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Changes in the proportions of types I and III collagen in hemorrhoids: the sliding anal lining theory



Carlos Sardiñas^{a,*}, Dilia Diaz Arreaza^b, Héctor Osorio^c

^a Hospital Universitario de Caracas, Unidad de Coloproctología, Laboratorio de Fisiología Anorrectal, Caracas, Venezuela

^b Universidad Central de Venezuela, Instituto Anatomopatológico “Dr. José Antonio O’Daly”, Caracas, Venezuela

^c Instituto Venezolano de Investigaciones Científicas (IVIC), Venezuela

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ABSTRACT

Objective: This study aims to determine changes in the proportions of types I and III collagen in hemorrhoids and to verify the sliding anal canal lining theory.

Patients and method: The study is focused on a sample of 17 patients, 9 females and 8 males (age range: 30–70 years), with grade III and grade IV hemorrhoids. Tissue from 4 fetuses (age: 16 weeks of gestation) was used as control sample. All the participants gave their informed consent. Samples were gathered in 2014. All patients underwent open hemorrhoidectomy by using the technique described by Milligan and Morgan, published in Lancet journal in 1937. The hemorrhoid samples were stained with hematoxylin–eosin for the histologic study to confirm the hemorrhoidal tissue diagnosis. The picosirius red staining protocol was used after the histologic analysis. The method used for image processing is described in the text. Images were imported to the Image Tool for Windows software. The same process was used on the embryonic tissue. Data resulting from the analysis of images were processed using STATISTICA, a software for statistical analysis.

Results: When compared, it was found that the two tissues presented very different values, with hemorrhoids containing the highest type III collagen values.

Conclusion: Our results seem to imply that hemorrhoids have a larger proportion of type III collagen than fetal tissue. They also suggest a possible age-related deterioration of the tissue.

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* Corresponding author.

E-mails: carloseduardosardinas@gmail.com, carlosardinas@yahoo.es (C. Sardiñas).

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Mudanças nos percentuais do colágeno dos tipos I e III em hemorroidas: teoria do revestimento anal deslizante

R E S U M O

Palavras-chave:

Hemorroidas

Anal

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Colágeno

Tipo I

Tipo III

Feto

Deterioração

Objetivo: Esse estudo tem por objetivo determinar mudanças nos percentuais do colágeno dos tipos I e III em hemorroidas e verificar a teoria do revestimento de canal anal deslizante.

Pacientes e método: O estudo está focado em uma amostra de 17 pacientes (9 mulheres e 8 homes; faixa etária: 30-70 anos), com hemorroidas de graus III e IV. Utilizamos tecido de quatro fetos (idade: 16 semanas de gestação) como amostra de controle. Todos os participantes deram consentimento informado. As amostras foram reunidas em 2014. Todos os pacientes passaram por uma hemorroidectomia aberta; para tanto, foi empregada a técnica descrita por Milligan e Morgan, publicada no periódico *Lancet* em 1937. As amostras de hemorroida foram coradas com hematoxilina-eosina com vistas ao estudo histológico para confirmação do diagnóstico de tecido hemorroidal. Após a análise histológica, o material foi corado com o protocolo de *picrosirius red*. O método empregado para o processamento das imagens está descrito no texto. As imagens foram importadas pelo software Image Tool for Windows. O mesmo processo foi empregado no tecido embrionário. Os dados resultantes da análise das imagens foram processados com o programa STATISTICA, um software para análise estatística.

Resultados: Por comparação, constatamos que os dois tecidos apresentavam valores muito diferentes, e as hemorroidas continham os mais altos valores de colágeno do tipo III.

Conclusão: Nossos resultados parecem implicar que hemorroidas possuem um percentual mais elevado de colágeno do tipo III *versus* tecido fetal. Os resultados também sugerem uma possível deterioração do tecido, relacionada à idade.

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Introduction

In 1950 Gass and Adams revealed that hemorrhoids resulted from degeneration of supportive tissue in the anal canal after observing connective tissue fragmentation in hemorrhoids specimens, and believed that their protrusion was related to a lax anus.¹ Later, Hughes (1957) and Patey (1972) supported that idea because it was in keeping with the presence of the hemorrhoid descent.^{2,3}

The presence of an important layer of smooth muscle tissue in the anal submucosa is relevant. It was first described in 1853 by Treitz, who noticed that a part of it arises from the internal sphincter, and the other from the conjoint longitudinal muscle, known today as Treitz's muscle. It is responsible for hemorrhoids retraction and elevation during defecation, and for the return of these structures to their normal position, together with the connective tissue.⁴

One of the arguments that have been debated the most is how the deterioration of connective tissue that supports hemorrhoids facilitates its prolapse, as proposed by Thomson in 1975.⁵ A research by Haas in 1984 showed the deterioration of the connective tissue and, consequently, the sliding of the anal structure in patients with hemorrhoids, a process which increases gradually with aging.⁶ Other factors that produce alterations in the elimination habits and traumas that cause such symptoms must be added to that. Hemorrhoids under constant local stress produced by the patient's effort would eventually lead to rupture of the Treitz's muscle and prolapse of the hemorrhoidal bundles.

