matrix metalloproteinase-1 as well as an increase of immature type III collagen can change the structure of the colonic wall and make it more susceptible to diverticulitis.[8]

The purpose of this study was to compare the quantity of type I collagen and type III collagen in diverticulitis patients against a control group, specifically looking at the type I/III collagen ratio. In addition, we wanted to see if younger patients (<50 y old) had a lower type I/III ratio.

Methods

This was a retrospective study using an institutional surgical database to identify all patients who underwent colon resections. The study group included patients who underwent sigmoid resection for diverticulitis, and the control group included patients who underwent sigmoid or left sided colectomy for cancer. The patients identified were then categorized into three cohorts: those with diverticulitis under the age of 50 y, those with diverticulitis over the age of 50 y, and a control group. Exclusion criteria included patients with inflammatory bowel disease or those patients in the control group that had evidence of diverticulosis. The pathologic specimens were then reviewed and prepared for analysis by two independent pathologists.

Histological methods

Tissue samples from the resected colon of each patient was embedded in paraffin, sectioned at 5 μm and stained with hematoxylin. Slides were then washed with tap water and stained with 0.1% fast green FCF for 10 min followed by acetic acid washing. The slides were then stained with picrosirius red F3BA for 1 h. The slides were subsequently washed with acidified water, dehydrated, cleared and mounted. Sirius red/fast green stain was used to differentiate between type I/III collagen. Sirius red is a strong anionic dye that stains collagen by reacting with sulfonic acid groups. The dye molecules attach to the collagen fibers in such a way the long axes is parallel. When viewed under cross-polarized light, the collagen fibers appear different from one another, with the type I collagen fibers appearing a bright yellow orange and the type III collagen fibers appearing green-blue (Figs. 1 and 2). Slides were examined under cross-polarized light microscopy with an axioskop 40 microscope (Carl Zeiss, Thornwood, NY) equipped with a Zeiss Axiocam zt 400X magnification. A total of 10 high-resolution images were captured of each slide. Care was taken not to overlap the images. The slides were then stored as multidimensional ZVI files for analysis.

Image analysis

The high-resolution images were analyzed using the automated measurement feature of Carl Zeiss' Axiovision software. The area (μm^2) stained red (type I collagen) and the area stained green (type III collagen) were quantified for each slide. The scaling was adjusted to correlate to the magnification of the digital image, with 0.16 μm/pixel. After the quantity of type I and type III collagen was recorded, the collagen I/III ratio

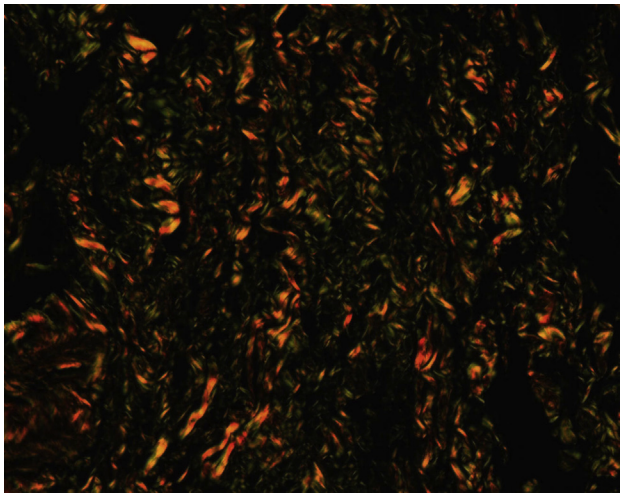

Fig. 1 – Digital images of Sirius red/fast green stained slides viewed through cross-polarized microscopy. Predominately type I collagen (red). (Color version of figure is available online.)