Original article

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Haemorrhoids – a collagen disease?

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Abstract

Objective The cause of haemorrhoidal disease is unknown, epidemiological data and histopathological findings support the hypothesis that reduced connective tissue stability is associated with the incidence of haemorrhoids. Therefore the aim of this study was to analyse the quantity and quality of collagen formation in the corpus cavernosum recti in patients with III°/IV° haemorrhoids in comparison with persons without haemorrhoids.

Method Haemorrhoidectomy specimens of 31 patients with III°/IV° haemorrhoids were examined. The specimens of 20 persons who died a natural death and who had no haemorrhoidal disease served as the controls. The amount of collagen was estimated photometrically by calculating the collagen/protein ratio. The collagen I/III ratio served as parameter for the quality of collagen formation and was calculated using cross polarization spectroscopy.

Results Patients with haemorrhoids had a significantly reduced collagen/protein ratio (42.2 ± 16.2 μg/mg *vs* 72.5 ± 31.0 μg/mg; $P = 0.02$) and a significantly reduced collagen I/III ratio (2.0 ± 0.1 *vs* 4.6 ± 0.3; $P < 0.001$) compared with persons without haemorrhoidal disease. There was no correlation with patients' age or gender.

Conclusions There is a fundamental disorder of collagen metabolism in patients with haemorrhoidal disease. It remains unclear whether this is due to exogenous or endogenous influences.

Keywords Haemorrhoids, collagen, extracellular matrix

Introduction

Haemorrhoidal disease is the consequence of distal displacement of the anal cushions. Haemorrhoids are common; their prevalence was estimated between 4.4% [1] and 86% [2]. Stelzner described them as a complex compound of arteriovenous links in the rectal mucosa with an interlace of unstriated muscles, elastic fibres and connective tissue, calling it the 'corpus cavernosum recti' [3]. This cavernous vascular padding is an important component of the continence mechanisms ensuring gas-tight seal. Although the aetiology of haemorrhoidal disease is widely unknown; constipation and abnormal bowel habit are commonly blamed despite largely contrary evidence [4]. The most consistently demonstrated physiological abnormality is an increased maximum resting anal pressure. This led to the hypothesis that haemorrhoids are a hyperplasia of the corpus cavernosum recti due to the strong tonic contraction of the internal anal sphincter [5]. Some evidence, however, points to this being a secondary phenomenon rather than the cause of haemorrhoidal disease itself [6].

According to Thomson's studies, haemorrhoids are the consequence of a disintegration of muscular and elastic components, leading to a distal shift of the vascular padding [7]. Anatomic studies by Lierse showed that the dilated haemorrhoidal vessels and the surrounding muscle fibres lie within a lattice of collagen and elastic fibres, which are arranged in longitudinal direction. Degradation processes of this extracellular matrix (ECM), especially of the protein elastin during ageing were thought to be a decisive pathway in the development of haemorrhoidal disease [8]. Elastic fibres in haemorrhoids are always linked to collagen fibres. While elastic fibres are responsible for the elasticity of the tissue, the collagen fibres are responsible for its tensile strength. They provide elastic fibres from overexpansion and sustain the original tissue configuration. Accordingly collagen metabolism might also be an import factor in the aetiology of haemorrhoidal disease. Therefore the aim of the present study was to analyse the quantity and quality of collagen formation in the corpus cavernosum recti in

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patients with III°/IV° haemorrhoids in comparison with persons without haemorrhoids serving as the controls.

Method

Patients

All patients having been operated on III° or IV° haemorrhoids consecutively during the last 3 years were evaluated. A Milligan–Morgan haemorrhoidectomy was performed in all of them. Patients who had undergone Longo procedures, malignancies, hernias, aortic aneurysms, varicosis or other connective tissue diseases were excluded from the study as well as patients on medical treatment with corticosteroids or cytostatic substances. Therefore only 31 patients could be included. The resected haemorrhoidal specimens had been fixed in 10% formalin immediately after the operation and had then been embedded in paraffin for further investigation.