In 1988 Morgado studied the microscopic anatomy of the anal canal in a group of fetuses with an average age of 32 weeks of intrauterine life.⁷ He found that the muscular tissue was clustered in either grooved or smooth bundles with collagen fiber agrupations of homogeneous, regular and non-fragmented appearance. This confirmed that hemorrhoids were connected to the rest of the anal canal wall by a thick, homogeneous and well-defended bundle of fibers that are not fragmented, producing firm adherence between them and the wall that surrounds them. This allows to establish that human fetuses are the comparative pattern for the evaluation of possible deterioration of muscular structure and collagen in adult patients with hemorrhoid problems.

However, in 2009 Willis presents a study where he compares quantity to quality of collagen among adult patients with or without hemorrhoid prolapse, finding no correlation with age or sex, as well as with endogenous or exogenous causes for alterations of collagen concentrations.⁸

This study aims to determine changes in the proportions of types I and III collagen in patients with hemorrhoids using human fetuses as comparison group.

Materials and methods

Patients and methods

The study is focused on a sample of 17 patients (9 females (♀) and 8 males (♂), age range: 30-70 years) diagnosed with grade III and grade IV hemorrhoids. Tissue from 4 fetuses (age range:

16 weeks of gestation) was used as control sample. All the participants gave their informed consent. Samples were gathered in 2014. All patients underwent open hemorrhoidectomy by using the technique described by Milligan and Morgan, published in Lancet journal in 1937.⁹

Histological study and picosirius red staining

The hemorrhoid samples were fixed in 10% formalin for 24 h, then processed and embedded in paraffin using standard histological techniques. 5-Micron thick sections were taken and placed on glass plates. The cuts were stained with hematoxylin-eosin to do a histological study using an Olympus CX31 microscope and to confirm the hemorrhoidal tissue diagnosis (Fig. 1).

After the histological analysis, a picosirius red staining protocol was performed. The selected cuts were deparaffinized and hydrated by immersion in xylene (twice), descending graded alcohol solutions (100%, 95%, 70%, 50%), and then washed with distilled water 4 times. Next, the cuts were stained with picosirius red and left to rest for 1 h. The excess stain was removed with two rinses of distilled water and then they were dehydrated, first with ethanol at 100% (3 changes) and then with a xylene treatment.

The picosirius red stained cuts were examined with an Olympus CX31 microscope (using a polarized light analyzer). The observation of stained tissues with this method allows differentiating type I and type III collagen (Figs. 2 and 3). Bearing this in mind, many photographs of every cut were taken with an 8.0 megapixel HP Photosmart R927 camera.

The method used for image processing is described in Fig. 4. Images were imported to the *Image Tool for Windows* software.¹⁰ Then images were turned into gray scale figures. Objects of interest were chosen depending on their brightness in the gray scale. After that, the pixels of the resulting binary images were counted. The derived values represent the assessment on proportionality between collagen type 1 and type 3 (CIII/CI). Data resulting from the analysis of images were processed using STATISTICA, software for statistical analysis.¹¹

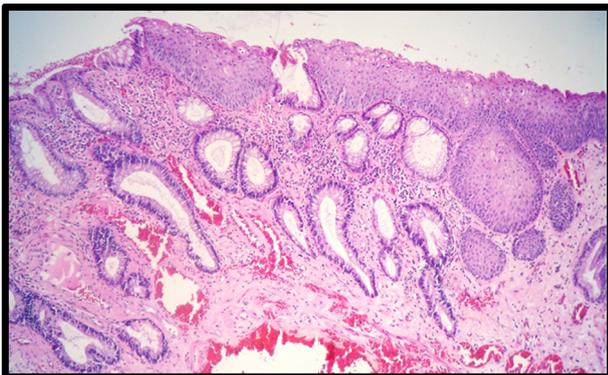


Fig. 1 – Hemorrhoids (hematoxylin-eosin). Stratified flat epithelium of the mucous type corresponding to the anus and the glandular epithelium of the rectum. The submucosa evidences dilated, thin-walled and thick-walled vessels corresponding to venous plexuses in the region.

