

Long Term Clinical Results in Laser Reconstruction of Spine Discs

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Abstract

Purpose: Determine the long-term clinical outcome of the laser reconstruction of intervertebral discs (LRD) in patients with chronic degenerative spine diseases.

Methods: Ninety-seven patients with chronic back and neck pain caused by single and multi-level spinal discs degeneration were treated with non-ablative laser irradiation (1.56 μm Er:glass fiber laser) of the nucleus pulposus and the inner third of the annulus fibrosus through percutaneous needle puncture. The results were analyzed during five years after LRD by the means of clinical observation, radiological and biomechanical testing. Three surgical biopsies of the laser-irradiated disks were examined by the morphological methods. Subjective estimation of LRD influence of the patients' life quality and back pain intensity has been performed with validated questionnaires of SF-36 and VAS.

Results: Majority of the patients, who underwent LRD procedure, demonstrated an essential improvement in their health state, including decreasing of spine discs instability, pain relief and the general quality of their lives. There were no any complications related to the use of LRD. Five-year outcome have shown positive dynamics of MRI features of the treated discs in 77% of patients as well as an improvement in the SF-36 total score and VAS in 92 and 95.9% of patients respectively. The histological results have proved the growth of hyaline like cartilage in laser-treated zone.

Conclusions: Five years outcome observations demonstrate stable positive structural changes in the intervertebral discs as well as the significant improvement in subjective feelings of the life quality and pain relief for the majority of the patients.

Keywords: Cartilage repair; Pain relief; Laser; Clinical outcome; Histology

Introduction

Discogenic degenerative spine diseases are still a serious problem as they are a major cause of back pain that deteriorates the quality of life of patients and often leads to disability [1]. The reviews of current treatment modalities of the lumbar intervertebral therapies show that conservative treatment of back pain can be insufficient [2,3]. Mini-invasive intradiscal puncture techniques including electrothermal therapy (IDET) [4], as well as mechanical and laser decompression of the discs [5] are FDA approved and used in the clinics. The main objective of these methods is dereception (destroying) of pathological nervous structures in the Annulus Fibrosus (AF) and / or decompression of intervertebral discs through removal of a part of the Nucleus Pulposus (NP). These interventions however do not repair disc cartilage and frequently their effect is short lasting [6]. Ablative and destructive methods in treatment of chronic discogenic pain may lead to the development of fibrous scar tissue in the disc cavity, which favors the formation and persistence of the pain generator in the disc lesion. Secondary effects and pain return can be observed in one-two years after surgery [7]. Therefore the search for the new effective and safe methods for treatment of spine discs diseases is essential.

In 2000 we have introduced a new low invasive approach - Laser Reconstruction of Discs (LRD), which is based on thermo mechanical effect of non-destructive laser radiation on the NP [8]. In vivo animal studies in rabbits have shown that LRD allows activation of the cartilage regeneration processes in the degenerative intervertebral discs [8,9]. Optimal laser settings have been established to provide the growth of hyaline type cartilage in the laser affected zone only [9-11]. The results of histological examination have demonstrated the development of new cartilage tissue of hyaline-like type in the intervertebral discs in response to non-ablative irradiation by an Er: glass fiber laser [10,11]. The mechanisms of laser-induced regeneration and repair of cartilage are presented elsewhere [11-13]. Clinical trials of LRD performed in

2002-2006 in 90 patients have shown positive results for majority of treated patients without any complications and secondary effects within one year follow-up observations [10,11]. In 2006 LRD has been approved for clinical use by the Russian Federal Service of Supervision in the health and social development. In 2007-2013 LRD was applied for more than 2000 patients [13].

Although most of the patients underwent LRD shown significant improvement, there is lack of solid data on long term-stability of the obtained results. Evaluation and the long term stability of positive LRD results are the main subjects of this study. Ninety-seven patients were available to contact during the follow-up period of 5 years after LRD procedure. A combined analysis of the data of MRI and biomechanical examination as well as the results of SF-36 and VAS questioning was used to find out how LRD treatment did effect in structure and functioning of the intervertebral disks and in pain relief and the life quality of the patients.