Persons having donated their body to the Institute of Anatomy of the RWTH Aachen and who had died of a natural death served as the control group. They were examined immediately after arrival in the institute and the accompanying medical chart was reviewed. A total of 20 consecutive persons without haemorrhoidal disease and without any of the above mentioned exclusion criteria were selected. The anal and deep rectal skin and submucosa were resected in analogy to the Milligan–Morgan procedure. The resected specimens were fixed in 10% formalin and then embedded in paraffin, too. Haematoxylin/Eosin staining was performed in all specimens to ensure that representative haemorrhoidal tissue was included for further examinations.

The median age in the control group was 76 (46–90) years. This was significantly more than in the patients' collective with a median age of 50 (28–83) years. Therefore this group was subdivided in patients under 60 years (n = 19; 38 (28–57) years) and those with 60 or more years (n = 12; 66 (60–83) years). There was no significant difference in gender (control m/f 10/10; haemorrhoids m/f 17/14).

Collagen/protein ratio

The relative amount of collagen was estimated by the collagen/protein ratio. Fifteen-micrometre-thick specimens of the paraffin embedded tissue samples were obtained from each group and placed in test tubes. After deparaffination, the slices were stained with Sirius red and Fast green (Polysciences, Warrington, Pennsylvania, USA). The specimens were rinsed several times with distilled water until the supernatant was colourless. Subsequently, the dyes were eluted from the sections by incubation with 0.1 N NaOH in absolute methanol. The fluid was read immediately in a spectrophotometer at the wave-lengths corresponding to the maximal absorbance of Sirius red (535 nm) and Fast green (605 nm). Results are expressed as the ratio of collagen (μg) to noncollagenous protein (mg) [9].

Collagen type I/III ratio

The quality of collagen was evaluated by the collagen type I/III ratio using cross polarization microscopy. Five micrometre sections were stained for 1 h in Picrosirius solution (0.1% solution of Sirius Red F3BA in saturated aqueous picric acid, pH 2) according to Junqueira [10]. The sections were washed for 2 min in 0.01 N HCl, dehydrated, cleared and mounted in synthetic resin. Thicker collagen type I fibres were stained in red-orange shades, whereas thinner collagen type III appeared as pale-green shades. For each sample, 10 regions within the interface (400×, area 50 × 50 μm) were captured by a digital camera (Olympus C-3030, Hamburg, Germany). Collagen I/III ratios were obtained by analysis of the amount of collagen type I and III using a digital image analysing software (Image-Pro Plus, Media Cybernetics, Silver Spring, Maryland, USA). Results are expressed as ratio of area of collagen type I and III (Figs 1, 2).

Statistics

After acceptance of normal distribution by the Kolmogorov–Smirnov-test all results were reported as means ± standard deviation (SD). Differences between both groups were evaluated using the unpaired Wilcoxon-rank sum test. For comparison of subgroups, analysis of variance was performed and *post hoc* comparison was performed using the Bonferroni multiple comparison test. Correlations

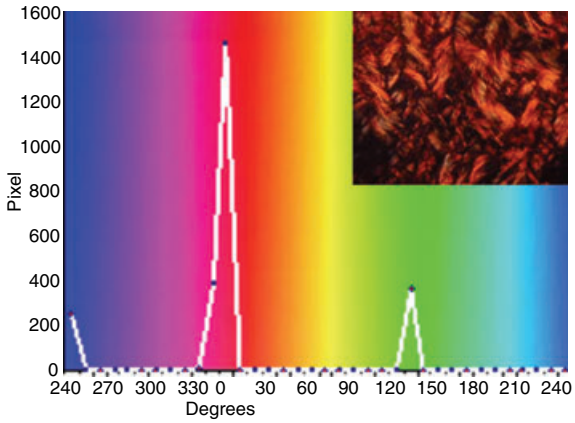

Figure 1 Sirius red – staining of collagen I (red) and collagen III (green) in a healthy control person.

