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Clinical, biomechanical and morphological peculiarities of the rectum in patients with obstructed defecation syndrome

Summary of the Doctoral thesis
for obtaining a doctoral degree (*Ph.D.*)

Sector – Clinical Medicine

Sub-Sector – Surgery

Rīga, 2020

The Doctoral thesis was carried out at Rīga Stradiņš University, Department of Surgery

Surgery was performed at the Ambulatory Surgery Centre of Paul's Stradins Clinical University Hospital and Riga *East Clinical University Hospital*, *Clinical* Centre "*Gailezers*", Department of Proctology

Biomechanical analysis was performed at the Institute of Anatomy and Anthropology, Rīga Stradiņš University and Scientific Laboratory of Biomechanics

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Defence of the Doctoral thesis will take place at the public session of the Doctoral Council of Medicine on 26 June 2020 at 12.00 in Hippocrates Lecture Theatre, 16 Dzirciema Street, Rīga Stradiņš University

The Doctoral Thesis is available in RSU Library and on RSU website: www.rsu.lv

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INTRODUCTION

Obstructed defecation syndrome (ODS) is a difficulty or inadequate rectal emptying for the last 3 months with symptom onset and at least 6 months prior to diagnosis, resulting in the need for straining at defecation and chronic constipation (Podzemny, Pescatori and Pescatori, 2015).

Obstructed defecation syndrome (ODS) often manifests with chronic constipation (CC), which affects about 17 % of the general population revealing significantly higher levels in the elderly, especially people above the age of 70 – 20.6 % men and 25 % women (Choung *et al.*, 2007). Approximately half of the patients with CC suffer from ODS (Rao, 2001).

Constipation is a very common problem in general population. The estimates of the prevalence of constipation in North America ranged from 12 % to 19 %. Prevalence estimates by gender support a female-to-male ratio of 2.2:1 (Higgins and Johanson, 2004).

2.5 million physician visits for constipation have also been reported every year in the United States, leading to high financial costs (Sandler, Jordan and Shelton, 1990). The average cost of diagnosing an outpatient patient with constipation in the United States is approximately 2752$ (Rantis *et al.*, 1997), but in hospital even more (Martin, Barghout and Cerulli, 2006).

CC diagnosis and treatment have been previously shown to carry a high burden in terms of financial costs, and also on work performance and impact on the individual's quality of life.

A Canadian survey of a weighted sample of 1000 adults was conducted to determine the prevalence of gastrointestinal symptoms over the previous three months. 13.2 % of respondents missed work or school and 28.8 % were less productive, but nearly 10 % reported missing work or having been forced to leave work (Hunt *et al.*, 2007).

Psychological distress is linked to having persistent gastrointestinal symptoms and physiology of CC. Prolonged emotional stress can be considered a cause of CC and CC itself could be triggered and exacerbated by stress. It has been proven in studies that patients with functional bowel disorders have a higher risk of psychological disorders than the control group, and 40–50 % of such patients have confirmed psychiatric diagnoses (Koloski, Talley and Boyce, 2003).

Constipation is frequently multifactorial. Constipation can be classified in three broad categories: normal-transit constipation, slow-transit constipation and disorders of defecatory or rectal evacuation (Lembo and Camilleri, 2003).

Defecatory disorders can be a result of functional or anatomical pelvic floor alterations. Functional causes are mostly treated by conservative management, with surgery having a minor role only. In contrast, disorders with an underlying anatomical cause leading to ODS should be more considered for surgery (Riss and Stift, 2015).

Two most frequent lesions of ODS are rectocele and rectoanal intussusception

For treatment of ODS, both conservative and surgical approaches are used. Conservative management needs to be offered to all patients initially: fiber diet, plenty of water and bulking laxatives, biofeedback, rehabilitation and electrostimulation. Conservative treatment is ineffective only in 20 % of patients who subsequently can be considered for surgery (Podzemny, Pescatori and Pescatori, 2015). A great variety of operative techniques to treat patients with ODS exists there. There is no ideal technique and not every operation fits every patient and vice versa.

According to a surgeon's preference, the approach can be transabdominal (open or laparoscopic), transanal, transvaginal or transperineal. Additionally, resection or reconstructive surgery with or without mesh implantation could be also performed during each approach (Riss and Stift, 2015).

All techniques have their advantages and disadvantages; thus, satisfying functional outcomes can only be achieved by offering a tailored approach to each individual patient.

Each technique has also its risks and benefits; thus, careful patient selection is crucial to achieve optimal functional results (Janssen and van Dijke, 1994; Murthy *et al.*, 1996).

Morphological cause and pathological physiological mechanism of the development of ODS, as well as biomechanical justification of surgical treatment methods are still unclear. There are international studies about biomechanical properties of female minor pelvic organs (bladder, vagina and rectum); however, these studies were performed for women without rectal pathology (Rubod *et al.*, 2012). There is another study assessing biomechanical properties of the rectal wall *in vivo* with impedance planimetry, but only total biomechanical properties of the rectal and pelvic muscles and ligaments were analysed, not assessing the rectal wall biomechanical properties separately (Dall *et al.*, 1993).

Rectocele is one of the main clinical findings of ODS that is a bulging of the front wall of the rectum into the back wall of the vagina. A rectocele could be detected also in clinically healthy female patients (Shorvon *et al.*, 1989).

Up to 93% of women are found to have a small rectocele less than 2.5 cm (Palit *et al.*, 2014).

The study suggests that rectocele may be the result of ODS, but clear relationships should be still determined (Hicks *et al.*, 2013).

There are limited data on biomechanical analysis of the ODS surgical specimens, as well as pathophysiological explanation and justification of the surgery.

In general, understanding of the pathophysiological mechanism of rectocele's formation is still relatively weak.

Aim of the study

The aim of the study was to evaluate morphological changes and biomechanical properties of pathologically changed rectal wall (ODS) using uniaxial tensile test and compare the results with the control group.

As a result, a better understanding of physiological primary and secondary changes in the rectal wall of ODS patients could be achieved.

Objectives

Following the study aim, the objectives of the research were:

- collect data on clinical symptoms in ODS patients with chronic constipation before Contour Transtar surgery;
- collect data on clinical symptoms in the above-mentioned patient group at 12, 24 and 36 months after Contour Transtar surgery;
- evaluate efficacy of the resection type surgery in ODS patients with additional defecography findings – rectocele, *m.puborectalis* syndrome;
- perform measurements of biomechanical properties of the rectal anterior and posterior wall specimens in patients with ODS and control group;
- perform morphological analysis (conventional histopathology, histochemistry and immunohistochemistry) of the rectal anterior and posterior wall specimens in patients with ODS and from the control group;
- evaluate results of biomechanical properties and morphological changes between the patient and control group, as well as between the anterior and posterior rectal wall within the group;
- recommend new prevention and treatment methods of ODS based of the study results.

Hypothesis

1. Short- and long-term postoperative outcomes in patients with obstructive defecation syndrome are convincingly better and stable in

the long term compared to preoperative results and irrespective of the defecography findings

2. Biomechanical and morphological properties of the rectal anterior and posterior wall in patients with ODS are equal when compared between the anterior and posterior rectal wall, but different from the control group.
3. There is a connection between morphological changes and biomechanical properties of the rectal wall in patients with obstructive defecation syndrome. Observed changes of biomechanical properties of the rectal wall can be explained by detected morphological changes.

Novelty of research

Importance of the problem. As there are no publicly available convincing data on pathophysiological mechanism of the formation of a rectocele and ODS, the study aim was to obtain more data and evidence of the causes of a rectocele and the pathophysiological mechanism. These is a possibility to obtain data on primary, hereditary or secondary nature of the formation of a rectocele during the study. The results of the study could serve as a basis for the development of new surgical or minimally invasive treatment methods. The results of biomechanical and morphological changes in the rectum in ODS patients could justify the resection or reconstruction type of surgery. The study data could also help to develop more targeted preventive measures for ODS patients.

Until the beginning of the study, no data were found in the available literature on rectal biomechanical and morphological changes in the rectum wall in patients with ODS. There were some publications available on morphological changes in the rectum as well as biomechanical properties in patients with ODS, but there were no data on the connection between these findings. Till now, the characteristics of the rectal anterior and posterior wall in patients with ODS have not been studied and compared.

Study sites

1. The Ambulatory Surgery Centre of Paul's Stradins Clinical University Hospital – the stapled transanal rectal resection (Contour Transtar surgery)
2. Riga East Clinical University Hospital (RECUH), clinical Centre "*Gailezers*" – the stapled transanal rectal resection (Contour Transtar surgery)
3. Riga East Clinical University Hospital Pathology Centre – obtaining the rectum specimens from the control group
4. Rīga Stradiņš University, Scientific Laboratory of Biomechanics – biomechanical analysis of the rectal wall specimens
5. Rīga Stradiņš University Department of Morphology – morphological analysis of the rectal wall specimens

Technical equipment

1. Uniaxial tensile test Zwick/Roell (Germany) BDO-FB0.5TS in the combination with the testXpert 2 testing software, in order to control and process data, biomechanical analysis
2. Cathetometer MK-6 (LOMO, Saint Petersburg, Russia) to measure thickness of samples
3. Leitz DMRB bright field microscope for morphological analysis
4. *HiDef Detection™ HRP Polymer system* – a visualization system for immunohistochemical reactions
5. Digital camera DC 300F for morphological specimen imaging
6. Contour Transtar *curved* cutter-stapler procedure set

1. MATERIALS AND METHODS

1.1. Study design, patient characteristics, demographic data, selection criteria

The study was conducted between October 2010 and December 2017. Two independent prospective studies were performed to reach the study aims. New practical guidelines were developed, and new methods of treatment may be elaborated based on the study results.