Materials and Methods

A subject of this study was a group of 97 patients (51 women and 46 men) with back and neck pain during at least two years without essential remission. All patients have received LRD surgery and were available for contact during the follow-up period of 5 years after LRD.

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Cervical intervertebral discs were treated in 23 patients and lumbar ones in 74. From 2 to 4 discs of cervical spine and 1-5 discs of lumbar spine were treated in one session. The total number of intervertebral discs treated by LRD was 235, out of these 74 were in cervical spine and 161 in lumbar spine. The age of patients at the time of treatment was 14-78 years (Mean \pm St.Dev. 42.4 ± 11.2). Three sub-groups of patients were considered: (1) 23 patients with manifested degenerative lesions in the spine, orthopedic diseases and pathopsychological disorders and two aging groups of 74 patients which did not have aggravating features: (2) 36 from 14 to 42 years old, and (3) 38 patients from 43 till 78 years old. All the patients gave their informed consent to the therapy and passed pre- and post- operative examination.

Inclusion criteria for the study were chronic (lasting at least two years) pain in neck or back and chronic vertigo as a sign of vertebrobasilar insufficiency (resulting from degenerative lesion of cervical discs), as well as an instability of spine revealed by flexion-extension X-ray examination. The above pathological conditions in the patients included into the study were not controlled by complex conservative therapy. The degeneration of intervertebral discs has been documented by MRI examination as following features on the scans as the dark discs, decreasing of T2 signal and decrease in the high of the disc. The appearance of a hyperintensive zone (HIZ) [14] and induction of high to moderate concordant pain and presence of fissures in the AF [15,16] emerged during the CT-discographic examination were also considered as necessary indications for LRD surgery.

Exclusion criteria for LRD were absence of signs of degeneration of intervertebral discs at MRI and CT-discography, significant protrusion or extrusion of intervertebral discs associated with apparent compression of nervous structures, significantly reduced height of the intervertebral discs, local and general infections, blood coagulation disorders and uncontrolled coagulopathy, obvious psychological component in the pain syndrome, including extreme intolerance to discography.

Preoperative diagnostic work-up included physical and MRI examination, spondylogram (Rg) with functional tests as well as provocative evaluation of pain concordance and the extent of intervertebral disc lesions. Omnipaque-300 was used as a contrast agent for imaging of the discs. In spite of different opinion regarding applicability of the discography, we espouse the view that above-mentioned diagnostic procedures are reliable and sufficient for the evaluation of the patients' condition and the treatment results in clinical practice [17,18]. Segmental instability was evaluated as a clinical syndrome of decrease of normal range of motion (ROM) in lumbar spine due to appearance of pain [19]. We performed qualitative evaluation of the segmental instability by lumbar flexion test in standing position. Restriction of lumbar flexion at an angle of 90 degree or less together with sudden onset of pain considered as a positive sign of instability. Functional X-ray estimation of segmental instability was performed at the L4-L5 level before and after LRD. Sagittal-plane rotation of greater than 9 degrees (by Cobb measurement) at L4-L5 level referred to segmental instability [20].

The surgery procedure of LRD has been performed using Er:glass fiber laser (Arcuo Medical Inc., CA, USA) with modulated laser beam of 1.56 microns in wavelength and power of 1.5 W. LRD was given in the out-patient setting under local anesthesia. Cefazolin (2g) was injected i/m in 30 min prior to operation as prophylaxis of infectious complications. Posterior-lateral approach was used to puncture of the lumbar intervertebral discs in the Kambin triangle. Cervical discs were punctured with the use of anterior-lateral approach [17]. LRD procedure was performed through the 18G needle introduced into the disc under

X-ray guidance. In cervical spine, the central zone of the NP and two zones of the transition layer of the AF were irradiated. In lumbar spine, two central zones of the NP and two zones of the transition layer of the AF were irradiated [13]. Every zone was irradiated by 3 series of pulses (each series lasted for 30 sec with intervals of 20 sec between them; pulse duration - 2 sec, interval between pulses - 1 sec). The efficacy and safety of LRD were provided with a feedback control system based on the fiber optics measurements of the back scattered light and simultaneous computer treatment of the light signal dynamics [11].

