Both alleles in the control group had approximately the same frequency: 31.3% for the rs759853 polymorphism and 29.9% for the rs9640883 polymorphism. With increasing severity of pathological processes in DM without retinopathy (o NPDR, o PDR), the frequency of the minor allele of the rs759853 polymorphism gradually increased (to a maximum of PDR–59.8%). This made it possible to consider it as a marker of the severity of the pathological process and, at the same time, highlighted its role as a causative factor in the development of eye lesions in type 2 DM.

The A allele frequency of the rs9640883 polymorphism, on the contrary, gradually decreased (to a minimum of 12.5% in NPDR and 15.6% in PDR). That means that this allele can be regarded as protective factor in the development of pathological processes. It is proved by the fact of absence of homozygotes of the minor A/A allele of the rs9640883 polymorphism in all patients with DR (in this study – 128 people).

CONCLUSIONS. The increased G/A heterozygote frequency of the rs759853 polymorphism was associated with the development of type 2 DM without retinopathy; the increased frequency of the mutant A/A homozygote was associated with the development of DR, and, to a greater extent, its proliferative type. In general, patients with increasing severity of the pathological process and progression of DR showed a clear pattern – a decrease in the frequency of the ancestral G allele and an increase in the frequency of the mutant A allele of the rs759853 polymorphism of the AKR1B1 gene, which could be considered a risk factor for the development of DR.

Patients with increasing severity of the pathological process and progression of DR showed a clear pattern – an increase in the frequency of the ancestral G allele and a decrease in the frequency of the mutant A allele of the rs9640883 polymorphism of the AKR1B1 gene. The homozygous genotype of the mutant A/A allele was not detected in patients with DR at all, and this justified the idea of the protective effect of the minor A allele in the development of DR.

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CONNECTIVE TISSUE PATHOLOGY AS A RISK FACTOR FOR INTESTINAL FISTULA DEVELOPMENT

Summary. Retro- and prospective trial was based on data about 30 patients, suffering intestinal fistulas, who were treated in the Shalimov National Institute of Surgery and Transplantology during 2016-2019. There was revealed that the most informative phenotypical markers of undifferentiated dysplasia of the connective tissue (UDCT) in patients with intestinal fistulas are visceral (83,3%), vascular (70%), arrhythmic (70%) pathologies. It is established that a direct correlation between the level of biochemical markers of the collagen biodegradation and the UDCT degree may be applied for prognostication of development and course of complications in patients, suffering entero- colocutaneous fistulas. The presence of connective tissue dysplasia in patients with intestinal fistulas was proved to be the aggravating comorbidity factor, that is difficult to treat and is accompanied by high mortality rates.

Severe degree of UDCT in the patients, intestinal fistulas, constitutes unfavorable prognostic sign and enhances the mortality by 62.5%. The presence of UDCT in the patients with intestinal fistulas is an aggravating

comorbid factor that is difficult to be treated and accompanied by high rates of mortality, which must be taken into account, choosing the adequate surgical tactics and complex pathogenetically substantiated treatment.
Key words: undifferentiated dysplasia of connective tissue, intestinal fistula, phenotypic and biochemical markers, diagnostic criteria, comorbid factor.

Formulation of the problem. Despite the improvement of existing techniques and the development of new surgical technologies, intestinal fistulas are serious complications that are a real threat to the life of the patient. The lack of a single classification, a clear algorithm for surgical treatment and the diversity of the pathological process creates major problems for both the surgeon and the patient. So far, there is no single point of view in the surgical community regarding the causes of intestinal fistulas development and surgical tactics in the development of these complications.

Analysis of recent research and publications.

The incidence of the intestinal fistulas are 1-2% of all abdominal operations, but they create many problems both from the surgical point of view and treatment of the patient [1].

There is still no single generally accepted classification of intestinal fistulas. Practicing surgeons use classic classifications created by Oppel (1927), P.D. Kolchenogov (1957), T.P. Makarenko, A.V. Bogdanova (1986).

A simple and convenient classification based on anatomical, functional (flow rate in ml/day) and etiological characteristics of the intestinal fistulas [2,3] is often found in English sources.

