Biomechanical and morphological peculiarities of the rectum in patients with obstructed defecation syndrome

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Abstract
The morphological and biomechanical peculiarities of the rectum observed in obstructed defecation syndrome (ODS) are not completely understood. The biomechanical properties and morphological features of the rectum in patients with ODS in correlation with the status of the enteric nervous system (ENS) were evaluated. Uniaxial tensile tests on the rectum samples of patients with ODS and controls were performed; collagenous constituents were assessed by Reticulin and Masson’s trichrome stainings; the expressions of α-smooth muscle actin (α-SMA), S100 and CD117 labeling of interstitial cells of Cajal (ICCs) were investigated by immunohistochemistry. In both groups, the ultimate stress in the posterior rectal wall was statistically significantly higher compared to the anterior one. The ultimate strain was higher in ODS compared to controls. The tangential modulus of elasticity was significantly higher in the control group than in the ODS one, both in the anterior and posterior walls. A significantly higher density of collagen demonstrated throughout the wall was evidenced in controls compared to ODS. The mucosal muscular compartment was significantly thicker but more disorganized in the patients group. The enteric S100-positive glial cells were significantly reduced in number in the anterior wall, but elevated in the posterior wall of the rectum in ODS simultaneously demonstrating the higher numbers of ICCs within the entire muscular layer and myenteric. The biomechanical and morphological results show that the rectal wall in patients with ODS is more deformable and less rigid compared to controls. The results of biomechanical properties and morphological changes in the human rectum are essential when choosing the method of ODS treatment.

Keywords: obstructed defecation syndrome, rectal wall, biomechanical properties, morphology, neuroenteric circuitry.

Introduction
Obstructed defecation syndrome (ODS) often manifests with chronic constipation, which affects about 20% of the general population revealing significantly higher levels in the elderly, especially people above the age of 65 [1–3]. There have also been reports about females being affected more often than males manifested in the male to female ratio of 1:2.2 [3, 4].

For the treatment of ODS, both conservative and surgical approaches are used. A conservative therapy is ineffective only in 20% of patients, who subsequently need a surgical treatment [5–8]. There is a wide range of surgical methods for ODS treatment, however each method has its advantages and disadvantages [5, 6, 9]. A careful patient selection before an operation is essential. The evaluation based on specific criteria can help to achieve positive clinical postoperative outcomes [10, 11].

A rectocele is one of the main clinical findings of ODS detected also in the clinically healthy female patients [12]. A minor rectocele has been reported in as many as 93% of females [13]. Another study suggests that rectoceles are more likely to be the result of ODS, however the direct cause of ODS still remains unclear [14]. Some authors suggest that a rectocele and rectal intussusception may be associated with the affected rectovaginal innervation [15], specifically interstitial cells of Cajal (ICCs) and glial cells [16–18].

Some studies consider the alterations of the neuroenteric circuitry to be a pathophysiological ground [19, 20]. The enteric nervous system (ENS) and ICCs control gastrointestinal function, especially smooth muscle activity. ICCs form networks widely distributed in the submucosal (ICC-SM), myenteric (ICC-MY) nerve plexuses and within the muscular layer (ICC-IM), functioning as electrical pacemakers, contributing to neurotransmission and stretch sensing [21–23], and ICCs decrease in the gastrointestinal tract causes intestinal motility disorders [24, 25]. The pelvic ligaments displaying certain biomechanical properties have been shown to have a local supportive function [26].

Previous studies, reporting on biomechanical properties of the rectum in vivo using impedance planimetry, assessed the overall characteristics of the rectal wall and pelvic muscle as a whole, rather than distinguishing the properties of the coats and compartments within the rectal wall [27]. Concerning the biomechanical examination of ODS postoperative material or pathophysiological rationale for surgery, no data were found in the available literature.

Aim
This study aimed to deepen knowledge about the pathogenesis of ODS based on the evaluation of biomechanical properties and morphological features of the rectal wall and their correlation with the status of the ENS.