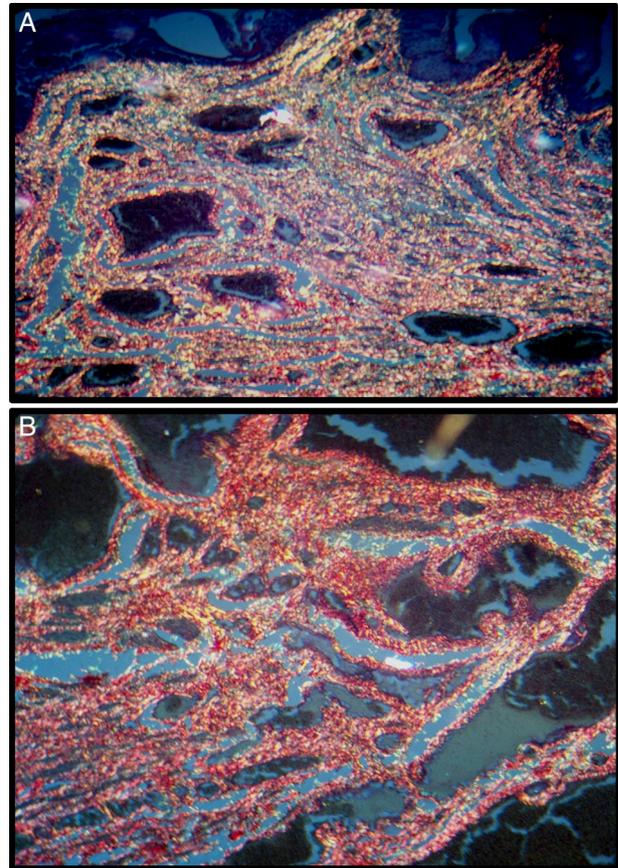


Fig. 2 – (A and B) Hemorrhoids (picosirius red). Stratified flat epithelium of the mucous type corresponding to the anus. The submucosa shows type I and type III collagen fibers stained in red and green with the polarized light, together with venous plexuses in the region.

Results

Fig. 5 shows a comparison between the CIII/CI values for fetal tissue and hemorrhoidal tissue. As it can be observed, when the two tissues are compared, they present very different values, with hemorrhoids containing the highest type III collagen values.

Discussion

Thomson's theory of the vascular cushions (1975) refers to discrete masses in the submucosa of the lower rectum that slide caudally during defecation. These blood-filled cushions work as protectors of the anal canal during defecation. Over time, the support of such structures in the muscular layer of the submucosa, known as the Treitz's muscle, can break and lengthen, producing prolapse, bleeding and other symptoms. Many authors, including Haas, consider that this marks the beginning of hemorrhoid problems, both symptomatic and asymptomatic, beginning the third decade of life. Such deterioration of the support structures at that age is reflected in values of up to 36% for the presence of hemorrhoidal prolapse, as opposed to other age groups.

