Histologic Changes At The Intervertebral Disc Tissue After Laser Nucleotomy
Arif ÖSÜN¹, Ali SAMANCIOĞLU¹, Peyker TEMİZ², Cüneyt TEMİZ³, Murat SAYIN³
¹Department of Neurosurgery, Faculty of Medicine, Dumlupınar University, Kütahya – Turkey
²Department of Pathology, Faculty of Medicine, Celal Bayar University, Manisa – Turkey
³Department of Neurosurgery, Faculty of Medicine, Celal Bayar University, Manisa - Turkey

Summary
Minimally invasive surgical techniques have become widely used for lumbar disc disease. Each of these techniques have a different physical effect in terms of retracting the prolapsed disc tissue and decompressing the adjacent nerve root by reducing the intradiscal pressure. Various types of laser generators with different wave lengths and electrode systems have been used for percutaneous laser disc decompression (PLDD). There are numerous studies regarding the clinical effectiveness of PLDD but histologic parameters which can prove the effectivity are few. Three patients with L4-5 disc prolapsus had undergone open microdiscectomy three months after PLDD because of the unsatisfactory results. The disc tissues were evaluated histologically. Fibrous fibrillary degeneration, integrity deformation of the fibres and chondrocyte proliferation were seen at the site of the microdiscectomy tissue. Previously laser irradiated disc materials had more severe fibrillary degeneration and the chondroid component degeneration was significant. In addition, degenerative calcification and massive necrosis of the chondrocytes were seen. Changes in fibronectin, tissue inhibitors of metalloproteinases (TIMP) and matrix metalloproteinases (MMPs) of the disc tissue were examined with immunohistochemical dyes. As a result, we found that PLDD causes a decrease in the water content of the disc tissue, hyaline degeneration, fibrillary destruction and chondrocyte depletion. Immunostaining showed that the extracellular matrix was effected, the metalloproteinase activity and protein compound of the extracellular matrix had changed. The findings were discussed in light of the literature.

Key words: Discectomy histology, laser discectomy, lumbar disc herniation, minimally invasive discectomy

Lazer Nükleotomi Sonrası İntervertebral Disk Dokusunda Histolojik Değişiklikler

Özet
Lumbal disk hastalıklarında minimal invazif teknikler oldukça yaygın olarak kullanılmaktadır. Tüm bu teknikler, değişik etkileri ile prolabe olan disk dokusunu retrakte ederek intradiskal basınç azaltmak suretiyle ilgili sinir köküni dekomprese ederler. Perkütan lazer disk dekompresyonu (PLDD) için çeşitli tip ve dalga boyutları ve elektrodları bulunan lazer jeneratörleri kullanılmaktadır. PLDD’nin klinik etkinliği ile ilgili çok sayıda çalışma olması rağmen etkinliğini ispat edebilecek histolojik parametrelerle dayalı çalışma çok azdır. Yetersiz sonuç alınması nedeniyle PLDD uygulamasından üç ay sonra açık disektomi yapılan üç hastadan alınan disk dokuları histolojik olarak incelendi. Mikrodisektomilerin materyalinde fibröz fibriller dejenerasyon, fibrillerde deforme entegrasyon ve kondrosit proliferasyonu gözlandı. Daha önce lazer uygulananmış disk materyallerinde ise çok daha belirgin fibriller dejenerasyon ve kondroid komponent dejenerasyonu saptandı. Bu bulgulara ek olarak

Anahtar Kelimeler: Diskektomi histolojisi, lazer diskektomi, lumbal disk hernisi, minimal invazif diskektomi

INTRODUCTION

The intervertebral disc is composed of at least three elements. The central portion of the disc contains the nucleus pulposus; the outer portion of the disc is the annulus fibrosis. A meningeal branch of the spinal nerve (sinuvertebral nerve) innervates the area around the disc space. The posterior longitudinal ligament is richly innervated by the nociceptive fibres from the major ascending branch of this nerve. Degenerated human lumbar discs have been shown to contain more nerve tissue and to be more vascular than normal discs(1,3) and pain causes the most disturbance for patients with disc hernias during both the pre- and post-operative periods.