Several medical institutions were involved in the study process. The study was approved by Latvia Independent Ethics Committee for clinical investigation of drugs and pharmaceutical products. Female patients, as statistically most ODS patients are females (Bassotti and Blandizzi, 2014; Higgins and Johanson, 2004), whom Contour Transtar surgery were planned for ODS clinical indications at the Ambulatory Surgery Centre of Paul's Stradins Clinical University Hospital or Riga East Clinical University Hospital, clinical Centre "*Gailezers*", were enrolled in the study. All patients were informed about the study aim, process and patient responsibilities before the enrollment. A written informed consent was obtained from all patients, who all had rectoceles and/or rectoanal intussusceptions, confirmed by defecography, and as a long-term conservative treatment was ineffective for all study patients that subsequently was considered as an indication for a surgical treatment.

Patients were asked to fill ODS Longo assessment questioner before the surgery. The long-term outcomes were assessed at 12, 24 and 36 months after surgery. All patients were contacted either through a telephone interview or during follow-up visits.

At the beginning of the study, rectal surgical specimens of a randomised sample of patients (after obtaining informed consent) were prepared for biomechanical and morphological evaluation. The study material for comparison was obtained from the anterior and posterior rectal wall. Biomechanical analysis of the rectal wall specimens was performed at Rīga Stradiņš University Scientific Laboratory of Biomechanics.

Morphological analysis of the rectal wall specimens was performed at Rīga Stradiņš University Department of Morphology by two independent pathologists.

The control group included females with no history of gastrointestinal disorders and pelvic organ prolapse during their lifetime; rectum specimens were obtained during autopsy. A written informed consent was obtained from control group relatives. Autopsy material was obtained at Riga East Clinical University Hospital Pathology Centre.

Table 1.1

Patient characteristics

Number of patients	n = 88
Gender	Female (100 %)
ODS diagnosis	Defecography and colonic transit time (100 %)
Additional diagnosis	Colonoscopy < 3 years before surgery (100 %)
Conservative treatment	> 12 months
Mean age	51.77 ± 12.54 years
Mean ODS score before surgery (max 37 points)	18.08 ± 7.09 points
Hospital	n = 40 (45 %) Paul's Stradins Hospital n = 48 (55 %) RECUH
Rectocele	n = 88 (100 %)
Average size of rectocele (cm)	4.92 ± 0.64 cm
Rectocele with residual content	n = 74 (84 %)
Recto anal intussusception	n = 34 (38 %)

Table 1.2

Study groups for biomechanical and morphological properties of the rectal wall

	ODS group	Control group
Size of group	n = 13	n = 8
Mean age (years)	46.83 ± 14.14	52.6 ± 9.6
Gender	Female (100 %)	Female (100 %)

1.2. Study design

1.2.1. Assessment of severity of ODS using Longo's ODS score before surgical treatment and in early and late Contour Transtar postoperative period

A prospective study of female patients with diagnosed obstructed defecation syndrome who underwent Contour Transtar surgery at the Ambulatory Surgery Centre of Paul's Stradins Clinical University Hospital or Riga East Clinical University Hospital was performed between January 2010 and September 2014. Patients prior to surgery were diagnosed with rectocele and/or rectoanal intussusceptions, in some case with enterocele, puborectalis syndrome and anal dilation confirmed by defecography.

All patients were female (n = 88), mean age 51.77 ± 12.54 years. All patients had an ineffective long-term conservative treatment that subsequently was an indication for a surgical treatment. Patients were informed about the aim of the study and a written informed consent was obtained from the study population.

Longo's ODS score system was filled in by all patients to assess symptoms and quality of life of patients (overall score ranging from 0 to 37). The score system is composed of the following indicators: defecation frequency, straining, sensation of incomplete evacuation, activity reduction, use of laxatives, enemas, digitation etc. (Table 1.3.).

Table 1.3

Longo's ODS score

<table>
<tr><td>Defecation frequency</td><td>1-2 def/
1-2 days</td><td>0</td><td colspan="2">2 def/week or
3 def or attempts/day</td><td>1</td><td colspan="2">1 def/week or
4 x or attempts/day</td><td>2</td><td>< 1 def/week or
> 4x or attempts/day</td><td>3</td></tr>
<tr><td>Straining - intensity (normal feces)</td><td>no, light</td><td>0</td><td colspan="2">moderate</td><td>1</td><td colspan="2">intensive</td><td>2</td><td></td><td></td></tr>
<tr><td>- extension (normal feces)</td><td></td><td></td><td colspan="2">short time</td><td>1</td><td colspan="2">prolonged</td><td>2</td><td></td><td></td></tr>
<tr><td>Sensation of incomplete evacuation</td><td>never</td><td>0</td><td colspan="2"><= 1x / week</td><td>1</td><td colspan="2">2x/week</td><td>2</td><td>>2x/week</td><td>3</td></tr>
<tr><td>Recto/perineal pain/discomfort</td><td>never</td><td>0</td><td colspan="2"><=1x/week</td><td>1</td><td colspan="2">2x/week</td><td>2</td><td>>2x/week</td><td>3</td></tr>
<tr><td>Activity reduction per week</td><td>never</td><td>0</td><td colspan="2"><25 % of activities</td><td>1</td><td colspan="2">25-50 % of activities</td><td>2</td><td>>50 % of activities</td><td>3</td></tr>
<tr><td>Laxatives</td><td rowspan="3">never</td><td>0</td><td rowspan="3"><25 % of defecations</td><td>1</td><td rowspan="3">25-50 % of</td><td>3</td><td rowspan="3">> 50 % of defec.</td><td>5</td><td rowspan="3">always</td><td>7</td></tr>
<tr><td>Enemas</td><td>0</td><td>1</td><td>3</td><td>5</td><td>7</td></tr>
<tr><td>Digitation</td><td>0</td><td>1</td><td>3</td><td>5</td><td>7</td></tr>
</table>

Longo's ODS score system was filled in by all patients prior to surgery, then at 12, 24 and 36 months after surgery. Postoperative surveys were conducted during follow-up visits or through a telephone interview. Longo's ODS score results were compared before and after surgery. The results were also compared for the patients with additional defecography findings 36 months after surgery.

1.2.2. Criteria and conditions for creating the study and control groups for assessment of biomechanical and morphological properties in the rectal wall

The rectal wall specimens for measurements of biomechanical properties and morphological analysis were obtained during Contour Transtar surgery. Surgery was performed at the Ambulatory Surgery Centre of Paul's Stradins Clinical University Hospital or Riga East Clinical University Hospital Proctology Department between January 2010 and September 2014 due to an ineffective long-term conservative treatment that was an indication for a surgical treatment.

Prior surgery patients were informed about the study process and a written informed consent was obtained from all patients. Patients were not subjected to any additional procedures in order to obtain study specimens during surgery. Study specimens were obtained from the rectal resection sample –the anterior and posterior wall.

The fixative 10 % buffered formalin was used to preserve specimens for morphological evaluation, but specimens for measurements of biomechanical properties were put in physiological saline and frozen

The control group included females with no history of gastrointestinal disorders and pelvic organ prolapse during their lifetime; rectum specimens were obtained during autopsy. A written informed consent was obtained from control group relatives. Autopsy material was obtained at Riga East Clinical University Hospital Pathology Centre

Study specimens were obtained from the anterior and posterior rectal wall 6 cm from *linea dentata*. Preparation and preservation of specimens for morphological and biomechanical analysis were performed as described above.

Studies to assess biomechanical properties of soft and hard tissues require the minimum study sample of 6 for a statistically significant result (Evans, 1973; Yamada and Evans, 1970).

ODS patient group (n = 13), mean age 46.83 ± 14.14 years, with previously diagnosed rectocele and/or rectoanal intussusceptions using defecography. Control group (n = 8), all female, mean age 52.6 ± 9.6 years.

1.2.3. Preparation and storage of ODS and control groups' rectal wall study samples

Samples of the rectal wall were prepared using a special punch with two parallel razor blades. Samples of the rectal wall muscle layers were at least 40–50 mm long and exactly 5 mm wide (Egorov *et al.*, 2002). 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 studies with soft biological tissues (heart valves, arteries), previously frozen and stored at low temperatures, that such storing conditions do not affect mechanical properties of the materials collected and could be recommended for the long-time storage of soft tissue samples (Chow and Zhang, 2011; O'Leary, Doyle and McGloughlin, 2014; Stemper *et al.*, 2007).

Moreover, reviewing the literature related to the descriptions of structural differences appearing in gastrointestinal tract samples obtained *in vivo* and postmortem (Bourgouin *et al.*, 2012; Rosen *et al.*, 2008), it was found that these do not affect generation of reproducible results due to similarity of mechanical properties demonstrated in proper storage conditions (Chan and Titze, 2003; Egorov *et al.*, 2002; Howes, 2013).