Post operation examination. The schedule for post operation examinations was: three, six and twelve months and then two and five years after LRD. The effect of LRD on the patients' quality of life was evaluated as a total score of the SF-36 health survey questionnaire designed for subjective evaluation by the patients of their physical and emotional wellbeing and quality of social adaptation prior to and after treatment [21]. The visual analogue scale (VAS) [22] was used to evaluate the pain syndrome prior to and after treatment. The effect of LRD evaluated by the SF-36 total score was considered as positive if it was higher after the treatment than prior to it. The comparison of the observed frequencies for the evaluated categories was done through analysis of cross-tables and 95% confidence intervals for the proportions. As most of the cross-tables do not have large numbers of observations in each cell we preferred to use Cramer's V test. To evaluate the statistical significance of the differences between the values of the total score and subscales' scores of the SF-36 and VAS prior to and after the treatment, Wilcoxon's Z-test for two related samples was used. Correlation analysis (Spearman's correlation coefficient, R_s) was used in the analysis of correlations of ordinal and qualitative features. Two-tailed criteria were used when testing statistical hypotheses; the critical level of significance was set at 0.05.

MRI examination was used to evaluate the structural alterations in the discs for the subgroup of 64 patients (randomly selected from the 97 patient's group) available for regular personal contacts. The total number of irradiated discs was 87. Axial and sagittal projections in T2 mode have been used for MRI examination [16]. The following criteria have been used: (1) the intensity of the T2 signal; higher T2 signal is associated with higher water content, manifests increase of hydrophilic cartilage matrix; (2) the size of disc protrusion; (3) the height of the disc and (4) the appearance of the internal disc structure: an increase of the T2 signal in the NP compared to the T2 signal from the AF was used as a sign of some advance towards normal disc structure. Flexion-extension X-ray tests before and after the laser treatments have been applied to evaluate changes in spine disc instability. The biopsy was taken from the intervertebral discs of 3 patients of the group 1 in approximately two years after LRD. The structural differences between the laser-irradiated and non-treated areas of the discs were evaluated morphologically using routine histological methods and the transmission electron microscopy.

Results

For most of the patients, pain began to decrease in two – three months after LRD and substantially diminished in 6-12 months subsequently. The results of one year follow-up observations are as follows.

Pre and post operation examination of the patients

Before LRD the typical complaints were: pain of mechanical pattern in the neck or lumbar spine increasing with physical activity (exercise stress), which diminish in horizontal position. Visual examination showed long muscle tension in the neck or back, palpatory tenderness of the paravertebral points at the level of affected or adjacent spine

discs. Neurological status was without pathology. Motion tests (lateral bending, extension, turning) increased local pain. In 12 months after LRD, the typical complaints included: temperate discomfort in the neck or lumbar spine during pronounced physical activity only; visual examination showed muscle tension in the neck or back; local palpatory tenderness did not reveal; neurological status was without pathology; motion tests (lateral bending, extension, turning) did not show perceptible painful sensation. Lumbar flexion test evaluated clinical signs of segmental instability in 39 of 74 patients with lumbar low back pain. In one year after LRD the number of patients with clinical instability decreased from 26 to 3. In these 3 patients the positive instability sign remained unchanged. Flexion-extension X-ray tests before and after laser treatments showed decrease in spine disc instability. Average rotational angle at X-ray ROM at L4-L5 Level in functional X-ray were 7.1 and 6.7 degrees before and after LRD treatment respectively. Before LRD the angle was greater than 9 degrees (10-13 degrees) in 6 patients and after LRD that was observed only in 3 patients (11-10 degrees). The Table 1 presents outcome results of LRD assessed by the tests of spine instability. The functional clinical signs of instability were improved for 59 percent of the treated patients, although no patients showed worsening of this parameter. The examination of the patients using rotational X-ray ROM at L4-L5 level showed statistically equal provability of positive and neutral outcomes, while the probability of negative outcomes was less than that for neutral one.