Materials and Methods
Based on reported data regarding females being affected by ODS more than males [3, 4], the frequency
of clinical findings interpreted as rectocele and intussusception as well as appropriate surgeries conducted, rectal tissues assessed in the study were obtained from female subjects exclusively. Female subjects who underwent a Contour® Transtar™ surgery for ODS between January 2011 and September 2014 were enrolled in the study. The surgeries were performed at the Ambulatory Surgery Centre of Pauls Stradins Clinical University Hospital, Riga, Latvia. The study was approved by the local ethical committee supervising clinical research of medicinal and pharmaceutical products and a written informed consent was obtained from all patients. The patients (n=13, all females), mean age 46.8±14.1 years, had rectoceles and/or recto-anal intussusceptions, confirmed by defecography, and did not reveal any objective and subjective evidences confirmed by records of intestinal/rectal pathology apart from ODS. The control group (n=6), mean age 52.6±9.6 years, included females with no history of gastrointestinal disorders during their lifetime; rectum specimens were obtained during autopsy.

The samples of the rectal wall were prepared using a special punch with two parallel razor blades. The samples were at least 40–50 mm long and exactly 5 mm wide. The planned stretching direction was transverse to the rectal wall. Before testing, the materials and tissue samples were stored for three to five days in frozen isotonic saline, at -20±1°C. It has been proven in the experiments with soft biological tissues (heart valves, arteries), previously frozen and stored at low temperatures, that such storing conditions do not affect the mechanical properties of the materials collected and could be recommended for the long-time storage of soft tissue samples [28–30]. Moreover, reviewing the literature related to the descriptions of structural differences appearing in the gastrointestinal tract samples obtained in vivo and postmortem [31, 32], we found that these do not affect generation of reproducible results due to the similarity of mechanical properties demonstrated in proper storage conditions [33–35].

The specimens in both groups were investigated with the help of a uniaxial tensile test using Zwick/Roell (Germany) BDO-FB0.5TS, equipped with the test load cell of 50±0.1 N. The testing machine was used in the combination with the testXpert 2 testing software, in order to control and process data. Before the testing of biomechanical properties, the thickness of all samples was measured by a cathometer. The measurement accuracy was ±0.01 mm. The samples were deformed with the speed of 5 mm/min until a rupture occurred. The ultimate (maximum) strain (ε*) and ultimate (maximum) stress (σ*) were calculated for each sample. The stiffness of the samples was expressed by the tangential modulus of elasticity (E) on the linear part of the stress-strain curve and calculated as a tangent of the angle between the strain axis and tangential line in its linear portion [36].

The data obtained using the uniaxial tensile testing machine were processed by the testXpert 2 software to determine ultimate stress, ultimate strain and tangential modulus of elasticity in the linear part of the stress-strain curve. The strain of the samples was calculated using the following formula:

\[ \varepsilon = \frac{l - l_0}{l_0} \times 100, \]

where l – the length of the sample at the time of the defined tension, \( l_0 \) – the original length of the sample.

The stress, which resulted during the tests, was calculated using the following formula:

\[ \sigma = \frac{F}{A}, \]

where F – axial force, A – the actual cross-sectional area of the sample, calculated assuming the sample is non-compressible.

All samples were routinely fixed, embedded, sectioned and stained. Masson’s trichrome staining was used to confirm the occurrence and distribution of collagenous and muscular constituents within the rectal wall, whereas RETICulin-Nuclear Fast Red staining – reticular fibers. Immunohistochemistry was performed conventionally using the following primary monoclonal antibodies: mouse anti-human α-smooth muscle actin (α-SMA, 1:100), which labels smooth muscle cells [37]; mouse anti-human S100 (1:100), which labels the glial and Schwann cells of the nervous system [38]; rabbit anti-human CD117, c-kit (1:300), which recognizes the tyrosine kinase receptor found in ICCs [39]. The amplification of the primary antibody and the visualization of reaction products were performed applying the HiDef Detection™ HRP Polymer system and 3,3′-diaminobenzidine (DAB) tetrahydrochloride substrate kit.