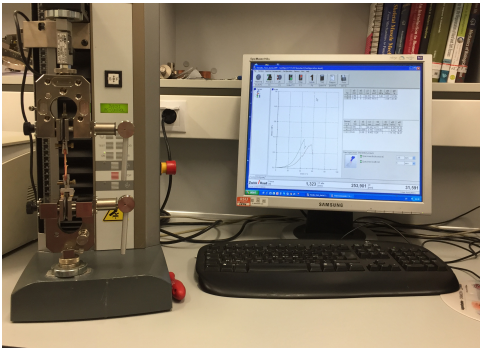
1.2.4. Determination of ultimate stress, ultimate deformation and tangential modulus of elasticity of the rectal wall in ODS patients and control group

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 (Picture 1.1).

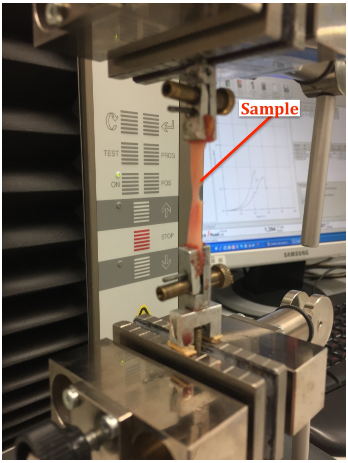
Before the testing of biomechanical properties, thickness of all samples was measured by a cathetometer (MK-6 (LOMO, Saint Petersburg, Russia). The measurement accuracy was ± 0.01 mm. Samples were deformed with the speed of 5 mm/min until a rupture occurred (Picture 1.2).

The ultimate (maximum) strain (ε^*) and ultimate (maximum) stress (σ^*) were calculated for each sample.

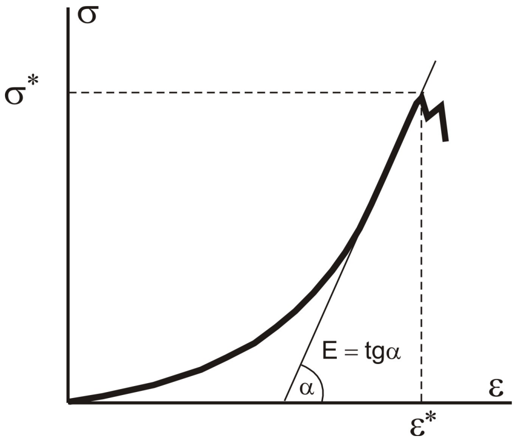
Stiffness of the samples was expressed by tangential modulus of elasticity (E) on the linear part of the stress-strain curve and calculated as a tangent of the angle (Picture 1.3) between the strain axis and tangential line in its linear portion (Barber *et al.*, 2001).



Picture 1.1. **A uniaxial tensile test Zwick/Roell (Germany) BDO-FB0.5TS**



Picture 1.2. **Test of study sample using the uniaxial tensile testing machine**



Picture 1.3. **Stress-strain curve and main mechanic parameters: σ^*- ultimate (maximum) stress, ε^*- ultimate (maximum) strain un E - tangential modulus of elasticity**

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 processed data by the testXpert 2 software were represented in the form of a table and on a graph as the stress-strain curve (Picture 1.4), the horizontal axis describing the deformation changes (%), but vertical axis describing stress changes (Mpa).

The strain of the samples in the deformation process was calculated using the following formula:

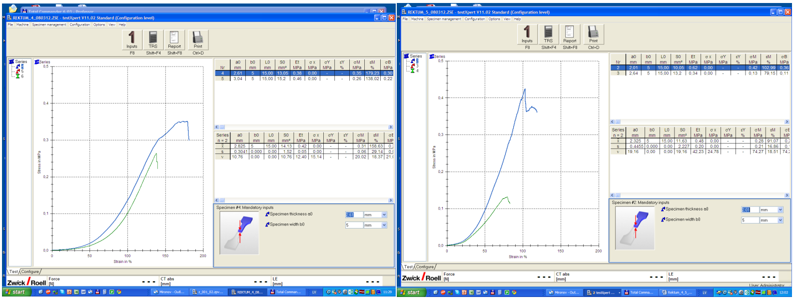
$$\varepsilon = ((l - l_0) / l_0) \times 100\ \%,$$

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 (Carew, Vaishnav and Patel, 1968).



Picture 1.4. **Stress-strain curve of the anterior and posterior rectal wall**

1.2.5. Morphological analysis of the ODS and control group rectal wall specimens

All study samples were routinely fixed (10 % buffered formalin), 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 (Skalli *et al.*, 1986); mouse anti-human S100 (1:100), which labels the glial and *Schwann* cells of the nervous system (Nakajima *et al.*, 1982), rabbit anti-human CD117, c-kit (1:300), which recognizes the tyrosine kinase receptor found in ICCs (Al-Shboul, 2013). Amplification of the primary antibody and visualization of reaction products were performed applying the HiDef Detection™ HRP Polymer system and 3,3'-diaminobenzidine (DAB) tetrahydro-chloride substrate kit.

The sections were counterstained with Mayer's Hematoxylin, washed, mounted, and covered with coverslips.

Immunohistochemical controls included 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.

Assessment of conventional histopathology, histo-chemistry and immunostaining was performed by two independent observers. 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 (Altman *et al.*, 2004), 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 semiquantitative and graded as: negative – with ≤ 5, weak – 6–20, moderate – 21–50, strong – > 51 %, stained as previously described (Roberts *et al.*, 2014). For S100 staining, the number of the submucosal (SP) and myenteric plexuses (MP) S100-positive glial cells was calculated, whereas, the intensity was assessed semiquantitative using the following scoring system: 0 – no staining; 1 – low; 2 – moderate; and 3 – intensive staining. The extent of 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. Density of ICCs was estimated at the submucosal aspect of *muscularis externa*, within the intermuscular region, and the entire circular and a longitudinal muscle layer following the recommendations of Hagger *et al.* (Hagger *et al.*, 1998; Yun *et al.*, 2010) assessing the results in 10 different microscopic fields.

1.3. Ethical considerations

The project was carried out in compliance with the Declaration of Helsinki, 1967. The protocol was approved by Latvia Independent Ethics Committee for clinical investigation of drugs and pharmaceutical products before patient recruitment.

All patients signed informed consent forms before enrolment.

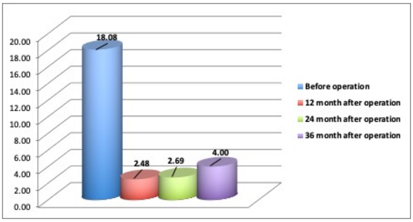
1.4. Statistical data analysis

Statistical data analysis, calculation and all graphs were made using program GraphPad PRISM version 6.0e (GraphPad Software Inc., San Diego, California, USA). Homogeneity of variances was tested using Brown – Forsythe and Bartiett's tests. In case of unequal distribution, the comparison of means between different groups of numerical variables was performed using non-parametric ANOVA or Kruskal – Wallis test followed by two–stage step-up method of Benjamini, Krieger and Yekutieli as post-hoc test. All values were presented as medians (Md) with interquartile range (IQR). Two values were considered as statistically significantly different if p – value was less than 0.05 ($p < 0.05$).

2. RESULTS

2.1. Evaluation of clinical symptoms using Longo score questionnaire before and after Contour Transtar surgery

Overall, a significant improvement in ODS scores (p < 0.01) was observed (Picture 2.1). Average ODS score before surgery was 18.08 ± 7.09 points, at 12 months follow-up – 2.47 ± 2.01, at 24 months follow-up – 2.69 ± 2.32 (p < 0.01), but at 36 months follow-up – 4.00 ± 3.55 (p < 0.01).



Picture 2.1. **Comparison of ODS scores at baseline (before surgery) and at a 12, 24 and 36-months follow-up (p < 0.01)**

Longo score results of the patients with additional defecography findings also demonstrated significant improvement. ODS score of patients with enterocele (n = 14) before surgery was 17, but at 36 months follow-up 3 (p < 0.01). ODS score of patients with puborectalis syndrome (n = 10) before surgery was 24, but at 24 and 36 months follow-up 3 (p < 0.01). There was one patient with anal dilation, ODS score of this patient before surgery was 23, but at 36 months follow-up – 22, although she noted significant clinical symptom improvement.

Distribution of patients' complaints by symptoms based on ODS questionnaire was evaluated to find out the most severe symptoms and to distinguish residual symptoms (Table 2.1).

Table 2.1

Distribution of patients' complaints by symptoms based on ODS questionnaire

	Before surgery n (%)	3 years after surgery n (%)
Defecation frequency	66 (75 %)	10 (11.3 %)
Straining intensity	73 (82.9 %)	12 (13.6 %)
Straining duration	80 (90.9 %)	15 (17.0 %)
Sensation of incomplete evacuation	78 (88.6 %)	12 (13.6 %)
Pain and discomfort during defecation	81 (92 %)	15 (17.0 %)
Activity reduction per week	76 (86.3 %)	12 (13.6 %)
Laxatives	44 (50.0 %) 22 (25.0 %) daily	10 (11.3 %) 1 (1.1 %) daily
Enemas	34 (38.6 %) 17 (19.3 %) daily	10 (11.3 %) 0 (0 %) daily
Digitation	37 (42.0 %) 17 (19.3 %) daily	3 (3.4 %) all daily

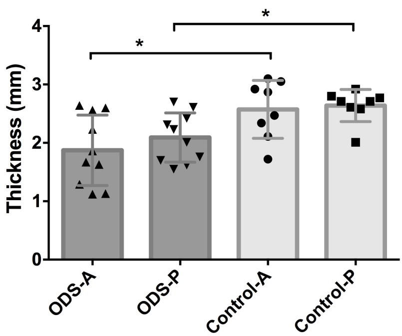
General disease symptoms were distributed evenly before surgery. Residual symptoms after surgery were also distributed similarly without maximum scores 11.3–17 %.

