RELATIONSHIP BETWEEN BIOMECHANICAL AND BIOCHEMICAL PARAMETERS OF SPINAL MOTION SEGMENTS AND RECURRENT LUMBAR DISC HERNIATION

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Objective. To identify natural radiological parameters of degenerative spinal motion segments and biochemical changes in intervertebral disc tissues, which have a significant connection with the hernia recurrence after microdiscectomy.

Material and Methods. The MRI, radiography and biochemical parameters of the nucleus pulpous and annulus fibrosus tissues from patients operated for L4—L5 and L5—S1 herniation were assessed and statistically analyzed. Two groups of patients were examined: Group I (n = 50) — with recurrent hernias, Group II (n = 50) — without recurrence during three years.

Results. Significant correlation was observed between recurrent lumbar disc herniation after microdiscectomy and the following biomechanical parameters: height of the intervertebral disc ($p = 0.001$; $r = 0.69$), segmental sagittal range of motion ($p = 0.001$; $r = 0.61$), lumbar lordosis ($p = 0.001$; $r = 0.78$), stage of the intervertebral disc degeneration ($p = 0.001$; $r = 0.46$), and type of hernia ($p = 0.001$; $r = 0.45$). The quantitative and qualitative characteristics of proteoglycans/glycosaminoglycans of the nucleus pulposus and annulus fibrosus differed significantly in patients of the studied groups, but significant correlation with recurrent hernias was not found ($r < 0.3$).

Conclusion. The preserved intervertebral disc height, hypermobility of the spinal motion segment, the smoothness of the lumbar lordosis, moderate intervertebral disc degeneration, and the disc protrusion have significant connection with the recurrence of lumbar intervertebral disc herniation after microdiscectomy.

Key Words: intervertebral disc herniation, microdiscectomy, recurrent disc herniation, glycosaminoglycans, proteoglycans.

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Human spine is a modal kinematic system of the musculoskeletal apparatus, withstanding the effects of gravity. L4–L5 and L5–S1 are the most vulnerable spinal motion segments (SMSs) in the lumbosacral spine.. These segments are the most prone to degenerative processes and draw the greatest attention of spine surgeons. Herniated lumbar intervertebral discs (IVDs) rank first among all the degenerative diseases requiring surgical treatment. Typically, surgical treatment of patients with herniated discs is effective. Excellent and good outcomes account for up to 90–95 % of cases [20]. However, despite the implementation of new techniques and improvement of surgical instrumentation, the recurrence rate of lumbar IVD herniation reaches 5–15 %, which is one of the most common causes of spinal revision surgery [1, 18, 19].

What is the reason for such a significant problem? Many researchers are trying to answer this question. Attempts are being made to identify the factors that negatively affect the outcome of surgical treatment. It is believed that these factors include abnormal development, high physical activity, patient's body weight, gender, age, stage of IVD degeneration, segmental instability, IVD height, and others [11, 19, 21]. However, there is currently no consensus on this problem. We believe that the study of the ultramicrostructure of IVD tissue, which determines the macroscopic stability of the SMS, is one of the most important issues.

IVD tissues have specific structure and include proteoglycans (PGs), collagen and water. PGs are complex protein-carbohydrate molecules that can retain and store the amount of water up to 50 times their weight. They play a crucial role in maintaining the resistance of IVDs to compression loads. Moreover, PGs support normal functioning of cells and tissue recovery after exposure to deforming loads [3, 9]. This function is performed by their carbohydrate moiety, glycosaminoglycan (GAG) chains linked by covalent bonds with the central protein chain. Biochemical analysis has shown that IVDs include several types of GAGs: chondroitin sulfates and keratan sulphates, whose ratio varies with age and IVD degeneration grade [13]. Quantitative and qualitative composition of PG/GAG is directly related to metabolic processes in the IVD tissues

and reflects its structural and functional state. Furthermore, the composition of PG/GAG affects the SMS stability, which can be assessed by radiological examination methods. Preoperatively detected signs of SMS instability allow a vertebral surgeon to apply differentiated approach to select the optimal type of surgery to achieve the most effective results.

