

The role of collagen type I/III ratio in the etiology of sacrococcygeal pilonidal

El papel de la proporción de colágeno tipo I/III en la etiología de la enfermedad del seno pilonidal sacrococcígeosinus disease

Arif Atay^{1*}, Ilknur Kucukosmanoglu², Hande Köksal³, Kemal Arslan³, and Osman Dogru³

¹Department of General Surgery, Izmir Katip Celebi University Atatürk Training and Research Hospital, Izmir; ²Department of Pathology, Saglik Bilimleri University, Faculty of Medicine, Konya Education and Research Hospital, Konya; ³Department of General Surgery, Saglik Bilimleri University, Faculty of Medicine, Konya Education and Research Hospital, Konya, Turkey

Abstract

Objective: The purpose of this study is to investigate whether there was a difference between the midline skin and the healthy skin in the lateral by means of total amount of collagen and Type I/III ratio which was the indicator of the collagen structure.

Material and methods: Fifty patients with pilonidal sinus disease were enrolled. Samples were prepared from the midline skin of the sinus where the holes were located and lateral skin of the resected material. **Results:** It was determined that the lateral line had significantly more collagen intensity and a higher collagen Type I/III ratio ($p < 0.001$). **Conclusions:** One of the reasons why hair mostly pricks into the midline in the intergluteal sulcus in pilonidal sinus disease is the fact that the amount of total collagen and collagen Type I/III ratio of the midline are lower than those of the lateral tissue. Complications are more common in cases with low Type I/III ratio and low total collagen rates.

Keywords: Pilonidal sinus. Etiopathogenesis. Total collagen. Collagen type 1/3 ratio.

Resumen

Objetivo: El propósito de este estudio es investigar si existía diferencia entre la piel de la línea media y la piel sana en el lateral por medio de la cantidad total de colágeno y la relación Tipo I/III que era el indicador de la estructura del colágeno.

Material y métodos: Se inscribieron 50 pacientes con enfermedad del seno pilonidal. Se prepararon muestras de la piel de la línea media del seno donde se ubicaron los orificios y de la piel lateral del material resecado. **Resultados:** Se determinó que la línea lateral tenía significativamente más intensidad de colágeno y una mayor relación de colágeno Tipo I/III ($p < 0.001$).

Conclusiones: Una de las razones por las que el cabello se pincha principalmente en la línea media en el surco interglúteo en la enfermedad del seno pilonidal es el hecho de que la cantidad de colágeno total y la relación de colágeno tipo I/III de la línea media son menores que las del tejido lateral. Las complicaciones son más comunes en los casos con una proporción baja de Tipo I/III y tasas bajas de colágeno total.

Palabras clave: Seno pilonidal. Etiopatogenia. Colágeno total. Relación 1/3 del tipo de colágeno.

Correspondence:

*Arif Atay

E-mail: atayarif@hotmail.com

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Introduction

Pilonidal sinus disease was first described by Mayo as "sinus containing hair" in 1833¹. Many factors have been blamed for the etiology of sacrococcygeal pilonidal sinus disease and various theories on the subject have been reported. There is no perfect theory that would explain the formation of pilonidal sinus even today². The theory that it is an acquired disorder has now become widely accepted³⁻⁵. According to this theory, researchers have argued that the hair falling off to the sacrococcygeal area prick into the skin and accumulate, causing chronic infection. For the disease to form, hair and skin where hair can prick into are needed. The place where hair generally pricks into in the sacrococcygeal area is the midline. Mechanical reasons may play a role in this pricking but the fact that hair always pricks into the midline suggests that the skin of the midline may be different. Studies on the subject have shown that one of the indicators of skin sturdiness in the collagen Type I/III ratio. Thus, the authors of a study, which was conducted with patients who had undergone surgery because of pilonidal sinus, found that the collagen Type I/III ratio was lower in some recurrent cases⁶.