The most severe indicators: laxatives, enemas and digitation, the daily usage of which is scored – 7 in maximum, were reported by half of the patients before surgery and half of them reported severe forms of daily usage. The severe symptom category (laxatives, enemas and digitation) was reported less after surgery – 11.3 % and severe forms were observed quite rarely (1–3.4 %), which suggests the wrong choice for treatment.

2.2. Biomechanical properties of the rectal wall specimens in ODS patients and control group

Analysis of the results showed that samples of the rectum are thicker in the control group compared to the ODS (Picture 2.2).

The thickness of control samples was 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 [Md = 1.77 mm (1.25; 2.57)]. Posteriorly, the thickness differed significantly ($p = 0.015$) when the control group was compared [Md = 2.71 mm (2.46; 2.78)] to the group with ODS [Md = 2.12 mm (1.68; 2.46)], respectively.



Picture 2.2. **Thickness of the anterior (A) and posterior (P) walls of the rectum found in patients with ODS and controls**

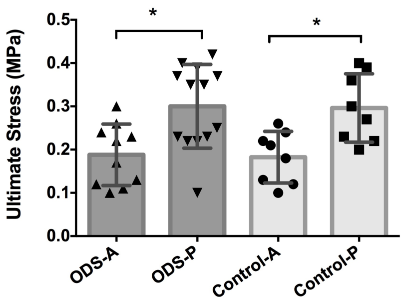
(* – statistically significant difference between two values, $p < 0.05$)

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.

Biomechanical experiments revealed a non-linear relationship between stress and strain of the rectal wall. The ultimate stress in the posterior wall (σ^*_P) appeared to be higher than in the anterior wall (σ^*_A) in both groups (Picture 2.3).

In the control group, the ultimate stress in the anterior [Md = 0.195 MPa (0.127; 0.225)] and the posterior [Md = 0.285 MPa (0.227; 0.392)] walls had statistically significant differences ($p = 0.011$).

In the group with ODS, ultimate stress in the anterior and posterior walls was Md = 0.195 MPa (0.117; 0.245) and Md = 0.35 MPa (0.225; 0.38), respectively ($p = 0.018$).

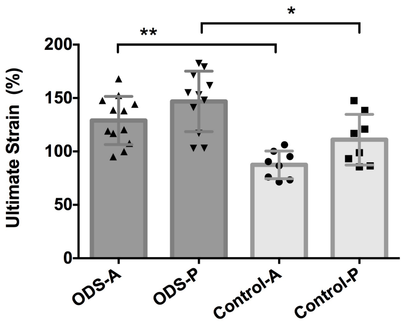


Picture 2.3. **Ultimate stress of the anterior (A) and the posterior (P) walls of the rectum found in patients with ODS and controls**

(* – statistically significant difference between two values, $p < 0.05$).

Regarding the ultimate stress σ^*_A 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 σ^*_P 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 (Picture 2.4). An ultimate deformation for the anterior [Md = 129.2 % (109.9–146.9)] and posterior [Md = 153.1 % (117.0; 171.6)] walls was not different ($p > 0.05$) either for the group with ODS, or the control groups ε^*_A [Md = 88.55 % (75.42; 96.45)] and ε^*_P [Md = 109.9 % (91.55; 140.7)].

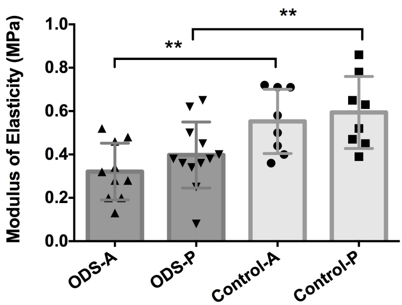


Picture 2.4. **Ultimate strain of the anterior (A) and posterior (P) walls for the control group and the group with ODS**
(* – statistically significant difference between two values, $p < 0.05$; ** – statistically significant difference between two values, $p < 0.01$)

The ultimate strain was statistically significantly higher for the group with ODS than for the control group, and ε^*_A in the group with ODS was statistically significantly higher than ε^*_A in the control group ($p = 0.001$). In the group with ODS, the ultimate stress of the posterior wall (ε^*_P) was higher than in the control group (ε^*_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 (Picture 2.5), which characterises the stiffness of the rectal wall, was statistically significantly lower for the group with ODS than for the control group ($p < 0.05$).

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



Picture 2.5. **Tangential modulus of elasticity of the anterior (A) and posterior (P) walls of the rectum found in patients with ODS and controls**
(* – statistically significant difference between two values, $p < 0.05$; ** – statistically significant difference between two values, $p < 0.01$)

Summarising the data collected using biomechanical tests, it has been suggested 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 pathogenesis of ODS, data reflecting biomechanical properties of the rectal wall were further correlated with the data obtained using morphology, including the status of the enteric nervous system.

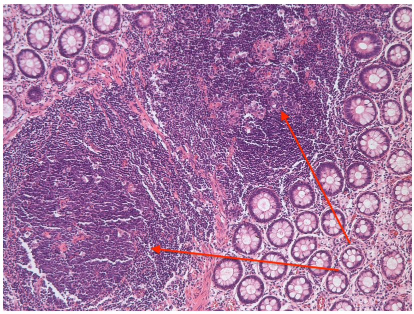
2.3. Morphological analysis of the rectal wall specimens in ODS patients and control group

All layers of the rectal wall in both, patients and controls, were analysed whenever it was possible, as sometimes not all layers were observed due to changes in the rectal wall architectonics.

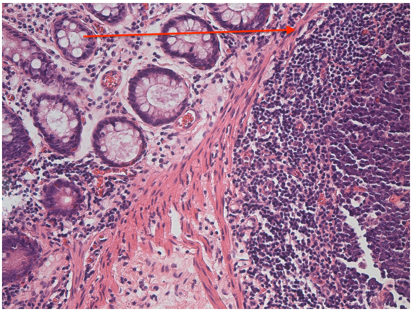
The slides were stained with hematoxylin and eosin and anti-CD 20 for evaluation of the lymphoplasma cells and lymphoid follicles.

Histopathological examination using hematoxylin and eosin staining method evaluated the basic structures of the rectum and determined which elements of the rectal wall should be given more detailed examination. Immunohistochemical examination, on the other hand, has already been carried out to clarify the changes detected in routine staining, to specify localization of muscle cells and elements of the nervous system.

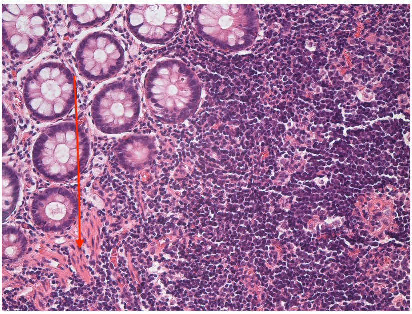
Focal lymphoplasma cells infiltration was observed for the control group in all slides, but in ODS patient group in 11 cases there was focal and in 2 cases diffuse lymphoplasma cells infiltration. Lymphoid follicles were found in 4 out of 13 patient slides (30.8 %), but in the control group they were not detected (Picture 2.6). The hyperplasia of the lymphatic follicles was so advanced that changes in the architecture of the rectal wall could be observed with a reduction in *lamina muscularis* (Picture 2.7) and even complete destruction (Pictures 2.8, 2.9 and 2.10). In ODS patients group, significant reduction of the mucosal crypts was observed in the rectal mucosa. Due to hyperplasia of the lymphatic follicles, crypto depression, reduction and even destruction could be observed.