The study was aimed at determining natural radiological parameters of degenerative spinal motion segments and biochemical changes in intervertebral disc tissues, which significantly correlate with recurrence of IVD herniation after microdiscectomy.

Material and Methods

A total of 1368 microdiscectomies for IVD herniation, which presented with compression radicular syndrome, was carried out at the department of neurosurgery No. 2 of the Novosibirsk RITO n.a. Ya.L. Tsivyan in 2008–2012, including 790 (57.7 %) surgeries at L4–L5 and 578 (42.3 %) surgeries at L5–S1. Microdiscectomy was carried out using the method proposed by Caspar [5] without IVD curettage.

During the follow-up period, 78 (5.7 %) patients were re-operated, including 50 cases (3.7 %) of recurrent IVD herniation at the same level on the ipsilateral side, 6 (0.4 %) – on the contralateral side, 7 (0.5 %) – recurrence of pain due to segmental instability and cicatricial-commissural epiduritis; 15 (1.1 %) patients were diagnosed with degenerative spinal stenosis at the level of surgery.

Inclusion criteria were as follows: IVD hernia at the level of L4–L5 and L5–S1 segments, recurrent IVD herniation at the same level on the ipsilateral side.

Exclusion criteria were as follows: other locations of herniated discs and herniation at more than one level, the combination of IVD herniation and other degenerative pathologies of the lumbar spine, concomitant non- degenerative lesions of the spine, pain recurrence caused by degenerative stenosis of the spinal canal due to the hypertrophy of the yellow ligament and/or facet joints in combination with or without segmental instability, and with the formation of contralateral IVD herniation.

The study included two patient groups: Group I (n = 50) with recurrent lumbar IVD herniation, Group II (n = 50) without recurrent IVD herniation within three years from the date of the operation (random sampling from the general population).

Both groups of patients were analyzed for preoperative radiological characteristics of the SMS as assessed by MRI and X-ray of the lumbar spine (Pfirrmann grade of IVD degeneration, disc height index, segmental sagittal range of motions, central angle of lumbar lordosis, Modic criteria, herniation type, presence of listhesis) and biochemical parameters of IVD tissues, which were sampled during primary microdiscectomy.

PGs from the samples of IVD hernia (nucleus pulposus and annulus fibrosus) were isolated by successive extraction with 0.14M NaCl solution, 4.0 M guanidinium chloride in the presence of protease inhibitors, and papain solution [13]. GAGs were separated from the protein core of PGs by treatment with sodium borohydride (0.15 M $NaBH_4$,/10M NaOH at a ratio of 10:1 at 60 °C for 4 hours) and purified by chromatography on the DEAE A-25 ion exchange resin. The amount of GAGs was determined by their structural units: uronic acids (UA), hexose, and the number of sulfate groups, sulfated GAGs (sGAGs) [3]. The results were calculated in micrograms of pure substances per mg of dry tissue weight. For this purpose, water content in the tissues was examined by drying to constant weight. We also determined the content of neutral hexoses in the tissues, which were not related to the PG structure, but rather to the end products of glycation, and characterized additional links in the collagen network.

Statistical analysis was carried out using SPSS 15.0 and Statistica 8.0 software. The obtained results were processed by calculating descriptive parameters (mean value – M, mean error – m; the results were reported in the form M ± m) and comparing the quantitative and qualitative characteristics in the study groups. The analysis was carried out using non-parametric methods. The differences between the compared mean values of the studied parameters in the groups were assessed using the nonparametric Mann – Whitney U-test. Relationship between the qualitative characteristics were studied using $\chi 2$ test. Differences between compared values were considered as significant, when the values did not exceed the threshold level determined as 0.05 ($p < 0.05$). Pairwise correlations between parameters were assessed using Spearman correlation coefficient (r).