Wound healing is a response to local trauma which results in the reformation of tissue integrity⁷. Type I collagen is very strong and enables distention. It is more stable and stronger than Type III. As the amount of collagen Type I/III ratio increases, recurrence rates decrease accordingly, especially in hernia cases. When the collagen Type I/III ratios in recurrent hernias were investigated, it was seen that these ratios were significantly low. High recurrence rates were observed in hernia cases with collagen tissue diseases also⁷⁻⁹.

The starting point of our study was the fact that hair frequently pricked into the midline in the sacrococcygeal area. We wanted to explore the possibility that sinus openings were always located in the midline could be explained by Type I/III ratio which was the indicator of the collagen structure and integrity of the skin in this area. Therefore, we aimed to investigate whether there was a difference between the midline skin and the healthy skin at the lateral with regard to the total amount of collagen and collagen Type I/III ratio. Further, we also aimed to investigate whether this amount of collagen in the midline was affected by such characteristics as the patient's gender and weight.

Methods

This study was conducted at Education and Research Hospital's General Surgery Clinic subsequent to the consent obtained from university, Faculty of Medicine, and Committee of Ethics for Clinical Trials (2012/118). The study was planned as a prospective clinical trial for the patients diagnosed with pilonidal sinus disease.

The study covered 50 patients who had presented between June 2011 and August 2011 because of pilonidal sinus disease. Patients with recurrent pilonidal sinus, with known collagen tissue diseases, who had received steroid treatment within the last year, accompanying malignant diseases, who had undergone surgery in the perianal area because of other reasons, older than 40 years of age, and those who had not want to participate in the study were excluded from the study. Thus, demographic data, BMI, and information on sinuses for a total of 50 patients were recorded.

All of the patients had undergone Limberg flap surgery. Samples 0.5×1 cm in size from the midline skin of the resection material containing the sinus openings and samples covering the skin and the subcutaneous tissue from the most lateral of the same resected material were placed in 10% formaldehyde. Following the fixation of 10% formaldehyde for 24 h, the samples were treated routinely pathological tissue processing procedure by a Thermo Scientific Shandon Excelsior ES tissue processor and embedded into paraffin blocks. A 5-6 micrometer-thick cross-sections were then respectively stained by H and E, Masson's Trichrome, and silvering methods with a separate staining of Type I and Type III collagen fibers.

Sinus tracts, foreign body type chronic inflammatory granulation tissues, and collagen fiber bundles were shown through hematoxylin-eosin staining. It was seen that the healthy tissue, samples from lateral part of resection material, contained much more collagen fibers than the samples from midline which contains lesions. Total (Type I and Type III) collagen fibers were stained in blue by the Masson trichrome staining method. The intensity of total collagen was evaluated for the midline and the lateral separately (Figs. 1A and B). Type III collagen fibers, which were observed in the form of thin black fibers by the silvering method, were identified intensively at midline sections of the specimen than those at the lateral sections (Figs. 1C and D). The collagen fibers stained in color from yellow to brown were identified as Type I collagen fibers and detected in high amounts at sections from the lateral