One year outcome results of LRD based on evaluation of the pain syndrome (VAS) and total score of the SF-36 health survey are presented in the Tables 2 and 3. In the majority of patients the intensity of pain following LDR decreased in all age groups (the rate of VAS-based positive outcomes was from 85% to 100%). The rates of positive outcomes based on the SF-36 total score and VAS did not depend on the gender of the patients, anatomical localization and number of irradiated discs. Both indicators showed positive results for 89.7 percent of the patients received LRD. A negative correlation between LRD outcome and additional diseases was observed using total score SF-36. The group 1 demonstrated the lower percentage of positive results for SF-36; and the group 3 showed the lower percentage of positive results for VAS. Although the group 2 of younger patients without additional diseases revealed the best outcome using both SF-36 total score and VAS (Table 2), the statistical significance of positive results on the patient age was not very substantial. Thus evaluation of one year effects of treatment showed statistically significant improvement including flexion-extension X-ray tests, pain relief and general quality of life for the overwhelming majority of patients.

Long duration results

Dynamics of long duration LRD results for different examination techniques is presented in the Tables 4 and 5. The observations during 5 years after LRD demonstrated positive dynamics of the results for all patients. No one of the patients with positive result obtained in the course of the first year showed any worsening during following years. MRI examinations demonstrated that LRD never led to negative changes of intervertebral discs. Evident improvement (increase of the T2 signal, growth of the height of the discs, better appearance of disc structure, or reduction of disc protrusion size) occurred in 77% of the discs as it was noted during the long-term observation, whereas in 23% of the treated discs the MRI dynamics was undetectable. At the same time, statistical analysis proved zero probability of worsening in structure of the discs after LRD as well as significant excess of improvement probability over the neutral and negative shifts (Table 4). The ratio of the patients with positive changes in MRI increased

in the course of five years of observation (Table 5). The typical MRI pictures are presented on the Figure 1. Pre-operation MRI showed a degenerative structure of the L4-L5 disc (A). No significant changes can be seen on the MRI in one year after LRD, although the patient demonstrated a substantial pain relief (B). The T2 signal increased in two years and three months after LRD (C). Reparation manifestations of the disc (increase of the disc height, appearance of the disc structure typical for normal disc) are clearly seen in five years after LRD (D).

Pain relief was observed for almost 96% of the patients, while 92% of the patients were satisfied with the LRD results. At the same time, MRI showed the visible positive alterations of the disc structure for nearly 64% of the patients treated. As it follows from the Table 5, the probability of positive shifts in terms of pain relief and quality of life surmounts the zero even at the early time check-point (3 months after LRD), while at the same time there are no patients demonstrating detectable positive changes of the discs' structural features on MRI. At the next stages (from 6 months up to 5 years after LRD) the evident progressive beneficial changes are documented by all the used assessment tools. As provided by the data presented in the Table 5, the major progress in pain relief was achieved during the first 6 months after the LRD procedure. Substantial outcomes in the life quality (SF-36) and structural normalization of the discs (MRI) observed during the first year after LRD. At subsequent period (from 2 to 5 years after LRD) all the examined parameters improved, however, these changes did not represent statistically significant shifts in comparison with 6 months (VAS) and 1 year (SF-36 and MRI) values.

Histological examination results

The histological results obtained for three patients have clearly demonstrated the signs of regeneration processes. The growth of hyaline-like and fibrous-hyaline cartilage was revealed in the laser treated zones only (Figures 2 and 3). Non-treated areas of the discs showed necrotic zones of the degenerated cartilage without any reparation processes.

Discussion

As the reasons of the back pain are not fully understood yet, an adequate evaluation of the clinical results can be performed on the base of a combination of different (subjective and objective) examination methods including long duration observations. A prospective, longitudinal study of the relative contribution of structural and psychosocial determinants of back pain has been carried out using MRI, provocative discography, physical examinations and psychometric testing was presented in the paper [23]. In our study, we applied similar methods of the patient's examination, which without any claiming to the absoluteness, from our point of view, allows evaluating the long-term results of the LRD. This paper presents the results of prospective cohort study of the effects of the LRD in patients with 5 years follow-up.