Results

Group I included 22 (44 %) males and 28 (56 %) females, mean age 43.6 ± 7.9 years. Group II included 18 (36 %) males and 32 (64 %) females, mean age 42.6 ± 12.7 years. Relapses of IVD herniation were observed within the period of 10.4 ± 1.2 months (1 to 24 months.). The results of statistical analysis of radiological parameters are shown in Table 1. All studied parameters differed significantly between the groups ($p < 0.05$). Significant correlation with recurrence was found for the following parameters: disc height index, segmental sagittal range of motion, central angle of lordosis of the lumbar spine, IVD degeneration, and type of IVD herniation ($z \geq 0.3$).

Relatively high IVD height and hyperangulation nature of the sagittal range of motion of the operated segment were observed in patients with recurrent IVD herniation (Group I). Analysis of the central angle of lordosis in this group also revealed a trend towards hypolordotic changes. Hernia type significantly correlated with recurrence ($p = 0.001$; $z = 0.45$). IVD protrusion prevailed in Group I (70.0 % cases), while Group II demonstrated greater number of extrusions (72.0 %). Type I Modic changes, which were significantly more common in Group I patients (62.0 %), were an indirect sign indicative of active consequences of biomechanical stress in the involved IVD, although they did not significantly correlate with recurrences ($p = 0.001$; $r = 0.19$). IVD degeneration grades differed significantly between the two

groups: the ratio of grades III and IV was about 4:1 in Group I and 1:2 in Group II. Statistical analysis of this parameter showed that patients with recurrent hernia had earlier stage of IVD degeneration, which was reflected in a significant positive correlation between this sign and unfavorable outcome of microdiscectomy (r = 0.42).

In our study, we found significant correlation between the recurrence of IVD herniation and IVD Pfirrmann degeneration grade. There was a relationship between MRI data and IVD biochemical profile [10]. For this reason, we analyzed the structure of IVD tissue from patients with grade III degeneration, which is one of the predictors of unfavorable outcome of microdiscectomy. The results of statistical analyzes of biochemical parameters of IVD tissue in both groups are shown in Table 2.

Water content in the nucleus pulposus is relatively high in both groups and demonstrates no significant differences. The amount of sGAGs, UAs, and galactose is significantly higher in Group II (p = 0.001); sGAG level is 1.2 times lower in Group I, than in Group II, which is a significant difference. sGAG/UA ratio in the nucleus pulposus tissue, which may partly characterize the level of GAG sulfation, amounts to 1.4 in both groups, which is characteristic of tissue aging or degeneration. The ratio of UAs and galactose, which are chemical constituents of chondroitin sulfates and keratansulfate, characterizes the qualitative composition of GAGs. This ratio was about 1.9 in Group I and 1.5 in Group II. The amount of neutral hexoses was significantly (about 1.9-fold) higher in the group without IVD hernia recurrence.

Water content and the amount of sGAGs in the annulus fibrosus did not differ significantly between the groups. In the group with recurrent hernias, the amount of UAs was significantly higher than in patients without hernia recurrence, while the level of galactose was comparable. sGAG/UA ratio did not differ between the groups and amounted to 0.6–0.7. The amount of UAs was almost two times higher than the amount of galactose, i.e. chondroitin sulfates dominated in the tissues of both groups. The amount of neutral hexoses was 1.6-fold higher in Group I compared to that in Group II.

The study included a correlation analysis aimed at identifying significant correlations between the biochemical and biomechanical parameters of the SMS (Table 3). The significant inverse correlation between the IVD height index and the amount of neutral hexoses was found in the nucleus pulposus and annulus fibrosus; the inverse correlation was found between the segmental range of motion and biochemical characteristics of the nucleus pulposus (the amount of neutral hexoses), as well as direct correlation with the content of galactose and hexoses, the substances involved in the strengthening of the crosslinks in the tissue, in the annulus fibrosus.

Annulus fibrosus forms the basis of the mechanical stability of the IVD and its condition affects the kinematic characteristics of the SMS. We have identified a specific pattern of changes in the biochemical composition of the annulus fibrosus in the studied groups. Significantly higher content of UAs and hexoses was observed in the case of grade III degeneration of the IVD in the group with recurrent hernias, however, these parameters did not significantly correlate with intervertebral hernia recurrence (r = 0.17; r = 0.24 respectively).