Postoperative intestinal fistulas account for 75-85% of all intestinal fistulas. Postoperative complications in the form of fistulas often develop after oncological surgeries, surgeries for inflammatory bowel disease and acute intestinal obstruction [4].

According to the literature, small intestinal fistulas open into the free abdominal cavity in 29-32%, through the abscess cavity in 24.3% of cases, through the eventrated wound - 9.3% [5]. Mortality in the development of intestinal fistulas in the early postoperative period is 16.5-57.5%, in the acute period (unformed intestinal fistulas) - 20.0-80.0%, with high intestinal fistulas - 82-90% [6]. The main causes of death are progressive peritonitis, sepsis, intoxication, malnutrition, fluid and electrolyte abnormalities, hepatic and renal failure, intestinal insufficiency [7]. Despite improvements in nutritional and metabolic support, antimicrobial therapy, improved wound care, improved surgical techniques, the mortality rate remains extremely high [3,5].

Therefore, the main two pathogenetic directions can be distinguished in the mechanism of development and progression of intestinal fistulas: 1) peritonitis, sepsis, intoxication, which require maximum mobilization of organism resources; 2) the fistula itself, which causes not only rapid loss of energy and nutrients due to malnutrition, but also makes it impossible to adequately replenish them due to impaired enteral nutrition and development of intestinal insufficiency.

Highlighting previously unresolved parts of a common problem.

In domestic and foreign sources, there are a small number of publications about the role of the pathology of connective tissue metabolism in the development of intestinal fistulas.

The prevalence of UDCT is controversial, which is related to different classification and diagnostic approaches. Several authors note that the prevalence of connective tissue dysplasia correlates with the frequency of major socially significant non-infectious diseases and ranges from 20 to 80% according to various literature [8].

Objective. Improving the results of treatment of patients with intestinal fistulas by determining the role of undifferentiated dysplasia of the connective tissue in the development of these complications, assessing the prevalence and development of informative criteria for the diagnosis of dysplasia of the connective tissue.

Material and methods. A retro- and prospective trial was based on data about 30 patients, suffering intestinal fistulas, who were treated at the Shalimov National Institute of Surgery and Transplantology during 2016-2019 and 20 practically healthy people (control group) who are comparable in age and gender with the subjects. 26 patients were referred from the surgical departments of other Ukrainian hospitals, in 4 patients fistulas appeared after operations performed in our clinic. Of the 30 patients in the experimental group, men were 18, women were 12. The age of patients was from 26 to 68 years, the average age was 49.3±7.18 years. All patients underwent a comprehensive examination, including general clinical, special laboratory and instrumental methods of examination. Special laboratory studies included serum procalcitonin and C-reactive protein. Serum hydroxyproline content was measured for connective tissue metabolism evaluation. The study of hydroxyproline metabolism was performed after *Bergman* and Loxley (1969). The level of glycosaminoglycans in the urine was determined using the method of precipitation in terms of grams of creatinine (1991). Material sampling (blood, urine) in the study group was performed after the elimination of peritonitis, in the absence of evidence of a systemic inflammatory reaction, which was quantified by procalcitonin and C-reactive protein levels. The statistical analysis of the survey results was performed using Microsoft® Office Excel (2017) spreadsheets and the statistical processing program Statgraphics Professional 16.0.03. To test the hypothesis of equality of averages, the Student's t-test for normally distributed samples and the Wilcoxon-Mann-Whitney test were used for samples whose distribution was different from normal. Checking the law of sampling for normality was performed using the Shapiro–Wilk test. The statistical dependence between the values was checked using Spearman's rank correlation coefficient.