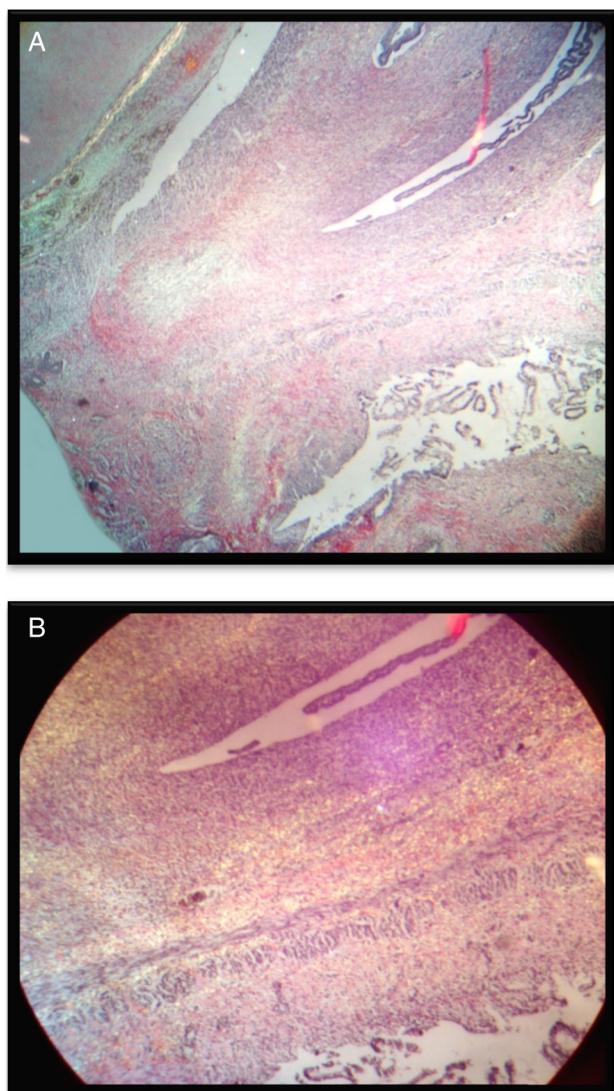


Fig. 3 – Embryo (*picrosirius red*). (A) Immature pubic bone is observed at a lower increase, followed by genital routes fibrous septum and rectum with venous plexuses in the region. (B) Fibers of the septum and rectum with collagen fibers are observed at a higher increase, which in the dark field seem to be yellow.

The vascular cushions of the submucosa are generally supported by the pectinate line and by the muscular layer of the submucosa. During defecation, the internal sphincter is relaxed and there is an eversion of the vascular tissue and the pectinate line. This eversion is produced at the anorectal union, while probably a disruption of this natural eversion and the lower rectum return is the fundamental mechanism for the production of hemorrhoids, as stated by Gass and Adams in 1950, when they considered that hemorrhoids resulted from degeneration of supportive tissue in the anal canal. This is known as the sliding anal lining theory. The factors that disrupt the normal eversion and return can be related to endocrine disruptions, age and constipation. As for constipation, no data are available so far as to consider the frequency

and time spent in the evacuation of fecal matter as a cause of hemorrhoidal disease.

Prolapse through the anus is considered a hemorrhoid from a folkloric point of view. The symptom of protrusion with spontaneous reduction, or through digital control of the masses inside the anal canal is one of the most frequent characteristics of the hemorrhoidal disease. Many times this signal tends to be confused with a hemorrhoidal thrombosis, or perianal folds are interpreted as prolapsed irreducible hemorrhoids. Hypertrophic papillae or polyps of the lower third of the rectum are rarely confused with hemorrhoidal prolapse because they can prolapse through the anus, and because they can be reduced. Data on the natural history of untreated hemorrhoidal disease are scarce. Therefore, there is no information available on the proportion of patients who, at some point, experience hemorrhage, prolapse, pain or itching, and those who present complications. It is also unknown how these complications are developed. In patients seeking consultation due to complications, prolapse accounted for 77%, thrombosis 45%, and bleeding 27% (Morgado. 1988).

Irregular elimination habits have been associated with hard and bulky stools that would demand a significant effort. This would mean pushing the vascular cushions out of the anal canal, producing an increase in the stress and congestion of the tissues during evacuation, and leading to much more intense sliding. If stretched and submitted repeatedly to such forces, the Treitz's muscle would suffer an imbalance that would produce imminent or permanent prolapse. This evidence allows to state that the vascular cushions prolapse is simply the result of the anal canal lining sliding downward, which suggests that the theory proposed by Thomson in 1975 is probably correct.

An interesting element introduced by Haas in 1984 is that the vascular cushions are formed during embryonic life and contribute to the anal canal closure mechanism. With this in mind it can be stated that the human embryo is the best comparison subject for studies like this, since it has been proved by Thomson and Haas that vascular cushions are anchored to the anal canal by collagen fibers of the connective tissue. Such fibers are dense, strong and undamaged in embryos, but weak, disrupted and broken in adults, as shown by Morgado in his 1988 comparative study between embryos and adults. The same process takes place in other parts of the human body due to aging. Therefore, it is necessary to add the theory of aging proposed by Strehler in 1963 and Bornstein in 1976 to what was said above. It would help explain the deterioration of the anchor as the disruption experienced by collagen fibers with age, which leads to an alteration of their functions, added to alterations in the collagen synthesis as a result of the individual's aging.

In 2009, Willis found that the quality of the connective tissue is determined mainly by the relation between the synthesizing proportion and the amount of type I and type III collagens that is deposited. A contrasting element between both collagens is that type I mature collagen forms dense bundles of fibers in the connective tissue and is responsible for its traction force; instead, type III collagens present thin fibers and they remain immature, being the type that dominates in the early stages of the healing process. When the proportions of type I and type III collagens are modified, collagen fibers