Discussion

This research is one of the few studies aimed at searching for characteristic correlations between tissue structure and biomechanical properties of the IVD, which can determine possible unfavorable outcomes of surgical treatment in patients with herniated lumbar IVDs.

In 90 % of cases, degenerative changes in the SMS start from IVD, the biomechanically important structure defining segmental stability of the spine. We assume that assessment of preoperative parameters of SMS, which depend on biochemical changes in the SMS, enable

Table 1
The results of statistical analysis of radiological parameters

Parameters		Group I (n = 50)	Group II (n = 50)	p	r
Disc height index		0.35 ± 0.05	0.26 ± 0.046	0.001*	0.69**
Segmental sagittal range of motion		9.80 ± 2.01	6.90 ± 1.86	0.001*	0.61**
Lordosis of the lumbar spine, degrees		33.40 ± 5.69	46.20 ± 6.78	0.001*	0.78**
Modic type I, n (%)		31 (62.0)	5 (10.0)	0.001*	0.19
Pfirrmann grade of intervertebral disc degeneration (III : IV), n (%)		38 (76.0):12 (24.0)	15 (30.0):35 (70.0)	0.001*	0.46**
Herniation type, n (%)	protrusion	35 (70.0)	13 (26.0)	0.001*	0.45**
	extrusion	13 (26.0)	36 (72.0)	–	–
	sequestration	2 (4.0)	1 (2.0)	–	–
Retrolisthesis, n (%)		19 (38.0)	9 (18.0)	0.026*	0.22

*the differences between the values are significant (p < 0.05);

**the coefficient of the correlation between the value and the fact of intervertebral disc herniation recurrence is significant (r > 0.3).

Table 2
The content of chemical components of glycosaminoglycans in the nucleus pulposus and annulus fibrosus in patients with recurrent and non-recurrent hernias and Pfirrmann grade III intervertebral disc degeneration

Parameters	Group I (n = 38)	Group II (n = 15)
Nucleus pulposus, H_2O,%	81.31 ± 0.69	83.34 ± 0.97
Nucleus pulposus, sGAGs, μg	38.33 ± 0.93	45.50 ± 0.96*
Nucleus pulposus, uronic acids, μg	27.20 ± 0.77	34.81 ± 1.81*
Nucleus pulposus galactose, μg	14.34 ± 0.68	23.50 ± 1.51*
Nucleus pulposus hexoses, μg	31.91 ± 1.03	57.11 ± 5.65*
Nucleus pulposus, uronic acids/galactose	1.93 ± 0.05	1.53 ± 0.07*
Nucleus pulposus, sGAGs/uronic acids	1.44 ± 0.07	1.42 ± 0.09
Annulus fibrosus H_2O,%	81.71 ± 0.56	81.14 ± 0.83
Annulus fibrosus, sGAGs, μg	44.61 ± 0.99	42.20 ± 1.42
Annulus fibrosus, uronic acids, μg	71.62 ± 0.88	64.10 ± 2.04*
Annulus fibrosus, galactose, μg	38.13 ± 0.79	35.22 ± 1.74
Annulus fibrosus, hexoses μg	191.42 ± 5.97	117.10 ± 5.88*
Annulus fibrosus, uronic acids/galactose	1.92 ± 0.04	1.93± 0.07
Annulus fibrosus sGAGs/uronic acids	0.61 ± 0.02	0.70 ± 0.04

sGAGs — sulfated glycosaminoglycans; calculated in micrograms of the substance — chondroitin sulfate C (for sGAGs), hexuronic acids, and galactose per mg of dry tissue weight;
*significant difference ($p < 0.05$).

predicting surgical outcome and taking preoperative measures to prevent adverse outcomes.