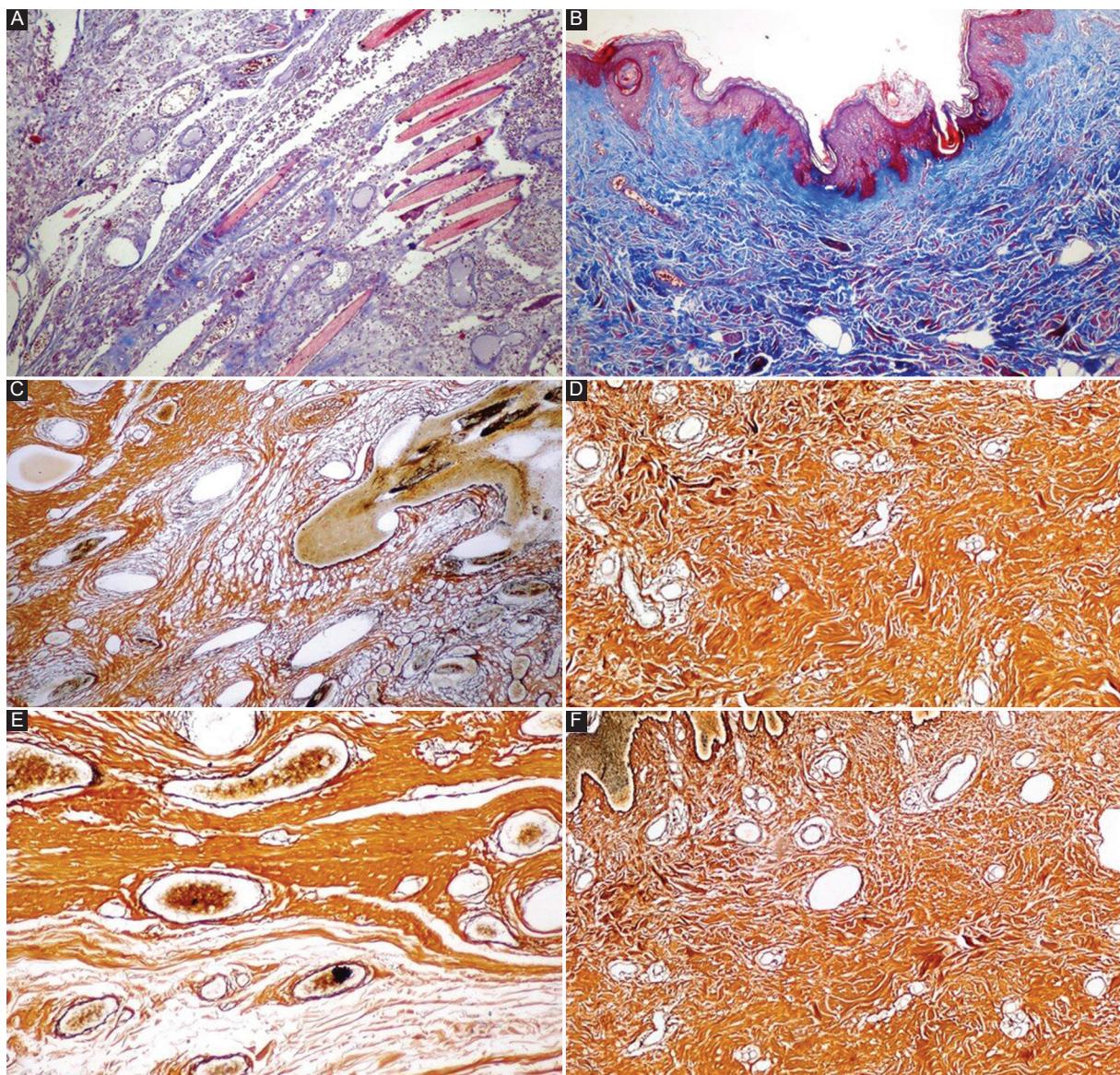


Figure 1. **A:** midline, collagen fiber bundles stained in blue are observed (Masson's Trichrome $\times 200$). **B:** lateral tissue, very dense collagen fiber bundles compared to the center painted in blue (Masson's trichrome $\times 100$). **C:** midline; thin black-stained collagen fibers (Type III) seem more intensive (Silvering $\times 200$). **D:** lateral; rare-thin Type III collagen fibers stained in black around vessels only (Silvering $\times 200$). **E:** midline; Type 1 collagen fibers stained in colors varying from yellow to brown (Silvering $\times 200$). **F:** lateral; intensive and thick bundles of Type 1 collagen fibers stained in yellow to brown in color (Silvering $\times 200$).

part of the specimen than the midline (Figs. 1E and F). The preparations were evaluated and photographed with the light microscope (Olympus BX-51).

The measurements were semi-quantitative and Type I/III ratios were recorded in percentages (%). The intensity of collagen, on the other hand, was measured by labeling values from 1 to 5 (+) based on intensity.

All patients were informed about the study before the operations and their written consents were obtained.