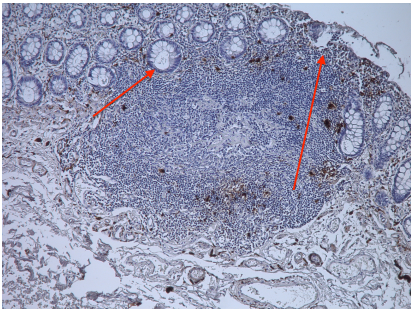
Picture 2.6. **Lymphatic follicles in the rectal wall *lamina propria* with muscle fiber suppression in ODS patients group. Hematoxylin and eosin staining x100**



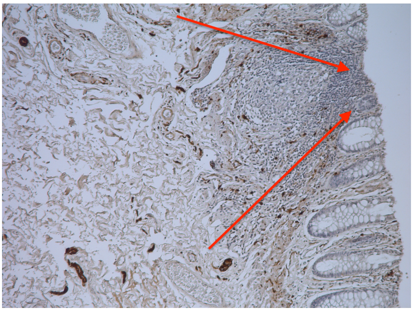
Picture 2.7. **Hyperplasia of lymphatic follicles in the rectal wall with observed alterations in architecture of the rectal wall and reduction of *lamina muscularis*' thickness in the patient group. Hematoxylin and eosin staining, x200**



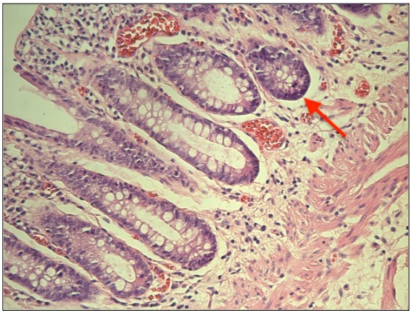
Picture 2.8. **Advanced hyperplasia of lymphoid follicles with observed alterations in the rectal wall architecture in the patients' group. The *lamina muscularis* has disappeared completely, with only a few muscle fibers remaining (arrow). Hematoxylin and eosin staining, x200**



Picture 2.9. **Hyperplasia of lymphatic follicles in the rectal wall in the patients' group with suppression and destruction of mucosal crypts. S-100, x100**



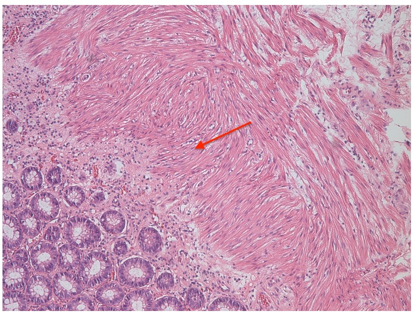
Picture 2.10. **Hyperplasia of lymphatic follicles in the rectal wall in the patients' group observed from the submucosa to the lumen of the rectum with suppression and destruction of mucosal crypts. S-100, x100**



Picture 2.11. **Increased mitotic activity in the crypts in the anterior wall of the rectum in ODS patients' group. Hematoxylin and eosin staining, x 200**

Mitotic activity was statistically significantly higher for the group with ODS (Md = 1.0 (1.0; 1.0)) than for the control group (Md = 0.0 (0.0; 0.75)). This confirms the enhanced recovery of the rectal wall.

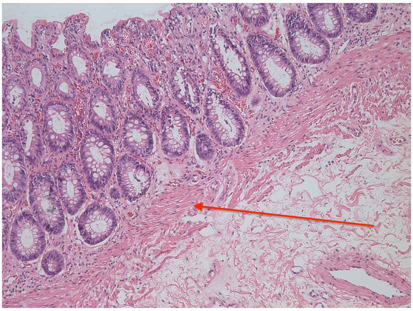
The mucosal *lamina muscularis* and *muscularis externa* myocytes as well as pericryptal myofibroblasts were labeled by anti-α-SMA, both study groups' samples showed high immunoreactivity, no differences were detected.



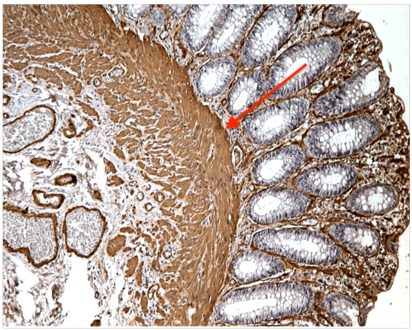
Picture 2.12. **Thickened *lamina muscularis* mucosae with enhanced fiber disorganisation. Hematoxylin and eosin staining, x100**

The mucosal *lamina muscularis propria* thickness was higher (p = 0.046), and disorganisation (Pictures 2.12 and 2.13) – more pronounced in the patients' group [Md = 2.00 (1.00; 2.50)] compared to controls [Md = 1.00 (1.00; 1.00)].

For a more accurate assessment of the pathology, myocytes of the rectal mucosa *lamina muscularis* and the muscular layers, as well as the pericryptal myofibroblasts were stained with anti-α-SMA antibody (Picture 2.14).



Picture 2.13. **Thickened *lamina muscularis* mucosa and submucosa. Hematoxylin and eosin staining, x100**



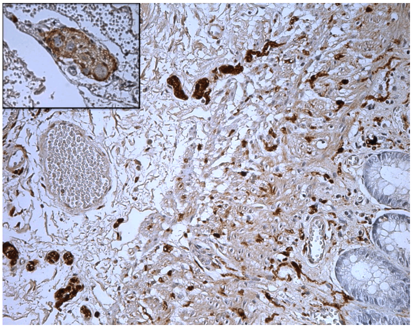
Picture2.14 **The anterior wall of the rectum of patient with ODS revealing the thickened mucosal muscular lamina, mucosal pericryptal myofibroblasts and positivity of vascular beds. α-SMA immunohistology-chemistry, ×100**

The connective tissue of the rectal wall was evaluated with particular attention to the connective tissue of the mucous membrane and the submucosa. 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), specifying pericryptal, submucosal, and intermuscular localisation, respectively. Similar results, estimating the same localisations, were demonstrated for the posterior wall – Md = 3.00 (3.00; 3.00) *vs.* Md = 1.50 (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 mentioned parameters compared using the Wilcoxon test revealed no differences within the groups estimating the staining values.

Picture 2.15. **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**

In ODS, a pericryptal mucosal reticular network was delicate, whereas the submucosal one was presented with the heavily stained fibers often inserted between mucosal *lamina muscularis* constituents (Picture 2.15.). Finally, reticular fibers appeared as ones tightly enveloping *muscularis externa* myocytes.

By contrast with the sum of collagen calculated, the estimations of reticular fibers only did not differ significantly anteriorly when controls were compared to ODS, whereas posteriorly, the pericryptal density of reticular fibers was significantly ($p < 0.001$) higher in controls compared to ODS – Md =2.00 (1.00; 2.00) *vs.* Md = 1.00 (0.00; 1.00), simultaneously revealing no differences in a submucosal and intermuscular distribution.



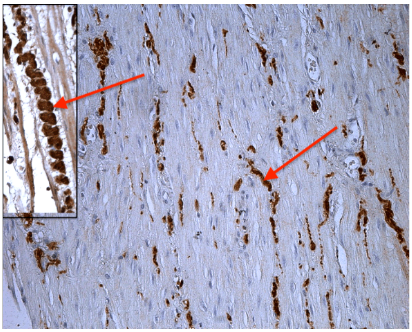
Picture 2.16. **S100 positivity demonstrated in delicate mucosal nerve fibers and those traversing muscular lamina continuously appears as strongly pronounced submucosal bundles found within the anterior rectal wall in case of ODS. Inset: Submucosal nerve plexus with the unstained neurons surrounded by S100-positive glial cells. S100 immunohistochemistry: ×200; ×250 (inset)**

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 (Picture 2.16.).

Both, straight and wavy S100-positive nerve fibers were demonstrated within the *muscularis externa* (Picture 2.17.).

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 = 0.150 (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].

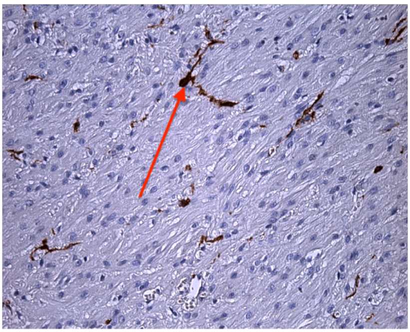


Picture 2.17. **S100 positivity within the muscularis externa appearing either as straight or wavy (inset) paralleled, heavily decorated nerve fibers found within the anterior rectal wall in case of ODS. S100 immunohistochemistry, ×200 (for both images)**

In order to obtain more detailed information about the rectal wall's innervation, Cajal cells of the rectal wall were also studied. ICCs presented as highly ramified cells bearing long, slender processes (Picture 2.18.) establishing intimate vascular and nerve plexuses contacts (Picture 2.19.).

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.

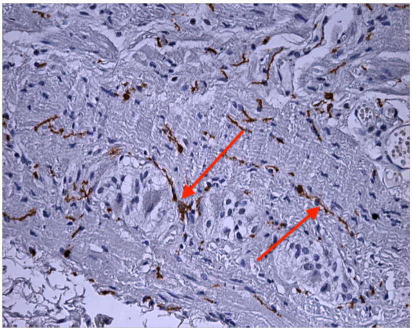
Estimations done for the posterior wall revealed similar results expressed as follows: ICC-IM [Md = 11.00 (10.00; 13.00) *vs.* Md = 5.00 (4.00; 6.75), $p < 0.001$], ICC-MY [Md = 9.00 (8.00; 10.00) *vs.* Md = 5.00 (4.00; 5.00), $p < 0.001$], and ICC-SM [Md = 3.00 (2.00; 4.00) *vs.* Md = 1.00 (0.75; 2.00), $p = 0.002$], for the patients and controls, respectively.



Picture 2.18. **The anterior wall of the rectum of patient with ODS revealing the CD117-positive ICC-IM displaying ramified processes. CD117 immunohistochemistry, ×250**

It is worth noting that CD117 and S100 estimations considered collectively within entire groups demonstrated that in the case of ODS ICC-IM, 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 cell count and estimation of S100 expression did not reveal any differences between the walls ($p = 0.918$; $p = 0.440$; $p = 0.621$, respectively).