The sections were counterstained with Mayer’s Hematoxylin, washed, mounted, and covered with coverslips. Immunohistochemical controls included the omission of the primary antibody. The sections from melanoma and gastrointestinal stromal tumor were used as positive controls for S100 and CD117, respectively. The internal vasculature staining was used for actin control. The sections were photographed by a Leitz DMRB bright field microscope using a digital camera DC 300F.

The assessment of conventional histopathology, histochemistry and immunostaining was performed by two independent observers. The density of connective tissue fibers appearing in Masson’s trichrome staining was graded as being: 0 – loose, 1 – minimally dense, 2 – moderately dense, 3 – markedly dense, and 4 – very dense [40], whereas, Reticulin staining – as being: 0 – lacking, 1 – low, 2 – intensive, specifying it as pericryptal, submucosal and intermuscular.

The levels of immunopositivity for α-SMA were defined semiquantitatively and graded as: negatively – with ≤5, weakly – 6–20, moderately – 21–50, strongly – >51%, stained as previously described [41]. For S100 staining, the number of the submucosal (SP) and myenteric plexuses (MP) S100-positive glial cells was calculated, whereas, the intensity was assessed semiquantitatively using the following scoring system: 0 – no staining; 1 – low; 2 – moderate; and 3 – intensive staining. The extent of the immunostaining defined as the percentage of positively stained areas was scored from 0 to 100%; finally, it was multiplied by intensity and defined as an expression. It was estimated in 10 properly stained and oriented microscopic fields for each region of interest. The density of ICCs was estimated at the submucosal aspect of muscularis externa, within the intermuscular region, and the entire circular and a longitudinal muscle
The ultimate stress in the posterior wall (\(\sigma^{*}\)) of the anterior wall, the difference between the control group and the group with ODS was not statistically significant (\(p>0.05\)). Likewise, there was no statistically significant difference between these two groups for ultimate stress \(\sigma^{*}\) in the posterior wall (\(p>0.05\)).

The ultimate strain in the anterior part of the rectum was lower than in the posterior part both in the control group and the group with ODS (Figure 3). An ultimate deformation for the anterior \([\text{Md}=129.2\% (109.9–146.9)]\) and posterior \([\text{Md}=153.1\% (117.0; 171.6)]\) walls was not different \((p>0.05)\) either for the group with ODS, or the control groups \(\varepsilon^{*}_{A} [\text{Md}=88.55\% (75.42; 96.45)]\) and \(\varepsilon^{*}_{P} [\text{Md}=109.9\% (91.55; 140.7)]\).

The ultimate strain was statistically significantly higher for the group with ODS than for the control group, and \(\varepsilon^{*}_{A}\) in the group with ODS was statistically significantly higher than \(\varepsilon^{*}_{A}\) in the control group \((p=0.001)\). In the group with ODS, the ultimate stress of the posterior wall \(\varepsilon^{*}_{P}\) was higher than in the control group \(\varepsilon^{*}_{P}\), \(p=0.02\).

The rectal wall in the case of ODS was less stiff compared to the control group, and the tangential modulus of elasticity (Figure 4), which characterizes the stiffness of the rectal wall, was statistically significantly lower for the group with ODS than for the control group \((p=0.05)\).

The tangential modulus of elasticity of the anterior wall \([\text{Md}=0.31\ MPa (0.2; 0.465)]\) was significantly lower in the group with ODS \((p=0.001)\) than in the control group \([\text{Md}=0.605\ MPa (0.43; 0.712)]\). Similarly, in the posterior wall \([\text{Md}=0.38\ MPa (0.345; 0.487)]\), it was significantly lower in ODS \((p=0.009)\) when compared to controls \([\text{Md}=0.585\ MPa (0.465; 0.8)]\).

Summarizing the data collected using biomechanical tests, we suggest that in the case of ODS the rectal wall becomes thinner, less stiff and more deformable compared to controls. In order to deepen knowledge on the pathogenesis of ODS, the data reflecting the biomechanical properties of the rectal wall were further correlated with the data obtained using morphology, including the status of the enteric nervous system.

**Results**

**Biomechanical results**

The analysis of the results showed that the samples of the rectum are thicker in the control group compared to the ODS one (Figure 1).