The strength of the lumbar disc is related to the fluid and proteoglycan content of the disc. Both the fluid and proteoglycan components of the disc tend to decrease with age(1,2). Fibronectin is a high molecular weight glycoprotein of the extracellular matrix which binds to membrane-spanning receptor proteins and extracellular matrix components such as collagen, fibrin and heparan sulfate proteoglycans. Insoluble cellular fibronectin is a major component of the extracellular matrix. It is secreted by various cells, primary fibroblasts, and plays a major role in cell adhesion, growth, migration and differentiation, and it is important for processes such as wound healing(3).

The MMP family are involved in the breakdown of the extracellular matrix in normal physiological processes, such as arthritis and metastasis(6). Most MMPs are secreted as inactive proproteins which are activated when cleaved by extracellular proteinases. This gene encodes an enzyme which degrades type IV collagen, the major component of basement membranes. Intervertebral disc proteins such as thrombospondins regulate the effective levels of MMPs 2 and 9, which are key effectors of extracellular matrix remodelling. MMPs are also thought to play a major role in cell behaviours such as cell proliferation, migration and differentiation, angiogenesis, apoptosis and host defence. The MMPs are inhibited by specific endogenous TIMPs. Overall, all MMPs are inhibited by TIMPs once they are activated but the gelatinases (MMP-2 and MMP-9) can form complexes with TIMPs when enzymes are in the latent form. There is no information about the matrix changes in the literature. In this study, we wanted to document the matrix changes of the disc tissue after laser irradiation.

It has been widely held that symptoms of lumbar disc disease are the result of either herniation of the nucleus pulposus through a mechanically weak annulus fibrosis or from tearing of the annulus itself(7). Patients who present with cauda equina syndrome, profound motor weakness and bony structural changes such as stenosis, spondylolisthesis and fragmented disc herniations need to be treated with open
surgical procedures. Percutaneous techniques are most effective in treating patients with small to moderate contained disc herniations, with both clinical and radiographic findings suggesting radicular nerve root irritation and compression\(^{(11)}\). These patients should have sciatica, with leg pain being greater than back pain, and some constellation of the classic findings of atrophy, paresis, sensory and reflex changes and positive tension signs. Chemonucleolysis, automated percutaneous lumbar discectomy, coblation nucleoplasty, ultrasonic percutaneous discectomy, hydropneumatic percutaneous discectomy and laser assisted nucleotomy are some of the percutaneous procedures.

Recent advances in technology, innovations in fibre-optics and miniaturization have brought laser technology into the realm of medical therapeutics. Laser energy is formed from focused light emitted from a medium which has been excited by an external power supply. This energy must be absorbed by biologic tissue in order to produce an effective surgical result. This effect is primarily one of ablation, necrosis and cautery.

Histologic changes after laser irradiation to the disc tissue have been studied by many researchers in cadaveric animal models\(^{(4,8,12,10,13)}\). Decomposition, homogenization and denaturation in the cartilagenous matrix, swelling of the nuclei and cytologic denaturation are some of the changes in the early period. Two months after laser irradiation of the intervertebral discs of rabbits, regeneration signs were seen in the inner layers of annulus fibrosus and in the nucleus pulposus. It has been observed that the shape and ultra-structure of chondrocytes are close to the cells of hyaline cartilage. The cartilaginous tissue is thought to have originated from poorly differentiated chondroblasts activated as a result of laser radiation\(^{(8)}\).

This paper aims to present the histologic changes in the human intervertebral discs three months after percutaneous laser disc decompression.

### MATERIAL AND METHODS

We used a 980-nm wave length multi-diode laser generator for percutaneous laser disc decompression (PLDD). Out of the patients on whom PLDD had been performed, three of them did not show signs of recovery after the application and we decided to operate on them with open discectomy three months after the PLDD technique was used. All three patients were operated on at the L4-5 disc level by the same surgeon. The disc materials were sent for histologic examination. To create a control group, 25 simple discectomy materials, which had not been irradiated previously, were also examined by the same pathologist.