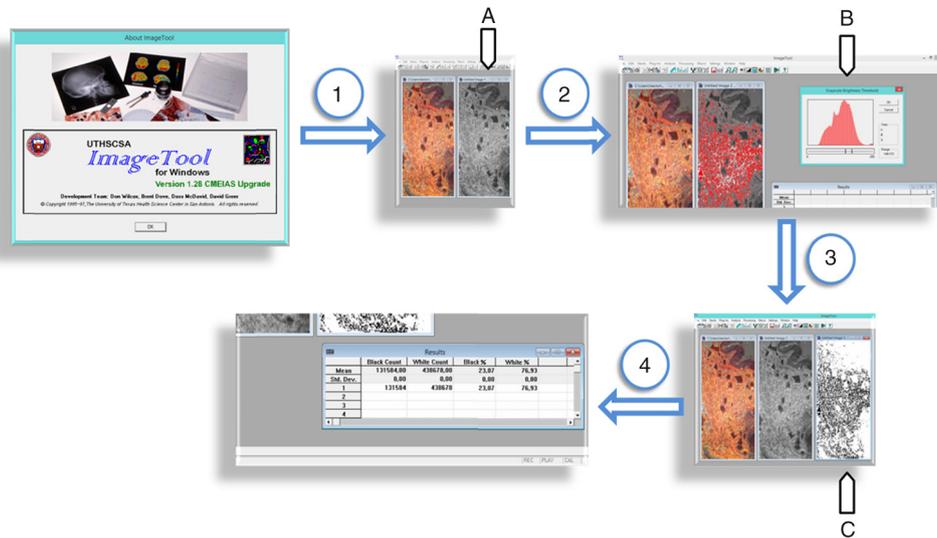


Fig. 4 – Image processing. Step 1. Images were imported to the *Image Tool for Windows* software, where images (16, 24 or 32 bpp) were modified to 8 bpp gray scales using the processing options (A). Step 2. Applying the “Threshold” setting functions (B), the key elements were chosen on the image after modifying the wide range of the value spectrum presented by the program. As a consequence, the elements chosen on the image are automatically marked in red. Step 3. After pressing the OK button, the program created a black and white copy of the image (C); in it, the black color corresponded to the chosen elements on the previous image. Step 4. Using the “black and white pixel count” tool, which will allow to count the amount of black and white pixels on the binary image. The results were shown in black and white pixel numbers and percentages, therefore quantifying the chosen structures.

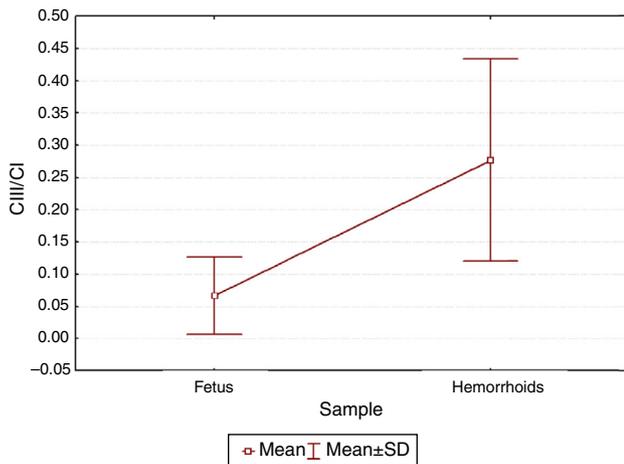


Fig. 5 – Range chart with standard deviation for CIII/CI index vs. fetus and hemorrhoids samples. Whiskers, standard deviation; marker: arithmetic mean.

subjects for the relation between type I and type III collagens, following research by Strehler in 1963, Bornstein in 1976 and Morgado in 1988.

Fig. 5 shows a comparison between the CIII/CI values for fetal tissue and hemorrhoidal tissue. When compared, it was found that hemorrhoids contained the highest type III collagen values. This seems to indicate that hemorrhoids have a larger proportion of type III collagen than fetal tissue. Taking this into account, it could be hypothesized that these changes in the collagen proportions could be associated to an age-related deterioration of tissue and/or to the process of tissue repair that is linked to the damage inflicted on the collagen fibers that anchor the vascular cushions of people with irregular evacuation habits due to hard stool. Therefore, we believe these findings could offer greater support to the research done in this subject.

Conflicts of interest

The authors declare no conflicts of interest.

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experience changes in their geometric arrangement and the fiber diameter, which causes their loss of motion and reduces the mechanical stability of the connective tissue, as proposed by Wiedemann in 1975 and Fleischmajer in 1990. Similarly, Willis shows in his research a disruption in the collagen metabolism in patients with hemorrhoidal prolapses and states the hypothesis of stability reduction as a key factor in the incidence of hemorrhoidal prolapse.

This allowed to develop a study that takes into consideration some differences, with embryos as the comparison

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