Presenting the main material. In the study group of patients with intestinal fistulas treated in our clinic, the vast majority of patients (86.6%) had operated in other medical institutions of Ukraine, who were admitted to the Shalimov National Institute of Surgery and Transplantology to determine the tactics and further surgical treatment. After urgent surgeries for acute intestinal obstruction and generalized peritonitis, intestinal fistulas occurred in 15 patients. In 46% of cases (7 out of 15), fistulas developed due to the anastomotic failure and mechanical damage of the intestinal wall during viscerolysis. In other cases, no compelling reasons were identified. After planned operations on the hollow viscera, fistulas occurred in 8 cases, the main reason was the anastomotic failure. In 2 cases, fistulas occurred in patients with giant recurrent ventral hernias (strangulation of intestine in the hernial sac - 1 case, bowel fixation to the mesh graft - 1 case); 2 colonic fistulas in patients with destructive pancreatitis (pressure sores from drainage - 2 cases); damage of the duodenum during right-sided nephrectomy - 1 case, perforation of the colonic diverticula - 2 cases.

Exclusion criteria from the study group were fistulas in patients with ulcerative colitis (4), Crohn's disease (3), and radiation-induced intestinal fistulas (3). Somatic status of patients and operative risk were assessed by ASA physical status classification system: 21 patients were classified as ASA III, and 9 patients were ASA IV. After additional examination and preoperative preparation, mostly reconstructive surgical interventions were performed in the gastrointestinal surgery department. At high fistulas, the possibilities of endoscopic techniques were actively used: stenting, clipping, EndoVac vacuum therapy.

In the examined patients with the anastomotic failure of the hollow viscera, signs of UDCT were found in 27 (90%) patients. The following phenotypic pathologies of UDCT were most commonly encountered (Figure 1).

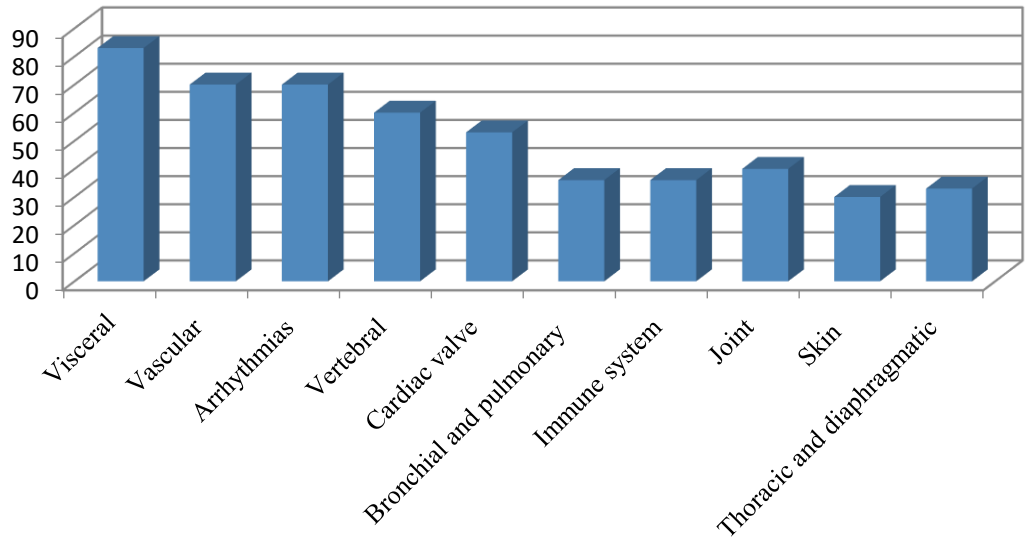

Diagram 1. Phenotypic pathologies of UDCT in patients with intestinal fistulas (%)