Picture 2.19. **The posterior rectal wall 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 immuno-histochemistry, ×250**

Table 2.2.

Summary of morphological analysis

		Norma		Pathology		p
		Anterior wall	Posterior wall	Anterior wall	Posterior wall	
Lymphoid follicles		-	-	+	+	
Mitotic activity		-	-	+	+	0.001
Anti SMA		+	+	+	+	
Thickness and disorganisation of *lamina muscularis propria*		1.00	1.00	2.00	2.00	0.046
Density of collagen fibers	Pericryptal	3.00	3.00	2.00	1.50	< 0.001
	Submucosal	3.00	3.00	1.00	1.00	< 0.001
	Intermuscular	3.00	3.00	1.00	1.00	< 0.001
Reticular fibers	Pericryptal	+	2.00	+	1.00	
	Submucosal	+	+	+	+	
	Intermuscular	+	+	+	+	
S100 - MP		29.50	+	45.00	+	0.005
S100 - SP		8.50	+	17.00	+	0.002
S100 expression		0.675	0.225	0.15	0.6	
ICC - IM		5.00	5.00	13.00	11.00	< 0.001
ICC - MY		5.00	5.00	13.00	9.00	< 0.001
ICC - SM		1.50	1.00	3.00	3.00	0.029

+ - no statistically significant difference between the study groups.

3. DISCUSSION

3.1. Evaluation of Contour Transtar (CoTr) surgery results using Longo's ODS score

Different symptoms assessment scores are used for assessment and measurement of constipation. The most popular is the Rome II criteria for diagnosis of chronic constipation (Table 3.1), but these criteria do not assess severity of constipation. More complicated constipation scoring systems have been introduced. The Rome II criteria were also updated on Rome III and Rome IV criteria.

When assessing a patient with chronic constipation, irritable bowel syndrome constipation type should be excluded.

Cleveland constipation scoring system (CCSS), severe symptoms score (SSS) and Longo's ODS score have also been offered for a more accurate evaluation of chronic constipation.

Table 3.1

Rome II criteria for diagnosis of chronic functional constipation

Two or more of the following for at least 12 weeks (3 months), which need not be consecutive, in preceding 12 months: 1. Straining during > 25 % of bowel movements 2. Lumpy or hard stools for > 25 % of bowel movements 3. Sensation of incomplete evacuation for > 25 % of bowel movements 4. Sensation of anorectal blockage for > 25 % of bowel movements 5. Manual maneuvers to facilitate > 25 % of bowel movements (e.g., digital evacuation or support of the pelvic floor); and/or 6. < 3 bowel movements per week Loose stools are not present, and there are no insufficient criteria for irritable bowel syndrome.

Cleveland subjective constipation score is calculated based on a detailed questionnaire that included over 100 constipation-related symptoms. A simplified version with 8 questions (Table 3.2), which is called Wexner or

Agachan Score more often is used in daily practice (Sharma, 2012). CCSS score > 15 is considered as a criterion for surgical treatment.

Severe symptoms score consists of nine parameters including need laxatives/enemas, unsuccessful attempts to open bowels, low frequency of bowel movements, pain on opening bowels, bleeding on bowel opening, incomplete bowel opening, increased time or straining to open bowels, incontinence/soiling, difficulty to withstand urge to open bowels which are graded on a scale of 0–4 with a maximum score of 36.

Other constipation assessment scales based on clinical symptoms and/or quality of life questionnaire are offered in literature. The most recently used score is Longo's ODS score. This scoring system was given by Italian surgeon A. Longo after ODS definitions have been introduced at the end of the 1990s, who also devised STARR procedure for treatment of ODS. Original Longo score (0–40) is a 8 point score, but several authors offer more simple modified Longo ODS score (Caetano *et al.*, 2018; Renzi *et al.*, 2013; Sharma, 2012).

Constipation scores most commonly are used to assess the efficacy of a surgery. There is a lot of discussion about cut off point for intervention (surgical treatment) in ODS patients. There is no consensus till date on cut off score. A cut-off point of CCSS score is < 15 (50 % of maximum), but in case of ODS: 7–9 points (20 % of maximum).

The exact cut-off point is difficult to set, multiple factors impact recommendations for surgery and usage of constipation score could be as an additional tool. Results of investigations, anatomical findings, patient's objective and subjective condition, quality of life and effectiveness of conservative treatment should be taken into account in addition to constipation score.

Table 3.2

Cleveland Clinic Constipation Scoring System (CCSS)

(min score – 0, max – 30)

Frequency of bowel movements	
1–2 times per 1–2 days	0
2 times per week	1
Once per week	2
Less than once per week	3
Less than once per month	4
Difficulty: painful evacuation effort	
Never	0
Rarely	1
Sometimes	2
Usually	3
Always	4
Completeness: feeling incomplete evacuation	
Never	0
Rarely	1
Sometimes	2
Usually	3
Always	4
Pain: abdominal pain	
Never	0
Rarely	1
Sometimes	2
Usually	3
Always	4
Time: minutes in lavatory per attempt	
Less than 5	0
5–10	1
10–20	2
20–30	3
More than 30	4
Assistance: type of assistance	
Without assistance	0
Simulative laxatives	1
Digital assistance or enema	2
Failure: unsuccessful attempts for evacuation per 24 hours	
Never	0
1–3	1
3–6	2
6–9	3
More than 9	4
	0

Table 3.2 (continuation)

Cleveland Clinic Constipation Scoring System (CCSS)

(min score – 0, max – 30)

History: duration of constipation (years)	
1–5	1
5–10	2
10–20	3
More than 20	4

When evaluating postoperative results, two extremes can be detected: a more accurate measurement of surgical efficiency or a simpler use for the doctor/patient. The extended version of the CCSS score is more used in academic studies. In everyday practice, however, the full version of CCSS is not convenient, so other assessment scores are usually used. Studies have shown that the most commonly used evaluation scales (CCSS, SSS, Longo ODS) are equally effective in evaluating postoperative results (Yu *et al.*, 2017).

Since the study patients underwent Contour Transtar surgery, Longo ODS score was chosen, which was adapted to local needs. As the study patients had underwent a complete course of conservative therapy with laxative, physiotherapy and biofeedback prior to the surgery, patients had a relatively high pre-surgery score of 18.08 ± 7.09 points. In the postoperative period, stable good results were observed in both short term (12 months) and long term (36 months) – 2.47 ± 2.01 and 4.00 ± 3.55, respectively. Patients with markedly higher scores prior to surgery felt subjectively better. Patients with lower scores before surgery without statically significant differences after surgery felt subjective less satisfied, although there was an improvement in constipation scores. In the current experience, however, the indication for surgery should be advised if the Longo ODS score is > 15 (38 % of the maximum).

In a similar international study, data from 22 European coloproctology centers (n = 100) on the effectiveness of the medium-term Contour Transtar operation were collected during the year. The mean ODS score before surgery and 12 months after surgery was 15 vs. 4; $p < 0.01$, (Ribaric *et al.*, 2014), which does not differ significantly from our data. Equally good results were also presented by the authors in other studies from the ODS register in Germany: n = 379, 11.14 vs. 6.45; $p < 0,01$ (Schwandner, Furst and German, 2010), Italy: n = 2171, 16.7 vs. 5.0; $p < 0.01$ (Stuto *et al.*, 2011) and China: n = 75, 18.39 vs. 8.55; $p < 0.01$ (Zhang *et al.*, 2013).

Table 3.3.

Comparison of constipation scores

	Authors	**Num. of quest.**	**Target auditory**	**Max. score**	**Point value of one item**	**Consti-pation score**
Constipation assessment scale (CAS)	McShane McLane	8	Oncology patients, pregnant women	16	0–2	> 1
Wexner Cleveland constipation scoring system (CSS)	Wexner, Agachan	8		30	0–4	> 15
Severe symptoms score (SSS)		9	Large clinical trials	36	0–4	
Patient Assessment of Constipation – Symptom (PAC-SYM		12	Patient self-assess-ment	48	0–4	no
Patient Assessment of Constipation-Quality of Life Questionnaire (PAC-QoL		28	Assess-ment of the quality of life	96		no

Table 3.3. (continuation)

Comparison of constipation scores

Visual Scale Analog Questionnaire (VSAQ)		5		40	1-5, 0-10	
Garrigues Questionnaire		21	Assessment of possibility of constipation		4	
Chinese Constipation Questionnaire		6	Functional constipation, widely used in Eastern countries			
Knowles Eccersley Scott symptom score		11	Diagnosis of constipation, differentiating between various subtypes of constipation	39	0–4	> 11
Longo's ODS score	A. Longo	8	Scoring system to diagnose and to choose the treatment strategy for ODS patients	40	0–7	> 9

Although indications for the Contour Transtar surgery are not *m. puborectalis* syndrome and enterocele, the surgery demonstrated positive long-term outcomes in these cases as well.

Most likely, *m. puborectalis* syndrome and enterocele have been additional findings and not the cause of the underlying disease. However, when planning surgery, it should be considered that a single operation may also help to eliminate the additional findings and improve the patient’s quality of life. Patient complaints about constipation should be regarded as a complex problem that can be caused by a simple anatomical pathology that can be corrected by surgery, but there may also be a multifactorial etiology requiring a multidisciplinary approach with the use of different treatment methods.