LRD allowed pain relief for majority of the treated patients. The long-term observation of the LRD results showed stable positive results. The quantitative evaluation using different examination techniques (Table 5) demonstrated different, but correlating one to another data. Although pain relief and SF-36 questionnaire gave the best outcome, they are quite subjective. More unprejudiced examination methods (including MRI, X-Ray, the visual examination by the doctor, and motion tests) showed also positive results for majority of the treated patients. Today MRI is the most used technique for spine disc examination, but its spatial sensitivity cannot clearly reflect early stages of tissue repair. MRI shows the substantial changes usually after one year of LRD application. The development of MRI equipment and

Test		Type of outcome			Total
		Improvement	No change	Negative	
Functional clinical signs of instability	n	23	16	0	39
	%	59.0	41.0	0.0	100
	CI95%, %	41.0; 76.9	23.1; 59.0	0.0; 2.5	-
Rotational X- ray ROM at L4L5 level	n	13	22	4	39
	%	33.3	56.4	10.3	100
	CI95%, %	16.0; 50.6	38.3; 74.5	0.0; 22.3	-

Table 1: A one-year outcome of LRD assessed by the tests of spine instability.

Assessment tools/ Health		PRE-LRD	POST-LRD	CHANGE	p
SF-36	domains				
	Physical function	46.80±33.32	78.30±18.25	31.49±33.92	<0.001
	Role, physical	8.25±21.26	52.58±39.93	44.33±42.00	<0.001
	General health	26.52±13.75	48.33±16.71	21.81±17.56	<0.001
	Vitality	41.08±19.28	60.57±18.14	19.48±20.98	<0.001
	Social function	46.55±23.15	72.93±21.46	26.31±26.20	<0.001
	Role, emotional	25.76±39.83	65.96±43.59	40.19±50.24	<0.001
	Mental health	52.95±17.90	68.99±16.92	16.04±17.98	<0.001
	Total score	34.20±15.05	63.49±17.94	29.30±22.17	<0.001
	VAS	7.04±1.87	2.98±1.90	- 4.02±2.653	<0.001

The data presented as Mean±Std. Dev. (for PRE-LRD and POST-LRD), paired differences Mean±Std. Dev. (for CHANGE). Statistical significance of the paired differences represented as **p** value.

Table 2: A one-year outcome results of LRD for 97 patients based on the VAS and SF-36 questionnaire.

Groups of patients	A number of treated patients		Pain relief, VAS	SF-36 total score
I. With additional diseases	n	23	21	20
	%	100	91.3	87.0
	CI95%, %		75.5; 100	68.9; 100
II. Without additional diseases. Age from 14 to 42	n	37	35	36
	%	100	94.6	97.3
	CI 95%, %		84.7; 100	89.4; 100
III. Without additional diseases. Age from 43 to 78	n	38	32	34
	%	100	84.2	89.5
	CI95%, %		70.0; 98.4	77.1; 100

Table 3: A number/percentage of positive results of LRD based on the VAS and SF-36 questionnaires for three groups of patients with one year follow up.

Number of treated discs	Intervertebral disc changes					
	Positive				Neutral	Negative
	Protrusion decrease	T2 signal increase	Disc height increase	Appearance of normal disc structure	No changes	Negative changes
N	10	41	7	9	20	0
%	11.5	47.1	8.0	10.3	23.0	0
CI95%, %	3.7; 19.3	35.5; 58.7	1.2; 14.9	2.8; 17.9	13.0; 33.0	0.0
Total, n	67				20	
%	77.0				23.0	
CI95%, %	67.0; 87.0				13.0; 33.0	

Table 4: MRI examinations results of 87 treated discs (64 patients) before and after LRD for a five-years follow-up.

technique, and the use of other examination techniques will probably allow better monitoring of the repair processes in spine discs.