The thickness of control samples was \(\text{Md}=2.89\ mm\ (2.28; 3.06)\). It differed significantly \((p=0.016)\) from the thickness of the anterior part of the rectum of the group with ODS \([\text{Md}=1.77\ mm \ (1.25; 2.57)]\). Posteriorly, the thickness differed significantly \((p=0.015)\) when the control group was compared \([\text{Md}=2.71\ mm \ (2.46; 2.78)]\) to the group with ODS \([\text{Md}=2.12\ mm \ (1.68; 2.46)]\), respectively. There were no statistically significant differences between the thickness of the anterior and the posterior rectal walls, either in the control group or in the ODS one.

The biomechanical experiments revealed a non-linear relationship between the stress and strain of the rectal wall. The ultimate stress in the posterior wall \(\sigma^{*}\) appeared to be higher than in the anterior wall \(\sigma^{*}_{A}\) in both groups (Figure 2). In the control group, the ultimate stress in the anterior \([\text{Md}=0.195\ MPa \ (0.117; 0.245)]\) and the posterior \([\text{Md}=0.195\ MPa \ (0.117; 0.245)]\) walls was not statistically significant \((p>0.05)\) either for the group with ODS, or the control groups \(\sigma^{*}_{A} [\text{Md}=0.605\ MPa \ (0.43; 0.712)]\) and \(\sigma^{*}_{P} [\text{Md}=0.31\ MPa \ (0.2; 0.465)]\).

In the group with ODS, ultimate stress in the anterior and posterior walls was \(\text{Md}=0.35\ MPa \ (0.225; 0.38)\), respectively \((p=0.018)\). As regards the ultimate stress \(\sigma^{*}_{P}\), of the anterior wall, the difference between the control group and the group with ODS was not statistically significant \((p>0.05)\). Likewise, there was no statistically significant difference between these two groups for ultimate stress \(\sigma^{*}\) in the posterior wall \((p>0.05)\).

**Figure 1** – The thickness of the anterior (A) and posterior (P) walls of the rectum found in patients with ODS and controls (* = statistically significant difference between two values, \(p<0.05\)).

**Figure 2** – The ultimate stress of the anterior (A) and the posterior (P) walls of the rectum found in patients with ODS and controls (* = statistically significant difference between two values, \(p<0.05\)).
Results of the structural analysis

Conventional histopathology and histochemistry

All layers of the rectal wall in both, patients and controls, were analyzed. The anterior wall of controls revealed the significantly \( p<0.001 \) higher density of collagen fibers when compared to ODS – \( Md=3.00 \) (3.00; 4.00) vs. \( Md=2.00 \) (1.00; 2.00); \( Md=3.00 \) (2.25; 4.00) vs. \( Md=1.00 \) (0.00; 1.00); \( Md=3.00 \) (3.00; 4.00) vs. \( Md=1.00 \) (0.00; 1.00). For both walls, the aforementioned parameters compared between the two values, \( p<0.05 \); ** – statistically significant difference between two values, \( p<0.01 \).

Interestingly, but S100 expression was pronouncedly compared to ODS – \( Md=5.00 \) (4.00; 6.00) vs. \( Md=1.00 \) (1.00; 1.00). For both walls, the aforementioned parameters compared between the two values, \( p<0.05 \); ** – statistically significant difference between two values, \( p<0.01 \).

Immunohistochemistry

The mucosal muscular lamina and muscularis externa myocytes as well as pericryptal myofibroblasts were labeled by anti-α-SMA (Figure 6). The mucosal muscular lamina thickness was higher \( p=0.046 \), and disorganization – more pronounced in the patients group \( Md=2.00 \) (1.00; 2.50] compared to controls \( Md=1.00 \) (1.00; 1.00). The muscularis externa was properly organized and similarly thickened in both groups.

S100-positive glial cells found in the patients’ and control group were diffusely and delicately distributed within the mucosal coat but tightly packed within the larger submucosal bundles. Moreover, these enveloped neurons of the nerve plexuses (Figure 7).