The Longo ODS score is a convenient and simple test to measure the severity of constipation, assess the need for surgery and evaluate the effectiveness of the surgery in the short and long term. This score could be also used to easily evaluate not only surgical outcome but also conservative treatment’s result before surgery.

Longo ODS score questions could be included taking a medical history that will improve communication and allow the doctor to gain a patient's confidence.

3.2. Comparison of biomechanical properties and morphological changes of the anterior and posterior rectal wall in the patient and control group

Obstructed defecation causes constipation either functional, or mechanical. The impaired function gradually brings morphological changes causing a mechanical blockage to the fecal passage and accentuating ODS (Rashid and Khuroo, 2014). ODS often is attributable to the muscular dysfunction of the pelvic floor, and multiple risk factors have been mentioned. There are publications on parasympathetic innervation deficiency and dysfunction of regulation centers in the case of certain constipation (Fukuda and Fukai, 1982; Varma, 1992). Recently published data on experimental colitis studies have strengthened our knowledge of biomechanical and histopathological research into the pathophysiology of disease. However, biomechanical properties and morphological features of the constituents of the human rectal wall analyzed collectively have not been studied before as far.

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

At the time of the study, no data were available in literature on biomechanical and morphological studies of the rectum in patients with ODS. From previous studies, it is known that biomechanical properties of colon are determined by the muscle layer (muscularis) and the submucosa, but the serosa and mucosa are irrelevant (Egorov *et al.*, 2002). Another publication demonstrated that the ultimate stress and the ultimate deformation of the rectal wall of healthy people are lower than the vaginal wall, but larger than the bladder wall (Rubod *et al.*, 2012). However, previous publications have not explained the structural differences between the anterior and posterior walls of the rectum and their biomechanical properties.

Analysing the biomechanical results of the study, it was found that in patients with ODS, the rectal wall is thinner than the control group, the ultimate deformation or deformability is greater, and the tangential modulus of elasticity or tissue stiffness is lower. In contrast, the ultimate stress or strength does not differ between comparable groups. The difference between the two groups is only by comparing the anterior and posterior rectal walls.

From the study data of the measurements of biomechanical properties of the rectal wall, it can be concluded that in the pathology group there is no difference between the rectal anterior and posterior walls, and comparing to the control group, some stiffness element in the rectal wall has disappeared.

Performing morphological analysis of the rectal wall, the amount of collagen fibers was analysed and the confirmation of biomechanical findings data received. In addition, since morphological analysis and accurate statistical calculations showed that the control group had a higher density of collagen fibers in the rectal anterior wall than in the ODS group, it would be thought that loss of connective tissue would be associated with a larger stretch of the front wall. Comparing two histochemical staining methods for collagen density determination, it was concluded that Masson's staining method, which more precisely indicates the density of all collagen elements, is more accurate for the current study than Reticulin-Nuclear Fast Red staining, which reveals only collagen III, which forms the network, fixes the components of the wall and tightly covering myocytes. Such assumptions were made about the histologic techniques based on McGeivin's histotechnical guidelines (McGavin, 2014). Undoubtedly, loss and weakening of connective tissue architectonics in the rectal wall in ODS patients leads to further deterioration of the situation. From this point of view, the pronounced inflammatory cell proliferation in mucosal crypts and lymphoid follicles infiltration in the mucosa could also be explained, as well as the mucosal *lamina muscularis* adaptive increase in thickness with myocyte disorganisation that was observed in the patient group compared to the control group. In this case, direct correlation between the reduction of rectal stiffness and the histopathological changes can be explained.

Recent advances in gastrointestinal research made a better understanding of ICC function and their role in the gastrointestinal tract, and studies based on different types of techniques have shown that ICC, as an integral part of the GI neuromuscular apparatus, transduce inputs from enteric motor neurons, generate intrinsic electrical rhythmicity in phasic smooth muscles, and have a mechanical sensation ability (Al-Shboul, 2013). In addition, the colon ICC has a more pronounced role in the *muscularis externa* feeding than in the stomach and small intestine (Iino and Horiguchi, 2006). ICC are found decreased throughout the colon from caecum, to the ascending colon, to the transverse colon and up to sigmoid colon in slow transit constipation (Lyford *et al.*, 2002). However, there are many discussions about the role of ICC in various colonic regions and, in terms of the role of ICC in etiology of a particular pathology, ICC count should be evaluated in the isolated colonic region rather than in the entire colon (Horisawa, Watanabe and Torihashi, 1998). By visualising with CD117 staining, ICC presented as highly ramified stellate-shaped cells bearing long, slender processes in *muscularis externa* of the anterior rectal wall. Similar observations have been made and proved by other authors as well (Hagger *et al.*, 1998).

It is worth noting that publications on the role of ICC in the human rectum are quite limited. In early results of research, rectal ICCs are mentioned as regulators of electrical activity in the rectal wall (Shafik *et al.*, 2004). Other studies of the count of ICC in the human rectum have proven that in case of constipation both increased and reduced ICC count in the rectal wall may be indicated (Bananzadeh *et al.*, 2013). Studies on the count of ICC in patients with rectal prolapse also demonstrated increase in the number of ICC cells (Sileri *et al.*, 2016). It seems that the count of ICC plays a role in etiology of pathology.

In the current study, evaluating the number of ICC cells in both the anterior and the posterior rectal walls, the number was greater in the group of patients compared to the control group. Despite the fact that the study group consisted of patients aged 50–60 years and this study did not aim to detect changes in ICC and glial cells of the aging colon, it should be taken into account that enteric neurodegeneration is considered a likely cause for the development of constipation in the aging gut in animal models (Wiskur and Greenwood-Van Meerveld, 2010). It can also be noted that ICC count in gastrointestinal tract tends to decrease with age (Gomez-Pinilla *et al.*, 2011). Since the enteric nervous system consists of many cell types, there are some difficulties in counting each cell's characteristic changes in every pathology (Saffrey, 2014). In some studies, authors report that gut cell aging processes are determined by diet and gut microbiome (Saffrey, 2013). Other authors, on the other hand, support hypothesis that disorders of paracrine neurotrophic signaling play an important role in the aging processes of enteric nervous system (Korsak *et al.*, 2012).

In the study, with accurate estimates, unequal distribution of S100 expression between the anterior and the posterior rectal walls was found. These results partly confirm the results of other studies, in which different effects of advanced physiological aging on the enteric nervous system's different subpopulations of neurons were analysed and regional and species specific differences noted (Bitar *et al.*, 2011). When evaluating the neuromuscular apparatus as a whole, it was found that CD 117 and S100 positive structures are more likely to be found in the anterior rectal wall.

Enteric glial cells were most commonly found in the colonic *myenteric plexus*, especially in the anterior rectal wall of ODS patients. In contrast, S100 expression in ODS patients in the anterior rectal wall was statistically lower compared to the control group. This indicates close cooperation between the glial nervous system and ICC. It is also worth noting that in some studies on novel functional roles for enteric glia in the gastrointestinal tract, it has been shown that glia within enteric ganglia are activated by synaptic stimulation, suggesting an active role in synaptic transmission (Gulbransen and Sharkey, 2012). Knowing the limitations of our study, the conclusions were based on the count of ICC together with enteric glial cells, as the evaluation of similar results was reported before (Knowles *et al.*, 2010).

3.3. Primary and secondary changes in the rectal wall in patients with ODS

The current study showed statistically significant differences in biomechanical and morphological properties in the rectal wall compared to the control group. Initially, it is difficult to understand which are primary and which secondary changes in the rectal wall.

However, a series of logical conclusions could help here. The changes that are observed in the pathology group in both the anterior and posterior rectal walls are primary. These changes are not affected by the rectal adjacent organs and their topographical location. The anterior rectal wall is a relatively weak point. From the back, the rectum is supported by the ventral surface of the lower half of the sacrum and the adjoining coccyx, but from the front it is fixed by the posterior wall of the intra pelvic vagina, *m. levator ani* and connective tissues of partitions. Pregnancy and parity are considered to be one of the factors contributing to rectocele, when the pelvic ligaments and musculoskeletal apparatus are stretched (MacLennan *et al.*, 2000). Consequently, changes in biomechanical properties in the rectal wall, which have developed in both the anterior and posterior rectal walls, despite the different stability of the adjacent organs, should be considered as primary changes. Whereas, changes that are found only in the anterior rectal wall of the study group compared to the posterior wall, not detected in the control group, could be considered secondary changes or compensatory mechanism.

From the above it is concluded that biomechanical changes: ultimate deformation and the tangential modulus of elasticity are associated with primary changes in the rectal wall. These changes are also confirmed by morphological examination, where reduction in the density of collagen structures in both the anterior and posterior walls was detected.

An increased number of ICC in the *myenteric plexus* of the rectal anterior wall and intramuscularly in ODS patients could be defined as secondary changes. In this case, cells are likely to act as pacemaker cells to compensate for reduced rectal rigidity, but to increase rectal smooth muscle myocyte tone.