Demographic data of patients were recorded. Those with a body mass index (BMI) of 30 or higher were recorded as obese while those with a BMI of less than 30 were recorded as non-obese. The patients' presenting complaints and duration of symptoms were questioned. The patients' disease were recorded as acute, chronic, and abscessed. Whole body hair development was recorded as mild, medium, and severe. The group with mild hair development refers to those patients with individual and sparse hair

distribution all over their bodies, while medium hair development refers to those with intensive hair development in clusters in normal localization all over their bodies, and severe hair development refers to those with frequent hair development covering their whole bodies starting from the shoulders including the lumbar region. The patients were also recorded as blonde, auburn, or brunette based on their skin color. Whether they had to sit for long hours because of their professions and whether they had a family history or not were questioned and those who had close relatives with the same disease were recorded. Moreover, the patients were asked if they had addictions such as smoking, alcohol, and drugs and recorded. Data on post-operative complications such as hematoma, seroma, infection, and wound dehiscence were also collected. Those patients with distinct purulent content and positive bacterial cultures were regarded as infected. Patients with hematoma or seroma as designated by puncture accompanied or spontaneous fluid content were recorded also. Duration of follow-ups, and recurrence, if any, were also recorded through regular controls. The controls were conducted on the 10th post-operative day, at the end of the 1st month and year. Thereafter, yearly controls were conducted.

Statistical analysis

Initially, the Shapiro-Wilk normality test was conducted and if even a single group among the groups, formed according to the variables, did not enable normality hypothesis, non-parametric testing methods were chosen. The Mann-Whitney U-test was used to compare the variables obtained through measurement in two independent groups, while Chi-square and Fisher's exact test were utilized for the analyses of relationships regarding categorical variables or differences between the groups. The statistical analyses of the study were conducted with SPSS 15.0 (Statistical Package for the Social Sciences, SPSS Inc., Chicago, IL, United States) and the statistical significance limit was set at $p < 0.05$.

Results

The mean age of the patients was found to be 28.26 years (21-39). Male/female ratio was 43/7. The mean BMI was $26.6 \pm 3.5 \text{ kg/m}^2$. The evaluation also revealed that 30% ($n = 15$) of the patients had normal weight (BMI between 18.5 and 24.9 kg/m^2), 48% ($n = 24$) were overweight (BMI between 2 and

529.9 kg/m^2), and 22% ($n = 11$) were obese (BMI between 30 and 39.9 kg/m^2). The other demographic data are given in table 1.

When the samples taken from the skin containing midline openings resected from the sinus material by surgery were compared to the samples taken from the most lateral part, it was determined that the lateral line had significantly more collagen intensity and a higher collagen Type I/III ratio ($p < 0.001$) (Table 2).

The patients were divided into two groups based on their body mass indices. Total collagen intensity in the lateral tissue and midline collagen Type I/III ratios of obese patients were significantly lower than those of non-obese patients (Table 3).

The collagen Type I/III ratios of patients with wound dehiscence were significantly lower. When the patients who had wound infection were evaluated, it was seen that these patients' midline total collagen amounts were significantly lower (Table 4).

Within a 41-43-month follow-up period, four out of 50 of our patients had recurrence. When the midline and lateral tissues of these patients were studied, no statistically significant difference was found either in collagen intensity or in the collagen Type I/III ratio.

Discussion

When we evaluated the cases of patients with pilonidal sinus, we observed that the disease was located in the intergluteal sulcus for the most part and the hair always pricked from the midline. Literature review we conducted for the explanation of this condition revealed no study that explored the differences between skin on the midline where hair pricked and its lateral healthy part.

Our literature review revealed that a study only investigated the effects of collagen Type I/III ratio on wound healing in patients with pilonidal sinus⁶.

Our study proves to be the first of its kind in literature as it compared the amount of total collagen, which is one of the indicators of skin integrity in pilonidal sinus disease, and collagen Type I/III ratio at the midline, where the hair pricked, and the lateral healthy tissue. Moreover, our study is the only study in literature which compared midline and lateral tissues with regard to the amount of total collagen and collagen Type I/III ratio to the etiological characteristics of the disease and the complications of treatment.