LRD is a low-invasive approach like a number of other techniques. The monotonous improvement is established for patients underwent LRD with 5 years follow up observations. Moreover, the important achievement of LRD is also the growth of hyaline-like cartilage in laser affected zone of spine discs which was confirmed for humans as

it was previously established for animals [10]. These features of LRD are advantageous compared to other low-invasive techniques for treatment of spine diseases which mainly provide heating of the AF and effect on the nerves innervating the disc. As it was established in the course of animal and cadaver's studies, the heating of AF in the course of LRD is quite low; the main reason for activation of reparative processes is controllable thermo mechanical effect of spatial and

Assessment tool	Total number of the patients examined	The part of the patients with positive outcome		Time periods after LRD				
				3 months	6 months	1 year	2 years	5 years
VAS	97	n	32	73	87	86	93	
		%	33.3	76.0	90.6	89.6	96.9	
		CI95%, %	22.9; 43.8	66.5; 85.6	83.8; 97.5	82.5; 96.7	92.4; 100	
SF-36 total score	97	n	28	68	87	88	89	
		%	29.2	70.8	90.6	91.7	92.7	
		CI95%, %	19.1; 39.3	60.7; 80.9	83.8; 97.5	85.1; 98.2	86.5; 98.9	
MRI	64	n	0	9	30	38	41	
		%	0.0	14.1	46.9	59.4	64.1	
		CI95%, %	0.0; 1.5	4.0; 24.1	33.1; 60.6	45.8; 72.9	50.8; 77.3	

Table 5: The long-term dynamics of LRD outcomes.

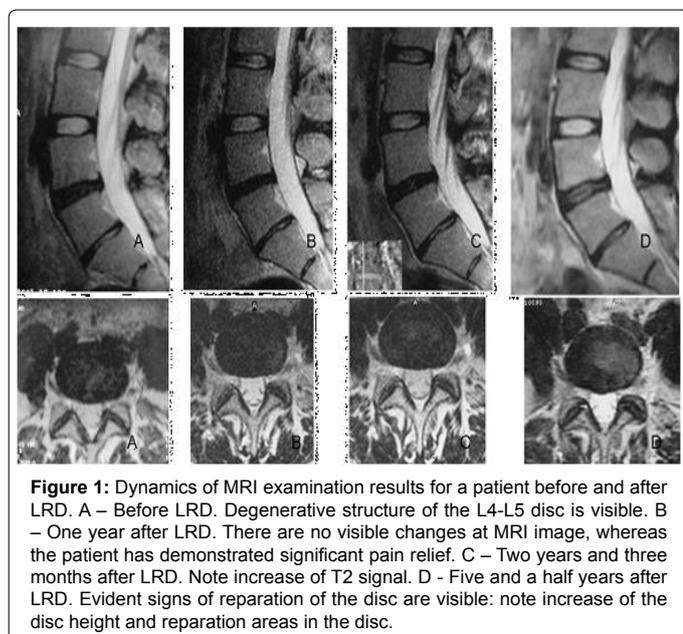


Figure 1: Dynamics of MRI examination results for a patient before and after LRD. A – Before LRD. Degenerative structure of the L4-L5 disc is visible. B – One year after LRD. There are no visible changes at MRI image, whereas the patient has demonstrated significant pain relief. C – Two years and three months after LRD. Note increase of T2 signal. D - Five and a half years after LRD. Evident signs of reparation of the disc are visible: note increase of the disc height and reparation areas in the disc.

temporal modulated laser radiation [11-13]. So, this study proves the long-term stability of positive results of LRD which can be considered as a promising, effective and safe technique for minimally invasive treatment of degenerative intervertebral discs diseases. Further studies are required to specify indications and limitations for percutaneous LRD. We suppose that prospective fields of effective application of the LRD will include laser-induced regeneration in the patients with spine instability; as well as the patients required to fill the defect in the AF after hernia removal. The present study estimates the effects of nonablative laser irradiation both on the morphology and the function of intervertebral discs. Morphological regenerative changes are strictly proved by the series of previous animal studies [8-12]. They demonstrate the process of the new tissue formation that includes the features of hyaline and fibrotic cartilage and was named fibro-hyaline cartilage. Apparently this process can cover the whole disc structure or can be focal in the vicinity of primal irradiation points. This evolution of tissue reflects the changes of inner metabolic processes in the disc, such as cells nutrition, newly pores formation in the cartilage endplates [24], improvement of oxygen transportation, water and ions shifting, etc.