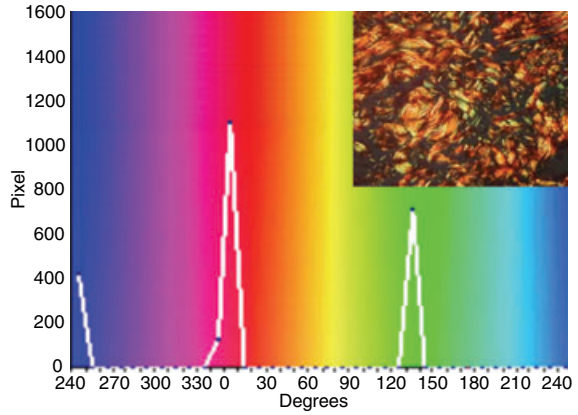

Figure 2 Sirius red – staining of collagen I (red) and collagen III (green) in a patient with III° haemorrhoids.

between age and the collagen/protein and the Collagen type I/III ratio were calculated using the Pearson correlation coefficient *r*. A *P* value of <0.05 was considered to indicate statistical significance.

Results

Collagen/protein ratio

Mean collagen/protein ratio was significantly higher in the control group (72.5 ± 31.0 μg/mg) than in patients with haemorrhoidal disease (42.2 ± 16.2 μg/mg; $P = 0.002$) (Fig. 3). There was no correlation with age in the patient group ($r = 0.09$) and in the control group ($r = -0.03$). The mean collagen/protein ratio in patients older than 60 years was 46.5 ± 17.3 μg/mg and not significantly different from patients under 60 years (39.5 ± 16.9 μg/mg; $P = 0.27$). There was no significant difference in the mean collagen/protein ratio between men and women (43.3 ± 15.7 μg/mg *vs* 41.5 ± 16.9 μg/mg; $P = 0.32$).

Collagen type I/III ratio

The mean collagen type I/III ratio was significantly higher in the control group (4.6 ± 0.3) than that in the patients with haemorrhoidal disease (2.0 ± 0.1; $P < 0.001$) (Fig. 4). There was no correlation with age in the patient group ($r = 0.02$) and in the control group ($r = -0.05$). The mean collagen type I/III ratio in patients older than 60 years was 2.0 ± 0.1 and not significantly different from patients under 60 years (1.9 ± 0.1; $P = 0.47$). There was no significant difference in the mean collagen type I/III ratio between men and women (2.0 ± 0.1 *vs* 2.0 ± 0.1; $P = 0.93$).

Discussion

Despite the proposal of three mechanisms that might underlie haemorrhoidal development – the varicose vein theory [11], the vascular hyperplasia theory [3] and the sliding anal-lining theory [7] – , none of these theories could explain the aetiology of haemorrhoids sufficiently [4]. Only few articles have addressed the changes of the ECM in haemorrhoidal disease. ECM is the defining feature of all connective tissue. Its main components are various glycoproteins. In humans and most animals, the most abundant glycoprotein in the ECM is collagen. Collagen consists of long, stiff, triple-stranded helical structures, which form by the association of three collagen polypeptide (α) chains into a superhelix. Up to now 34 different α chains have been characterized and 19 different combinations have been identified in the family of collagens [12]. The main collagen types found in connective tissues are the fibrillar types I, II, III, V and

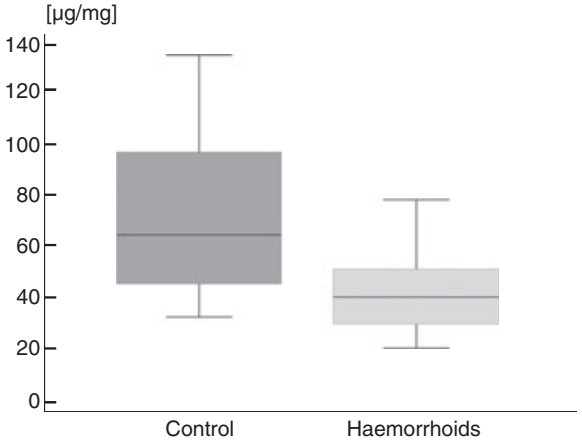

Figure 3 Collagen/protein ratio in patients with haemorrhoidal disease ($n = 31$) and healthy control persons ($n = 20$, $P = 0.002$).