According to Zheng et al.¹⁰, physical characteristics of collagen were impaired and the tensile force of the abdominal wall decreased in patients with a low Type I/III

Table 1. Demographic Features of the patients

	n (%)
Mean age ± SD	28.2 ± 5.3
Gender	
Male	43 (86)
Female	7 (14)
Mean BMI ± SD	26.6 ± 3.5
Smoking	
Yes	19 (38)
No	31 (62)
Long duration of sitting	
Yes	33 (66)
No	17 (34)
Skin color	
Blonde	10 (20)
Auburn	17 (34)
Brunette	23 (46)
Body hair	
Mild	10 (20)
Moderate	16 (32)
Severe	24 (48)
Family history	
Yes	8 (16)
No	42 (84)
Severity of disease	
Acute	8 (16)
Chronic	38 (76)
Abscessed	4 (8)
Symptom	
Pain	29 (58)
Discharge	13 (26)
Swelling	8 (16)

SD: standard deviation, BMI: body mass index.

Table 2. Comparison of total collagen amount and Type I/III collagen ratios of midline and lateral tissues

	Mean ± SD	p
Total collagen amount, midline	3.02 ± 0.91	<0.001
Total collagen amount, lateral	4.78 ± 0.41	
Type I/III collagen ratio, midline	1.93 ± 2.65	<0.001
Type I/III collagen ratio, lateral	21.73 ± 15.42	

ratio because of significantly high collagen Type III. It has, therefore, been argued that inguinal and abdominal incisional hernias were seen frequently. The results of our study also revealed that the collagen Type I/III ratio and the amount of total collagen in patients with pilonidal sinus, as in the case with hernias, were significantly lower than those of the lateral tissue.

Hesp et al.¹¹ showed that abnormal collagen metabolism could be inversely effect the healing of colonic anastomoses. In their experimental study, the authors demonstrated that the integrity of anastomoses could be related to collagen loss by measuring

Table 3. The relation between obesity and total collagen amounts and ratios

	BMI < 30 kg/m ²	BMI ≥ 30 kg/m ²	p
Total collagen, (mean ± SD)			
Midline	3.07 ± 0.92	2.81 ± 0.87	NS
Lateral	4.84 ± 0.36	4.54 ± 0.52	0.03
Collagen Type I/III ratio, (mean ± SD)			
Midline	2.06 ± 2.64	4.54 ± 0.52	0.03
Lateral	23.01 ± 16.22	17.18 ± 11.67	NS

NS: not significant.

bursting pressure and hydroxyproline levels following colonic anastomosis in rabbits. In another study, researchers found that abdominal aortic aneurysm was seen more frequently in patients with high amounts of collagen Type III on the aortic wall¹².

Various studies have also shown that collagen Type I/III ratio was the underlying reason for the failure to achieve healthy wound healing in such tissues as the fascia, skin, and colon^{10,11}.

Binnebosel et al.⁶ reported in their study that patients with wound healing complications or delays in wound healing had a significantly lower collagen Type I/III ratio in comparison to patients with favorable wound healing. The impairment in collagen metabolism could be an indicator of the delay in wound healing in some patients. The results of our study also revealed that patients with problems like wound dehiscence during wound healing had significantly low collagen Type I/III ratios. There are studies which reported that recurrence was seen more frequently in such patients as well¹³.

When we reviewed the studies showing the relationship between obesity and pilonidal sinus, we noticed that the study conducted by Franckowiak et al.¹⁴ reported that most of the students with pilonidal sinus were significantly overweight (45% vs. 26%) than healthy students. Sondena et al.¹⁵ also reported that the obesity rate was 37% among 322 patients with pilonidal sinus. Cubukcu et al.¹⁶, however, found no statistically significant difference between BMI and pilonidal sinus.

The results of our study revealed that the total collagen intensity of the midline tissue and the midline collagen Type I/III ratio of obese patients were significantly lower than those of non-obese patients. The molecular explanation of the relationship between obesity and pilonidal sinus disease as reported in literature is the low collagen Type I/III ratio of the midline tissue as demonstrated in our study.