1. Visceral pathology (digestive and pelvic viscera ptosis, nephroptosis, dyskinesia of hollow viscera, gastroesophageal and duodenogastric reflux, sphincter insufficiency, esophageal diverticula, hiatal hernia, ventral hernia, rectal prolapse; ptosis, prolapse of reproductive organs in women) - 25 patients (83.3%);
2. Vascular pathology (damage of elastic, muscular and mixed types of arteries: aneurysms, pathological arterial tortuosity; venous damage: pathological tortuosity, varicose veins of upper and lower extremities, hemorrhoids, esophageal varices, varicocele; telangiectasias)- 21 patients (70%);
3. Arrhythmias (ventricular extrasystoles of different degrees; atrial extrasystoles; paroxysmal tachyarrhythmias; pacemaker migration; atrioventricular and ventricular block; long QT syndrome) - 21 patients (70%);
4. Vertebral pathology (vertebral osteochondrosis and instability, vertebral hernia, vertebrobasilar insufficiency; spondylolisthesis) - 18 patients (60%);
5. Cardiac valve pathology (isolated and combined prolapse of cardiac valves, myxomatous mitral valve degeneration) - 16 patients (53.3%);
6. Joint pathology (joint hypermobility according to Beighton Scoring, clubfoot, flatfoot) - 12 patients (40%);
7. Bronchial and pulmonary pathology (tracheobronchial dyskinesia, tracheobronchomegaly, ventilation impairment: obstructive, restrictive, mixed) - 11 patients (36.6%);
8. Immune system pathology (allergic syndrome, syndrome of immunodeficiency, autoimmune syndrome) - 11 patients (36.6%);
9. Thoracic and diaphragmatic pathology (asthenic thorax, deformity of the thorax, vertebral deformity, diaphragmatic location and excursion) - 10 cases (33.3%);
10. Skin pathology (thin, vulnerable skin, atrophic striae, increased skin extensibility, hypertrophic and keloid scars) - 9 cases (30%).

UDCT was diagnosed by a well-established technique (utility model patent No. 120158 UA), which includes evaluation of the most informative phenotypic, visceral and ultrasonographic signs of connective tissue pathology [11].

The degree of dysplasia was evaluated according to the original clinical screening scale, based on the table of criteria for the severity of connective tissue dysplasia according to T.Y. Smolnova (2003). Evaluated phenotypic and visceral signs of connective tissue pathology were divided into small (1 point), medium (2 points) and large (3 points). A score of up to 8 corresponds to a mild UDCT; from 9 to 16 – moderate UDCT; from 17 and more – severe UDCT [9].

Investigation of the phenotypic traits of UDCT in the group of patients with anastomotic failure showed (diagram 2) that 5 patients (16.6%) had a mild degree of UDCT, 12 (40%) had a moderate and, accordingly, 10 patients (33, 3%) revealed a severe degree of UDCT. In 3 patients (10%) signs of connective tissue pathology were not detected.

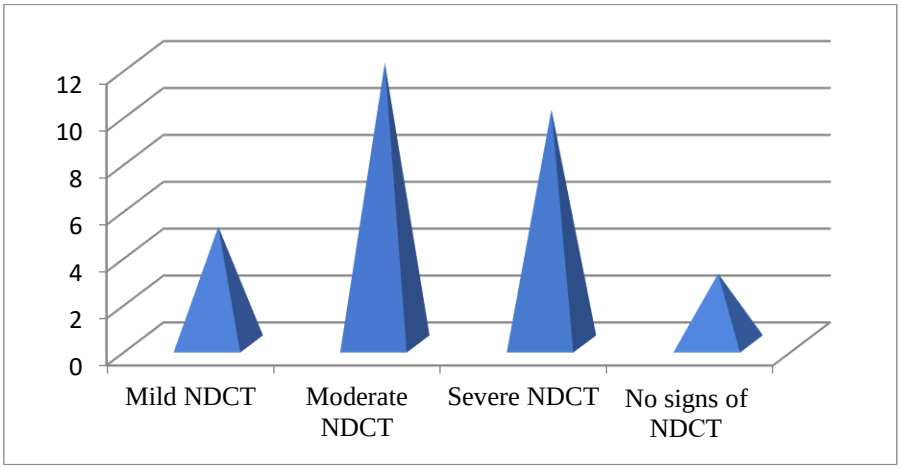

Diagram2. The severity of UDCT in patients with intestinal fistulas

The level of serum hydroxyproline in the group of patients without phenotypic signs of dysplasia was 37.4±4.7 μmol/l, which is 76% higher than the control group (21.2±0.8 μmol/l) (Table 1). These changes are apparently due to increased proteolytic activity in patients with intestinal fistulas and anastomotic failure. This confirms the data of several authors that in patients with intestinal fistulas persistent mismatch in the system of proteinase-protease inhibitors of the plasma developed. Hyperactivation of the proteolytic systems of the body amid the reduction of inhibitory potential is regarded as one of the key pathogenetic links of endogenous intoxication.