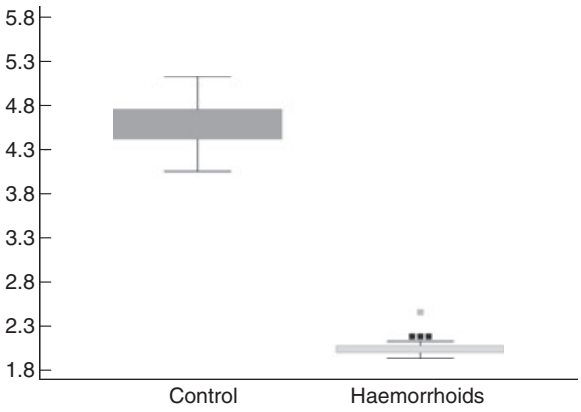

Figure 4 Collagen I/III ratio in patients with haemorrhoidal disease ($n = 31$) and healthy control persons ($n = 20$, $P < 0.001$).

XI, with collagen type I being the abundant in humans. Connective tissue quality is mainly determined by the amount and ratio of synthesized and deposited collagens type I and III. In particular, mature type I collagen, found in dense bundles in connective tissues, is responsible for tensile strength. By contrast, type III collagen fibres are thinner in diameter and are regarded as the immature collagen predominantly found in early wound healing. A reduced ratio of type I to III collagen is known to change the geometrical arrangement and diameter of collagen fibrils and to decrease the amount of cross-linking, with reduced mechanical stability of connective tissue [13,14].

Extracellular matrix in the anal submucosa varies with age. Smooth muscle predominates in the newborn, which is gradually replaced by fibrous tissue during ageing. The subepithelial space becomes thicker and more disorganized with time [15], indicating that the degradation of elastin possibly is not the only pathway which is responsible for the increase in haemorrhoidal prevalence with age [1]. Comparing autopsy specimens of the anal canal of foetuses and adults, Morgado *et al.* [16] described a higher number of collagen fibres with respect to muscle fibres in submucosal vessels and a less homogenous appearance of the collagen fibres in adults. Our data clearly demonstrate a reduced amount of collagen in haemorrhoidal disease. Furthermore, there is a decrease in the ratio of type I to III collagen, thus indicating a reduced mechanical stability of the perivascular tissue and of the anchoring connective tissue system. We therefore hypothesize that the collagen metabolism might also play an important role in the aetiology of haemorrhoidal disease. The physiological degradation of elastin during ageing in combination with a reduced tensile strength of the collagenous components of the ECM might lead to a loss of elasticity, a relative outflow obstruction and a pressure increase in the arteriovenous glomerula with concomitant vascular dilatation. With progression of the disease the collagenous attachment of the vascular padding tears and gives way for the corpus cavernosum recti to descend below the dentate line [4]. Therefore the present results are not in contradiction with Thomson's or Stelzner's hypothesis of haemorrhoidal development. Even the recent hypothesis of Aigner *et al.*, [17] supposing that intrinsic vascular sphincter mechanisms regulate filling and drainage of the anorectal vascular plexus and therefore contribute to haemorrhoidal disease, is not in contradiction to our results.

The demonstrated differences in this study do not depend on age, however, this could be due to the relative small numbers in both groups. With respect to the data of Morgado *et al.* [16], there might be a physiological replacement of smooth muscle fibres by collagen. However, this does not explain the significant differences in collagen quality and therefore other factors than physiological degradation alone must influence the amount and quality of collagen. Epidemiological data showed that haemorrhoidal disease is often associated with herniae and genitourinary prolapse, which might be linked by individual connective tissue abnormalities [6,18]. To exclude any influences by concomitant diseases, patients with herniae or other connective tissue disorders have been excluded from this study. Therefore the decrease of collagen amount and quality can be contributed to the haemorrhoidal disease alone. As a correlation of congenital collagen disorders with the development of herniae has been reported [19], a genetic predisposition of haemorrhoidal disease has also been discussed [4]. However, connective tissue must be regarded as a highly dynamic and complex system characterized by permanent cell turnover and remodelling of the ECM. The modulation of this network is influenced by numerous matrix molecules, e.g. MMPs, TIMPs, proteoglycans, hormones and cytokines. A tightly regulated balance of both collagen synthesis and degradation is the fundamental component determinating connective tissue quality. Whether the changes of collagen formation in haemorrhoids are caused by metabolic influences or whether a genetic background underlies this pathology remains unclear. Further research on collagen metabolism, on transcriptional control and on analysis of possible sequence polymorphisms in collagen genes has to be performed. The aetiology of haemorrhoidal disease seems to be multifactorial and it has to be kept in mind that social, cultural and psychological elements might also be as important as physiological factors.