Table 4. Comparison of complications according to collagen fiber properties at midline and lateral parts

	Total collagen				Collagen type I/III ratio			
	Midline	p	Lateral	p	Midline	p	Lateral	p
Wound dehiscence								
Yes	2.25 ± 0.50	NS	4.50 ± 0.57	NS	0.29 ± 0.24	0.02	14 ± 5.77	NS
No	3.08 ± 0.91		4.80 ± 0.40		2.07 ± 2.72		22.40 ± 15.84	
Infection								
Yes	2.00 ± 0.00	0.01	4.75 ± 0.50	NS	0.54 ± 0.36	NS	34 ± 17,32	NS
No	3.10 ± 0.90		4.78 ± 0.41		2.05 ± 2.73		20.66 ± 14.98	
Hematoma								
Yes	3.66 ± 0.57	NS	5.00 ± 0.00	NS	4.66 ± 4.04	NS	15.66 ± 5.77	NS
No	2.97 ± 0.92		4.76 ± 0.42		1.76 ± 2.50		22.12 ± 15.79	
Seroma								
Yes	2.75 ± 0.95	NS	4.75 ± 0.50	NS	1.77 ± 2.61	NS	26.50 ± 15.00	NS
No	3.04 ± 0.91		4.78 ± 0.41		1.94 ± 2.68		21.31 ± 15.55	

NS: not significant.

According to the results of our study, one of the reasons why hair always prick on the midline is both the low amount of total collagen and low collagen Type I/III ratio. Thus, a molecular response to this issue has been offered by our study.

Karydakis¹⁷ was the first physician who had both explained the etiology of the disease and suggested a treatment modality. Karydakis¹⁷ explained that one of the reasons why hair always pricked into the midline was the presence of vulnerable skin. Within the framework of the Karydakis procedure, he excised this skin on the midline and formed a midline with stronger skin from the lateral and achieved successful results. In our study, midline collagen Type I/III ratio and the amount of total collagen, which showed the strength of the skin, were found to be significantly lower than those of the lateral tissue. The molecular explanation of why the rate of recurrence was lower in Karydakis' procedure can be explained by the high amount of total collagen and high collagen Type I/III ratio in the lateral tissue in our study.

According to Karydakis¹⁷ and many other authors, the main reason for recurrence was the fact that suture line scar tissue was located on the midline^{17,18}. It was also reported that collagen Type I/III ratio was significantly low in the scar tissue, formed as a result of various surgical procedures^{9,10}. In support of our study, the fact that weak collagen Type I/III ratio made it easier for hair to prick into the skin in this area. Therefore, the commonly accepted surgical procedure for the treatment of this disease is the utilization of flap methods which keep the suture line of the defect, formed after the excision, out of the midline with more strong tissue from the lateral¹⁹.

We believe that the explanation of the underlying reason for the higher success rates of the methods which shifted the midline for the surgical treatment of pilonidal sinus, in line with literature, is high collagen intensity and the collagen Type I/III ratio of the lateral tissue instead of vulnerable and weak midline tissue.

In the light of the results of this study, we can argue that one of the reasons why hair mostly pricks into the midline in the intergluteal sulcus in pilonidal sinus disease is the fact that the amount of total collagen and collagen Type I/III ratio of the midline are lower than those of the lateral tissue. The wound dehiscence is more frequently seen in patients with low collagen Type I/III ratio, while infection is more commonly seen in patients with low amount of total collagen. The recurrence rates are lower in surgical procedures utilizing flap methods, during which the tissue on the midline is removed and the lateral tissue is shifted in its place, because of the replacement of collagen Type I/III ratio-poor skin with collagen Type I/III ratio-rich skin from the lateral.

The results of this study offer a molecular response to etiopathogenesis and treatment of pilonidal sinus disease.

Limitations

The study was limited by being a non-randomized study.

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Conflicts of interest

The authors declare that there are no conflicts of interest in connection with this paper.

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Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors have obtained approval from the Ethics Committee for analysis and publication of routinely acquired clinical data and informed consent was not required for this retrospective observational study.

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