When studying the dynamics of changes in serum hydroxyproline levels, it was found that the increase in collagenolytic activity of glycosaminoglycans and free hydroxyproline had a direct correlation with the severity of UDCT. Thus, at mild UDCT level, serum hydroxyproline level was (48.2±2.6) μmol/l, moderate UDCT (75.1±3.6) μmol/l and severe UDCT (114.3±3.9) μmol/l, which is 5.5 times higher than the control group and 3 times higher than patients with intestinal fistulas without clinical signs of dysplasia.

Table 1

Dynamics of some indicators of connective tissue metabolism in patients with intestinal fistulas depending on the severity of UDCT (M±m)

Survey groups	Severity of UDCT	Free serum hydroxyproline (μmol/l)	Glycosaminoglycans in the urine (μmol/l)
Experimental group (n=30)	mild (n=5)	48,2±2,6*	76,18±4,8 **
	moderate (n=12)	75,1±3,6**	111,62±5,5**
	severe (n=10)	114,3±3,9**	129,86±8,4**
	No signs of UDCT (n=3)	37,4±4,7 *	61,32±4,1*
Control group (n=20)		21,2±0,8	44,68±1,8

Note: only statistically significant differences are given (* p <0.05; ** p <0.01).

The study of the dynamics of changes in the level of glycosaminoglycans in the urine also revealed a direct correlation with the severity of UDCT. Thus, with a mild degree of UDCT, the level of glycosaminoglycans was 76.18±4.8 μmol/l, which is probably almost twice the level of the control group (44.68±1.8). With a moderate degree of 111.62±5.5 μmol/l and a severe degree of 129.86±8.4 μmol/l, which is almost 3 times higher than the control group and more than twice the rates of patients with intestinal fistulas without clinical signs of dysplasia.

Therefore, a direct correlation of the level of biochemical markers of collagen biodegradation and the severity of UDCT which diagnosis based on

phenotypic, visceral manifestations and instrumental examinations, can serve as an informative diagnostic criterion for UDCT and can be used to predict the development and course of complications in patients with intestinal fistulas.

Analysis of patients with varying severity of UDCT indicates that the severity of dysplasia was correlated with the severity and duration of the underlying disease. Thus, in the group with moderate (25.12±3.49 days) and severe dysplasia (28.19±4.06 days), the mean length of hospital stay exceeded the indices of groups without signs of dysplasia (15.43±2.17) and with mild dysplasia (18.34±2.9).

The overall mortality rate in the study group (n = 30) was 10%, which is slightly lower than the literature data - 16.5-57.5% [6].

In the group of patients with a moderate degree of dysplasia, 1 lethal event (8.3%) was recorded. In the group of patients with severe dysplasia were 2 lethal cases, which is 20%. It should be noted that in 4 patients with grade 4 ASA and a moderate degree of dysplasia 1 lethal event - 25% recorded, which almost corresponds to the literature data (22-30%) [6]. In patients (5) with a similar grade 4 ASA in the group with severe dysplasia, the mortality rate was 40%, which is almost twice as high.

This suggests that a severe degree of connective tissue dysplasia in patients with intestinal fistulas and the same somatic status (ASA 4) is an unfavorable prognostic sign and 1.6 times (62.5%) increases the incidence of lethal cases.

Thus, studies have shown that the presence of connective tissue dysplasia in patients with intestinal fistulas is an aggravating comorbid factor, which is difficult to treat and is accompanied by high mortality rates. The presence of UDCT signs in such patients should be considered in the choice of surgical tactics and complex pathogenetically substantiated treatment.

Conclusions and suggestions.

1. The most informative phenotypic hallmarks of UDCT in patients with anastomotic failure: visceral (83.3%), vascular (70%), arrhythmic (70%) pathologies.

2. The presence of connective tissue dysplasia in patients with intestinal fistulas is an aggravating comorbid factor, which must be considered when choosing adequate surgical tactics and complex pathogenetically substantiated treatment.

3. Severe connective tissue dysplasia in patients with intestinal fistulas is an unfavorable prognostic sign and 1.6 times (62.5%) increases the incidence of lethal cases.

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