There is a correlation between the type of IVD hernias and their recurrence. Morgan-Hough et al. [14] showed that IVD protrusions are associated with significantly higher risk of reoperation than extrusions or sequestered herniation. Such a relationship was also found in our study ($r = 0.45$): in the group with recurrent IVD hernias, protrusions were significantly more common than in the group without recurrence.

Currently, the significance of changes in the endplates (Modic criteria) in patients with degenerative spine lesions for tactics and outcome of surgical treatment is being actively discussed [18]. Toyone et al. [16] found that in the case of type I change, hypermobility occurs in 70 % of cases, type II – only in 16 % of cases. Based on the radiographic data, the authors concluded that pain in the lumbar spine and type I changes are often associated with instability that necessitates lumbar fixation [16]. The relationship between type I Modic changes and segmental instability is mainly supported by indirect evidence based on fusion results in the lumbar spine [6, 17]. Although no significant correlation between type I Modic changes and recurrences was found, their incidence was significantly higher in Group I ($p = 0.001$; $r = 0.19$).

Some authors believe that retrospondylolisthesis is a sign of segmental instability, which plays an important role in relapse prediction. Shen et al. [15] revealed a significant correlation between formation of IVD hernias at the level of L5–S1 and the presence of L5 retrospondylolisthesis. However, we found no correlation between the posterior displacement of the overlying vertebra and recurrence of IVD hernias despite the significant differences in the study groups ($p = 0.026$; $r = 0.22$).

Not only segmental biometric state of the spine, but also the condition of the whole segment can affect the outcome of surgical treatment. Prodan et al. [2] identified the pattern of the impact of hypolordotic posture on the risk of degenerative IVD lesions based on clinical and radiological examination of 100 patients with chronic lumbalgia and sciatica. We compared the parameters of the central angle of lumbar lordosis and found that patients with recurrent IVD hernia are prone to hypolordotic changes (the central angle of lordosis was 33.4° ± 5.69°) in contrast to patients without recurrence (46.2° ± 6.78°).

IVD height can serve as a predictor of microdiscectomy outcome. Yorimitsu et al. [20] found that intact IVD height during primary operation significantly correlated with recurrence of IVD hernias. This correlation was also observed in our study ($r = 0.69$).

It is known that the range of motion of the SMS depends on the structure of the IVD, articulate joints, and mechanical stress on these elements [8]. A relationship between degenerative changes in SMS structures and sagittal stability was found. Fujiwara et al. [8] showed that the segmental range of motion increases in the early stages of IVD degeneration and decreases in advanced stages. Bible et al. [4] reported that segmental range of motion decreases proportionally to degeneration stage. In our study, patients with recurrent hernias and grade III and IV IVD degeneration had significantly higher segmental angulation than patients without recurrence. Dora et al. [7] noted that the risk of IVD hernia recurrence was 6.8 times higher in patients with early stages of degeneration than in patients with advanced stages. We observed significant positive correlation between degeneration stage and IVD hernia recurrence ($r = 0.46$).

This study showed that IVD tissue in patients with and without recurrent hernias have characteristic structural features. Comparative analysis of biochemical parameters of the nucleus pulposus and annulus fibrosus in patients with Pfirrmann grade III IVD degeneration in both groups showed changes in the sulfation level of GAG chains in the nucleus pulposus tissues, which is one of its crucial properties and it is highly important for its functioning as a whole. This term refers to the negative charge density in polysaccharides. The reduction of this parameter usually occurs with aging or during degenerative process. The amount of neutral hexoses, which increases in

Table 3
Correlation between biochemical and biomechanical parameters in patients with Pfirrmann grade III intervertebral disc degeneration