Studies in animal models following rectal treatment with no ablative radiofrequency showed an increase in collagen type I and a statistically significant reduction in ICC compared to the control group (Herman *et al.*, 2015). In this case, ICC may decrease as the tissue architectonics stabilises. Comparing the number of ICC cells in the surgical specimens of the patients with different postoperative outcomes, it was reported that in patients with postoperative relapse and an unfavorable functional outcome a relatively lower number of ICCs in the rectal wall was observed (Lin et al., 2018). Pre-operative reduced amount of ICC cells in the rectal wall might predict for the unfavorable functional outcome following surgery. Whereas, the amount of ICC cells itself is genetically determined (Fontanesi et al., 2014).

Since studies on biomechanical properties of pelvic organs have shown that vaginal walls are tougher and less deformable compared to the rectum (Rubod *et al.*, 2012), the role of the vaginal wall and surrounding pelvic tissues in the development of ODS should be recognised.

In summary regarding to ODS etiopathogenesis, it can be concluded that ODS is caused by a collagen defect or deficiency in the rectal wall that results in ODS manifestation due to other external factors, but the increased amount of ICC is like a compensating mechanism, but not as an etiological factor.

Since the etiopathological mechanism of ODS is multifactorial with primary and secondary changes in the rectal wall, it is also difficult to define the most accurate surgical approach. Following this analysis of the results, it can be said that both resection and reconstructive type of surgery are appropriate. Resection type surgery results in elimination of the morphologically changed colonic segment. Reconstructive surgery despite primary morphological changes in the rectal wall, reduce the possibility of developing secondary changes in the rectal wall and discontinue the development of clinical symptoms. Each of these surgical techniques in the treatment of patients with ODS affects different phases of ODS etiopathological mechanisms.

As ODS is symptomatic only in a case when secondary changes in the rectal anterior wall have developed, selective resection of the anterior rectal wall is appropriate, that is also associated with less risk of injury and less risk of complications.

3.4. ODS preventive measures

To some extent, the whole range of conservative treatment is considered as preventive measures. The whole range of measures that delay surgical intervention and improve a patient's quality of life can be defined as preventive measures.

Patients who develop clinical signs of ODS should follow appropriate diet rich in fiber to make stools softer and easier to pass. Adequate water intake at least 30 ml/kg body is recommended. Regular meals and utilising natural postprandial gastrocolic reflex (the patient should be encouraged to attempt defecation shortly (30 min) after meals) will facilitate bowel emptying with minimal straining. Patients should also be recommended the accurate toilet culture: adopting the correct toilet position with the knees slightly above the hips (a semi-squatting position), spending time in toilet for no more than 5 minutes, do not occupy oneself with other activities (reading, phone and tablet use), preferably use a bidet instead of toilet paper after visiting the toilet (Emmanuel, 2011).

Different physical activities are recommended to strengthen muscles and ligaments. More focused activities on pelvic muscles and ligaments are aerobics, pilates, medical gymnastics, running and walking (Fabrizio, Alimi and Kumar, 2017; Rao, 2009; Sharma and Rao, 2017).

Adequate management of childbirth process should be recognised as an important preventive measure of ODS. A fast childbirth could damage the pelvic ligament and musculoskeletal apparatus, which later significantly weakens the rectovaginal septum, resulting in chronic constipation (Ferdinande *et al.*, 2018; Shin, Toto and Schey, 2015).

Following healthy diet and healthy exercise adherence will significantly improve patient quality of life and reduce the risk of surgical intervention.

3.5. Future treatment options for ODS

At present, different operative techniques are the ultimate ODS treatment options. There exists a great variety of operative techniques to treat patients with ODS. The operative approach can be transabdominal, laparoscopic, transperineal, trans anal and transvaginal; in addition, resection or reconstructive surgery with or without mesh implantation during each approach could also be performed. The results depend on the type of pathology, total surgical volume, accompanying diseases and experience of a surgeon (Riss and Stift, 2015).

Quite often despite the perfectly performed surgery, real results and improvement of a patient's quality of life are not as good as expected (Janssen and van Dijke, 1994; Murthy *et al.*, 1996). As secondary changes are observed only in the anterior rectal wall, a selective resection of the anterior rectal wall is sufficient. As a result, a patient has less injury and fewer risks of complications. In addition, from an economic point of view, such type of surgery requires a smaller number of staplers' magazines.

Modern medicine's trends are towards a more conservative treatment, prevention and minimally invasive operations. Future research should focus on the development of new minimally invasive surgery treatment methods of ODS. Based on the study results, one of the measures of prevention and elimination of ODS should be to increase the amount of collagen fibers and remodeling in the rectal wall. Studies in animal models following rectal treatment with no ablative radiofrequency showed an increase of the amount of collagen fibers in the rectal wall (Herman *et al.*, 2015). As an example, an application of laser in gynecology could be mentioned; laser treatment methods used in gynecology for the treatment of urinary incontinence and laser therapy for genitourinary syndrome of menopause have demonstrated to change the collagen structure in the vaginal wall.

The study results show that micro ablative fractional CO_2 laser can produce remodeling of the vaginal connective tissue without causing damage to surrounding tissue, and the process of mucosa remodeling while under wound dressings enables collagen to increase and the vaginal wall to become thick and tightened (Kwon *et al.*, 2018; Zerbinati *et al.*, 2015). In a similar way, this kind of regular rectal wall treatment could improve biomechanical properties of the rectal wall and delay disease progression at an early stage.

Focusing more on the development of compensatory mechanisms in the case of ODS, as therapeutic preventive measures, the improvement of neuromuscular impulse transmission in the colon could be advised. Percutaneous posterior tibial and sacral nerve stimulation and biofeedback for treatment can be offered. Increasing and improving the transmission of neural impulses in the rectal wall can help to improve the tone of the rectum and therefore the regression of the disease and the improvement of a patient's quality of life. The number of surgical interventions could be reduced below 20 % by developing new preventive measures and new minimally invasive surgery treatment's methods of ODS patients

CONCLUSIONS

1. Clinical symptoms in patients with ODS and chronic constipation have disappeared, and the quality of life was maintained until the end of the study (36 months) after Contour Transtar surgery
2. Constipation clinical symptoms in patients with ODS and additional defecography findings- rectocele and *m.puborectalis* syndrome have also disappeared after Contour Transtar surgery
3. Ultimate deformation or deformability of the rectal wall is statistically significantly greater in patients with ODS compared to controls
4. The rectal wall in the case of ODS was less stiff compared to the control group: the tangential modulus of elasticity was statistically significantly lower for the group with ODS than for the control group
5. The anterior and posterior rectal walls of ODS patients have statistically significantly lower density of collagen fibers compared to controls.
6. There is a correlation between morphological changes and biomechanical properties in the rectal wall of ODS patients. The relatively greater deformability and lower tissue stiffness can be explained by the reduced amount of collagen fibers in the rectal wall in the patient group.
7. An increased amount of glial and ICC cells has been observed as a compensatory mechanism in patients with ODS, especially in the anterior rectal wall

RECOMMENDATIONS

1. ODS score is an essential and simple tool for daily practice for assessing patients with chronic constipation and evaluating treatment effectiveness
2. Anterior semicircular resection of the rectal wall from etiopathological point of view would be recommended for ODS patients with an isolated rectocele
3. The development of new ODS treatment methods should be aimed at increasing synthesis of collagen and remodeling collagen structures in the rectal wall, especially in the anterior wall.
4. The development of neurogenic stimulation methods is purposeful and etiopathologically based.

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ACKNOWLEDGEMENTS

I would like to cordially thank my scientific supervisors Dr. med. Aigars Martinsons for the idea of this study, for his advice and time devoted; Dr. habil. sc. ing. Vladimirs Kasjanovs for faith in my abilities, the excellent pedagogical approach, motivation, persistence and endless source of energy and positivism. I would not have done it without you!

I would like to express my deep gratitude to Dr. habil. med., professor Valentīna Groma for her help in implementation and improvement of the morphological section of the Doctoral Thesis.

Thanks Dr. Katrīna Pekarska for summarising morphology results.

I would also like to thank all the staff of Rīga Stradiņš University Department of Morphology for their great work they have done.

Gratitude to my colleagues Dr. Aija Kusmane and Dr. Kaspars Snipe for the performed surgeries, engaging in the study process, and collegially friendly advice on evaluation of the study results and final presentations.

Thanks to Dr. Jurijs Nazarovas for the opportunity to obtain a control group material.

Thanks to reviewers Dr. med., professor Arnis Engelis, Dr. med., docent Paulius Žeromskas and Dr. habil. sc. phys., professor Jurijs Dehtjars for criticism and advice in elaborating my Doctoral Thesis.

Thanks to Liga Stirne for putting together the correct commas and long marks, as well as getting inadvertent mistakes.

Thanks to Panda for the contribution of literary brilliance to the medical text in the final steps of the work.

Thanks to all my colleagues and to the head nurse Solveiga Rolle for their understanding and their great help in particular when I was unable to fulfill my duties due to the studies.

Thanks to my friends for understanding and ability to help even in cases of most foolish requests, thanks to Inga Lotiša for “arrows”, thanks to the team “Return of Štepselētāji” for friendly support and a relaxed atmosphere during the studies.

My special thanks to my family: my wife Gundega Bruņeniece for her understanding, tenderness and love, children Līva, Elza and Jēkabs for their joy of life and motivation, my mother Vēsma for always being ready to support me in every situation and valuable non-medical advice, my sister Gundega Provais for willingness to help me in any situation.