Both, straight and wavy S100-positive nerve fibers were demonstrated within the muscularis externa (Figure 8).

The quantitative analysis of enteric glial cells revealed that the number of S100-MP and S100-SP positive cells in the anterior wall was significantly higher \( p=0.005 \) and \( p=0.002 \) in the patients’ group compared to controls – \( Md=45.00 \) (34.00; 59.00), \( Md=17.00 \) (10.00; 30.00) and \( Md=29.50 \) (24.25; 36.50), \( Md=8.50 \) (7.75; 10.50), respectively. In contrast with the posterior wall, the number of S100-MP and S100-SP positive cells did not differ significantly \( p=0.057 \) and \( p=0.105 \) when both groups were compared. Interestingly, but S100 expression was pronouncedly heterogeneous being significantly lowered in ODS anteriorly \( Md=1.50 \) (0.027; 0.200) when compared to controls \( Md=0.675 \) (0.300; 1.012), \( p<0.001 \), whereas elevated posteriorly \( Md=0.600 \) (0.400; 0.900) vs. \( Md=0.225 \) (0.150; 0.375), \( p<0.001 \).

ICCs presented as highly ramified cells bearing long, slender processes (Figure 9) establishing intimate vascular and nerve plexuses contacts (Figure 10). Both anteriorly and posteriorly, the number of ICCs within the entire musculature and myenteric was significantly higher in the patients’ group compared to controls – \( Md=13.00 \) (10.00; 19.25) vs. \( Md=5.00 \) (4.00; 6.00), \( p<0.001 \); \( Md=13.00 \) (9.00; 17.00) vs. \( Md=5.00 \) (4.75; 6.25), \( p<0.001 \); and \( Md=3.00 \) (2.00; 4.00) vs. \( Md=1.50 \) (1.00; 2.25), \( p=0.029 \), for ICC-IM, ICC-MY, and ICC-SM, respectively, and as revealed anteriorly.

The estimations done for the posterior wall revealed similar results expressed as follows: ICC-IM \( Md=11.00 \) (10.00; 13.00) vs. \( Md=5.00 \) (4.00; 6.75), \( p<0.001 \), ICC-MY \( Md=9.00 \) (8.00; 10.00) vs. \( Md=5.00 \) (4.00; 5.00), \( p<0.001 \), and ICC-SM \( Md=3.00 \) (2.00; 4.00) vs. \( Md=1.00 \) (0.75; 2.00), \( p<0.002 \), for the patients and controls, respectively.
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Figure 5 – The posterior wall of the rectum of patient with ODS revealing a delicate black mucosal network of reticular fibers enveloping crypts, and heavily decorated submucosal reticular fibers demonstrating haphazard patterning and insertions into the mucosal muscular lamina. Reticulin–Nuclear Fast Red staining, ×200.

Figure 6 – The anterior wall of the rectum of patient with ODS revealing the thickened mucosal muscular lamina, decoration of mucosal pericryptal myofibroblasts and positivity of vascular beds. α-SMA immunohistochemistry, ×100.

Figure 7 – S100 positivity demonstrated in delicate mucosal nerve fibers and those traversing muscular lamina continuously appears as heavily decorated submucosal bundles found within the anterior wall in case of ODS. Inset: Submucosal nerve plexus with the unstained neurons surrounded by S100-positive glial cells. S100 immunohistochemistry: ×200; ×250 (inset).

Figure 8 – S100 positivity within the muscularis externa appearing either as straight or wavy (inset) paralleled, heavily decorated nerve fibers found within the anterior wall in case of ODS. S100 immunohistochemistry, ×200 (for both images).

Figure 9 – The anterior wall of the rectum of patient with ODS revealing the CD117-positive ICC-IM displaying ramified processes. CD117 immunohistochemistry, ×250.