Parameters	Disc height index	Segmental sagittal range of motion
Nucleus pulposus, water content	0.01	0.02
Nucleus pulposus, sGAGs	-0.19	-0.26
Nucleus pulposus, uronic acids	-0.17	-0.04
Nucleus pulposus, galactose	-0.26	-0.13
Nucleus pulposus, hexoses	-0.30*	-0.32*
Nucleus pulposus, uronic acids/galactose	0.15	0.07
Nucleus pulposus, sGAGs /uronic acids	-0.008	-0.11
Annulus fibrosus, water content	0.12	0.22
Annulus fibrosus, sGAGs	0.13	0.13
Annulus fibrosus, uronic acids	0.11	0.17
Annulus fibrosus, galactose	0.19	0.31*
Annulus fibrosus, hexoses	-0.30*	0.39*
Annulus fibrosus, uronic acids/galactose	-0.12	-0.15
Annulus fibrosus, sGAGs/uronic acids	0.03	-0.03

sGAGs — sulfated glycosaminoglycans;
*correlation coefficient is significant ($r > 0.3$).

the tissue during active degenerative processes, is significantly reduced in the group with IVD hernia recurrence. GAG molecules also undergo significant changes in the annulus fibrosus tissue, which can be characterized as degenerative ones, and the cascade of changes is more severe in Group I patients.

The data on the content of hexoses, whose total level in the tissue is an order of magnitude higher, is of particular interest. Hexose levels in patients with recurrent IVD hernias is significantly higher compared to those in the group without recurrence. Accumulation of neutral hexoses along with decrease in the amount of GAGs and their sulfation level modifies the elastic properties of IVD tissue, which becomes more rigid and brittle, enhances tissue resistance to distraction load and therefore reduces the ability to retain water [12]. Thus, accumulation of neutral hexoses reflects the development of degenerative processes in the tissue accompanied by loss of its functional properties.

GAG changes in the nucleus pulposus and annulus fibrosus are associated with the features of the physiological processes under pathological conditions. Accumulation of low-sulfated GAGs significantly changes the properties of the tissue. On the one hand, stress-strain properties of the tissue are reduced, on the other hand, low-sulfated GAGs stimulate increased activity of degrading enzymes, which produce resident cells. In turn, increased degradation of the extracellular matrix material stimulates the biosynthetic potential of chondrocytes. This results in a metabolic vicious circle. The tissue becomes looser, since highly sulfated GAGs are required to form normal extracellular matrix and collagen fibrils. The nucleus pulposus accumulates soft and non-functional tissue, and the annulus fibrosus cannot serve as a full-featured fixing component of the IVD.

Correlations between the quantitative and qualitative characteristics of GAGs and biomechanical parameters (disc height index and sagittal range of motion) reflect the characteristic features of the structural organization of IVD tissue. Thus, in the case of grade III IVD degeneration, disc height is inversely proportional to tissue stiffness, which depends on the amount of PGs retaining water, and collagen network strength, which is determined by effective crosslinks between the fibrils, including glycation products. Stability of the annulus fibrosus results from strong crosslinks in various protein-carbohydrate structures, which determines the range of motion in the SMS. In the case of degenerative changes, IVD height is determined by the structure of the nucleus pulposus and the content of GAGs, water, and chondroitin sulfates. The content of GAGs and their sulfation level in the annulus fibrosus, which affect stabilization characteristics of the latter, are also important.

In summary, significant differences in biochemical parameters were observed between the groups with and without recurrent IVD herniation. Moreover, we found significant correlations between biomechanical and biochemical characteristics of IVD intercellular substance. However, no significant correlation was found between biochemical parameters, reflecting the qualitative and quantitative composition of GAGs both in the nucleus pulposus and annulus fibrosus, and recurrent herniation of lumbar IVDs.

Conclusion

1. Intact IVD height, SMS hypermobility, flattened lumbar lordosis, moderate disc degeneration, as well as its protrusions significantly correlate with recurrent herniation after microdiscectomy.

2. The amount and structure of PGs/GAGs in the nucleus pulposus and annulus fibrosus reflect the characteristics of metabolic processes in IVD tissues and are specifically altered in patients with and without recurrent herniation.

3. Biomechanical properties of IVD tissues significantly correlate with alteration of the structure and amount of GAGs.

4. Assessment of radiological parameters reflecting spine biomechanics and their relationships can be important when selecting the optimal type of surgical treatment in patients with herniated lumbar IVDs.

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