[11]. Also it is the stimulus of cartilage regenerative potential such as cells proliferation, matrix and fibers synthesis. The latter in the human discs are proved by single histologic investigation of this study. The serial study is not possible owing to ethic limitations and percutaneous puncture procedure is rarely followed by open surgery. That's why the main morphological markers are MRI changes. The usual scale reflecting degenerative disc changes is Pfirmann grading system [25]. However, it reflects very prominent structural changes disregarding the intra stage changes such as signal intensity level, focal cartilage changes, etc. [26]. The qualitative assessment of MRI of this study could be continued by quantitative further investigation of MRI signal intensity before and after LRD than could notice even slight metabolic and reparative changes. The estimation of function of intervertebral discs in this study included clinical assessment and functional X-ray. The ideal control group would be the one of needle disc puncture without laser irradiation. This was done by previous animal study but impossible in clinical practice due to ethical limitation [8-12]. This would be implemented in double blind randomized trial that could give us the sound conclusion of LRD liability in the treatment of degenerative disc disease. The present study is the pilot one demonstrating positive effects of LRD for degenerative disc disease (DDD) treatment. Of course the future study needs to be done separately for cervical and lumbar discs considering that low back pain and cervical pain are different entities though the disc changes are the pivotal point in the pain pathogenesis both in lumbar and cervical spine [27-29].

The usage of functional spine X-ray assessment is the attempt to catch the influence of LRD on spine segmental stability. The natural course of DDD includes the stage of destabilization and in some cases followed by restabilization. The role of disc in the spine segment stability is well-known [30]. Apparently the morphological changes in the disc after LRD can affect the segmental stability and the present study made the attempt to entrap this effect. The precise quantitative measurements of vertebrae movement should be done in the future study.

Conclusions

LRD is a promising, effective and safe technique for minimally invasive treatment of degenerative intervertebral discs diseases. Five years outcome observations demonstrate positive dynamics in the structural changes in intervertebral discs as well as the significant improvement in subjective feelings of the life quality and pain relief for

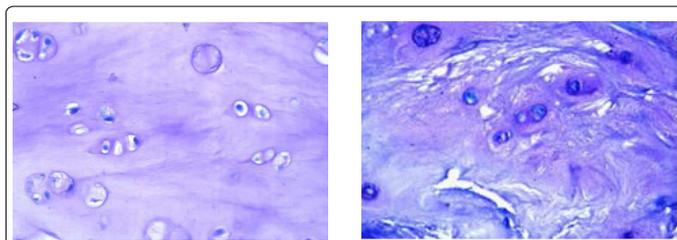


Figure 2: Histological structure of the laser treated zone of spine disc, two years after LRD. A – The result of replacement of degenerated tissue of NP by neogenetic hyaline-like cartilage; many multi-cellular clones and high content of glycosaminoglycans are visible. B – Fibrous-hyaline cartilage is replacing the necrotic areas of the AF. Toluylene blue staining, $\times 400$.

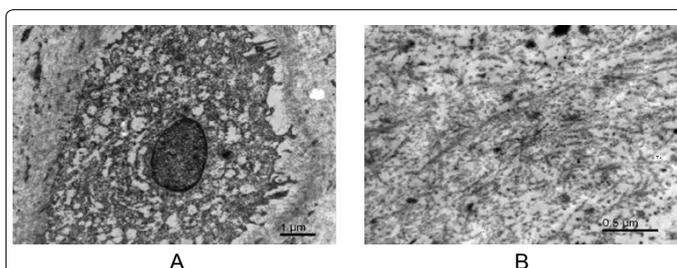


Figure 3: Transmission electron microscopy of the disc, which underwent laser treatment. A - Young chondrocyte (chondroblast) in the hyaline-like neogenetic cartilage emerged following to LRD. Note the well-developed granular endoplasmic reticulum and Golgi complex indicating an active synthesis of collagen and proteoglycans, $\times 15000$; B – Matrix of new grown tissue in the laser treated area is characteristic for hyaline cartilage, $\times 60000$.

the majority of the patients.

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