Figure 10 – The posterior wall of the rectum of patient with ODS revealing the CD117-positive ICC-IM and ICC-MY bearing long, slender processes running parallel to smooth muscle cells and enveloping CD117 negative constituents of the myenteric plexus. CD117 immunohistochemistry, ×250.
It is worth noting that CD117 and S100 estimations considered collectively within the entire groups demonstrated that in the case of ODS ICC-MY and S100-SP positive cells’ count was significantly higher in the anterior wall compared to the posterior one ($p<0.001$; $p=0.001$; $p=0.010$, respectively). Simultaneously, ICC-SM, S100-MP positive cells’ count and the estimation of S100 expression did not reveal any differences between the walls ($p=0.918$; $p=0.440$; $p=0.621$, respectively).

## Discussion

Obstructed defecation causes constellation either functional, or mechanical. The impaired function gradually brings morphological changes causing a mechanical blockage to the fecal passage and accentuating ODS [44]. ODS often is attributable to the muscular dysfunction of the pelvic floor, and multiple risk factors have been mentioned [45]. In this context, a deficit of parasympathetic sacral nerves and regulatory changes related to the putative pontine defecation center have been reported [46, 47].

Some recently published results deepen our knowledge about the relevance of the histopathology combined with the biomechanical tests in study of the experimental colitis [48]. However, the biomechanical properties and morphological features of the constituents of the human rectal wall analyzed collectively have not been studied before as far.

We performed a complex biomechanical and morphological study on the human rectal tissue obtained from the patients with ODS and compared them with controls. It was found out that a combination of the before mentioned methods was instructive enough, firstly, evidencing, and then extensively describing the major findings.

The analysis of literature data suggests that the ultimate stress and ultimate strain in the rectal wall is lower than in the vagina, which, in its turn, is lower than in the urinary bladder [17]. However, previous publications have not explained the structural differences between the anterior and posterior walls of the rectum and their biomechanical properties. In our study, we have tried to prove that the rectal wall is thinner, less stiff and more deformable in patients with ODS compared to controls. Analyzing the estimations of the density of collagen fibers done using morphology, we have found them in agreement with the biomechanical data obtained. However, morphology evidencing the higher density of collagen fibers in the anterior wall of controls compared to ODS provides superior suggestions based on the accurate estimations and statistics applied specifying that the loosening of connective tissue matter happens at a greater extent within the submucosal and intermuscular compartment of the wall.

Moreover, it is worth noting that estimating the relevance of two histochemical staining techniques used to assess density, the distribution and orientation of collagen fibers allowed us to suggest that Masson’s trichrome staining decorating the sum of collagenous elements, appears to be more favorable under the conditions studied compared to Reticulin-Nuclear Fast Red staining distinguishing exclusively the type III collagen created network, fixing the wall constituents and delicately enveloping myocytes. Regarding the aforementioned techniques, our evidences and suggestions are in agreement with the guidelines proposed for histotechniques by McGavin [49]. Undoubtedly, the loosening and weakening of connective tissue architecture found in the rectal wall of patients with ODS creates conditions for its’ further impairment. From this point of view, we may explain it either as a severe distortion of crypts by inflammatory cells and lymphoid follicles infiltrating mucosa or adaptive thickening accompanied by the disorganization of myocytes of the mucosal muscular lamina demonstrated in the patients’ group. A clear connection between the histopathological findings occurring in the rectal wall and biomechanical parameters suggesting on reduction of the wall stiffness in the case of ODS was found.

Recent publications have deciphered many aspects of ICCs function and their role in the gastrointestinal tract demonstrating that ICCs transduce inputs from enteric motor neurons, generate intrinsic electrical rhythmicity in phase smooth muscles, and have a mechanical sensation ability [39]. According to the results obtained by Iino & Horiguchi, in 2006 [50], the colonic ICCs carefully nursing the muscularis externa appear to be more abundant compared to those represented in the stomach and small intestine. Simultaneously, a decrease of the pan-colonic ICCs found within the wall of caecum, ascending colon, transverse colon, and sigmoid colon has been demonstrated in patients with slow transit constipation [51]. However, the number of ICCs appearing in different regions of the human colon is a subject of controversy [52]. The appearance of ICCs demonstrated by revealing CD117 positivity is mostly spindle-shaped but slightly more often ramified within the muscularis externa of the anterior wall. These observations are in accordance with the evidences confirmed by other authors [53].