Conclusion

Epidemiological data support the hypothesis that reduced connective tissue stability is associated with the incidence of haemorrhoids. This study is the first to demonstrate a fundamental disorder of collagen metabolism in patients with haemorrhoidal disease. It remains unclear whether this is due to metabolic or genetic influences.

References

1 Johanson JF, Sonnenberg A. The prevalence of hemorrhoids and chronic constipation. An epidemiologic study. *Gastroenterology* 1990; **98:** 380–6.
2 Haas PA, Haas GP, Schmaltz S, Fox TA Jr. The prevalence of hemorrhoids. *Dis Colon Rectum* 1983; **26:** 435–9.

3 Stelzner F, Staubesand J. Das Corpus cavernosum recti – morphologische Grundlage der inneren Hämorrhoiden. *Langenbecks Arch Chir* 1962; **229:** 302–12.
4 Bruch HP, Roblick UJ. Pathophysiology of hemorrhoids. *Chirurg* 2001; **72:** 656–9.
5 Stelzner F (1998) *Chirurgie an den Visceralen Abschlusssystemen*. Thieme, Stuttgart.
6 Loder PB, Kamm MA, Nicholls RJ, Phillips RK. Haemorrhoids: pathology, pathophysiology and aetiology. *Br J Surg* 1994; **81:** 946–54.
7 Thomson WH. The nature of haemorrhoids. *Br J Surg* 1975; **62:** 542–52.
8 Lierse W. Anatomie und Pathophysiologie des Haemorrhoidalleidens. *Langenbecks Arch Chir* 1989; (Suppl. II): 769–72.
9 Lopez-De Leon A, Rojkind M. A simple micromethod for collagen and total protein determination in formalin-fixed paraffin-embedded sections. *J Histochem Cytochem* 1985; **33:** 737–43.
10 Junqueira LC, Cossermelli W, Brentani R. Differential staining of collagens type I, II and III by Sirius Red and polarization microscopy. *Arch Histol Jpn* 1978; **41:** 267–74.
11 Burkitt DP. Varicose veins, deep vein thrombosis, and haemorrhoids: epidemiology and suggested aetiology. *Br Med J* 1972; **2:** 556–61.
12 Aumailley M, Gayraud B. Structure and biological activity of the extracellular matrix. *J Mol Med* 1998; **76:** 253–65.
13 Wiedemann H, Chung E, Fujii T, Miller EJ, Kuhn K. Comparative electron-microscope studies on type-III and type-I collagens. *Eur J Biochem* 1975; **51:** 363–8.
14 Fleischmajer R, Perlish JS, Burgeson RE, Shaikh-Bahai F, Timpl R. Type I and type III collagen interactions during fibrillogenesis. *Ann N Y Acad Sci* 1990; **580:** 161–75.
15 Haas PA, Fox TA Jr. Age-related changes and scar formations of perianal connective tissue. *Dis Colon Rectum* 1980; **23:** 160–9.
16 Morgado PJ, Suarez JA, Gomez LG, Morgado PJ Jr. Histoclinical basis for a new classification of hemorrhoidal disease. *Dis Colon Rectum* 1988; **31:** 474–80.
17 Aigner F, Gruber H, Conrad F *et al.* Revised morphology and hemodynamics of the anorectal vascular plexus: impact on the course of hemorrhoidal disease. *Int J Colorectal Dis* 2009; **24:** 105–13.
18 Heslop JH. Piles and rectoceles. *Aust N Z J Surg* 1987; **57:** 935–8.
19 Rosch R, Junge K, Lynen P, Mertens P, Klinge U, Schumpelick V. Hernia – a collagen disease. *Eur Surg* 2003; **35:** 11–5.