It is worth noting that studies reporting on the alterations and role of ICCs in the human rectum are less numerous. The rectal ICCs are mentioned as regulators of electric activity within the wall in early studies conducted by Shafik et al. [54]. Other authors [55], reporting on the quantitative estimations of ICCs in the human rectum, postulate that both decreased and increased numbers of ICCs may be demonstrated in chronic constipation. Observations done on the rectal ICCs of patients with rectal prolapse brought an evidence regarding the cell increase [56]. Therefore, quantitative estimations appear to be crucial. In our study, ICCs estimated both anteriorly and posteriorly appeared in significantly higher numbers in the patients’ group compared to the control group. Despite the fact that our patients in their 50-s and 60-s constituted the study group, and the given study did not attempt to investigate the changes of ICCs and glial cells demonstrated along with the aging of the gut, enteric neurodegeneration has been considered a likely cause for the development of constipation in the aging gut in animal models [57]. Since accurate quantification of the constituents of the ENS has certain difficulties reported [58], the changes demonstrated are characterized by greatly varying cell numbers. Some publications report on other factors, including diet and the microbiota, that may influence cellular aging in the gastrointestinal tract [59], whereas other scientists support the hypothesis that disruption of paracrine neutrophic factor signaling may play an important role in the aging of the ENS [60].

For the first time, we have evidenced heterogeneity...
of S100 expression within the human rectal wall displayed along the anterior-posterior axis and confirmed by accurate counting. From this point of view, our results are partly in accordance with the suggestions published by Bitar et al. [61], who reported that advanced age likely has differential effects on subpopulations of neurons in the ENS, which demonstrate regional- and species-specific differences. Interestingly, that regarding the neuromuscular apparatus collectively, we have found that CD117 and S100 positive structures are significantly more frequent findings within the anterior wall. The counting performed on enteric glial cells revealed the highest number of these within the myenteric nerve plexuses, especially, in the anterior wall of the rectum of patients with ODS. By contrast, the expression of S100 in the anterior wall was found to be significantly lowered in patients with ODS compared to controls, thus indicating the complexity of intimate glial-neuronal and ICCs interactions. It is worth noting that the evidences, discovering some novel aspects in the functions of enteric glial cells, demonstrate that glial cells are activated by synaptic stimulation and contribute to synaptic transmission [62].

Understanding the drawbacks of our study, we want to stress the relevance of findings regarding ICCs analyzed collectively with the estimations of enteric glial cells, since usefulness of such investigations was very much endorsed previously [63]. Accepting the known postulate that ODS if a multifactorial condition, which follows distinct pathogenic routes, based on either functional or mechanical causes, we understand that further studies should provide more data on the mechanisms involved.

**Limitations of our study**

We recognize limitations of our study, including a small number of the rectum tissue samples, both patients and controls, collected exclusively from females, and incompleteness of the uniaxial tensile tests performed, since biaxial measurements were not possible. Commonly used tissue sample preservation techniques should be investigated as important factors contributing to the final sample structure, and therefore, biomechanical tests applied. Finally, our knowledge about the ENS, analyzed in the rectum and affected in patients with ODS, remains insufficient and further studies in this field are needed.

**Conclusions**

This study presents for the first time the results of experiments done by measuring biomechanical parameters applicable throughout uniaxial tensile test and coupled to the histopathology findings revealed in the samples of the human rectum in patients with ODS. The study demonstrates that in patients with ODS the ultimate stress is equal throughout the wall. The ultimate strain is significantly higher in both parts of the wall in ODS patients compared to controls. By contrast, the tangential modulus of elasticity is higher in controls than in ODS. The significantly higher density of collagen demonstrated throughout the wall is evidenced in controls compared to ODS. Structurally, the rectal wall in ODS is a subject of tissue changes occurring within the entire wall, including even those distinguished as neuroenteric. Collectively, the results regarding the biomechanical properties and morphological changes found in the human rectum are essential in choosing the method of ODS treatment.

**Conflict of interests**

The authors declare that there is no conflict of interests regarding the publication of this paper.

**References**


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