### Histopathological examination:

The disc materials were fixed with 10% formalin solution and then kept in paraffin blocks. The blocks were cut into sections of about 3µ in thickness, perpendicular to the irradiated surface. Hematoxylin-eosin, Masson's trichrome, Alcian Blue and Van Gieson's dye were used for staining; Matrix Metalloproteinase-2 (MMP-2), Matrix Metalloproteinase-9 (MMP-9), Matrix Metalloproteinase-11 (MMP-11), TIMP and fibronectin were stained with immunohistochemical dyes. All the disc materials were examined by the same pathologist under the light microscope at 40X, 100X and 200x magnification.

### Parameters:

Degeneration of the chondroid components and fibrous tissue and cellular changes in the irradiated region were the histologic parameters for examination. The parameters were compared with the non-irradiated disc materials.

### RESULTS

Histologic examination of the disc tissue stained with Hematoxylin-eosin showed chondrocyte proliferation, granular/fibrillary and musinous
degeneration of the chondroid matrix and collagenous fibrillary degeneration of the fibrous component. Previously laser irradiated disc materials had more severe fibrillary degeneration and the chondroid component degeneration was significant. This group also had degenerative calcification and chondrocyte necrosis fields.

Van Gieson's staining caused the disappearance of the degenerated fields but Masson's trichrome dye stained the fields red. All the degenerated field was stained blue and became significant by Alcian blue (Figure 1).

At the immunostaining stage, while chondrocytes were positively stained, the MMP-2 staining was negative. However, at MMP-11, focal interstitial chondroid matrix staining was observed, and also the musinous degenerated fields were stained. The granular/fibrillar degeneration fields were dye negative. Fibronectin was significantly stained in both the musinous and granular/fibrillar degeneration fields. There was no stained field at MMP-9 and TIMP. (Figure 2)

Staining intensity and the patterns of the conventional discectomy materials which were laser irradiated were similar with both the histochemical and immunohistochemical dyes. (Table 1)

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**Figure 1:** Fibrillary/granular degeneration of disc tissue; A: HE X100. B: Alcian blue X40. C: Massson’s trichrome X40. D: PAS X40 E: Chondrocyte proliferation HE X200.

**Figure 2:** Immunohistochemical staining. A: Fibronectin positivity at the fibrillary/granular degeneration fields. B: Focal interstitial chondroid matrix and musinous degenerated fields with positive MMP-11 staining (X40) C: MMP-2 positivity in nucleus of chondrocyte but matrix is negative (X100)
Table 1: Staining results of the dyes.

<table>
<thead>
<tr>
<th>DYE</th>
<th>CHANGES AT THE DISC TISSUE</th>
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<tbody>
<tr>
<td>Hematoxylin-Eosin</td>
<td>Chondrocyte proliferation, granular/fibrillary and musinous degeneration, collagenous fibrillary degeneration</td>
</tr>
<tr>
<td>Van-Gieson’s</td>
<td>Disappearance of the degenerated fields</td>
</tr>
<tr>
<td>Masson’s Trichrome</td>
<td>Blue staining of degenerated fields</td>
</tr>
<tr>
<td>Alcian Blue</td>
<td>Significant blue staining of degenerated fields</td>
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Laser irradiated disc tissue had previously been shown to be similar but the results are more severe in comparison to the simple discectomy disc material at the fibrous component. The most significant finding was the degeneration of the chondroid component. Degenerative calcification, massive necrosis and cellular depletion of the chondrocytes were the main changes. Masson's trichrome staining confirmed the findings.

As a result, laser irradiation of the intervertebral disc tissue causes a decrease in the water content of the disc tissue, hyaline degeneration and fibrillary destruction, as well as chondrocyte depletion.

DISCUSSION

The first surgical use of a laser disc removal occurred in 1984 when a carbon dioxide laser was used as an agent during a standard open anterior cervical discectomy. Choy and colleagues reported the first clinical use in humans of neodymium:yttrium-aluminium-garnet laser disc decompression and nucleotomy in 1987.

Experimental studies have showed that the effectiveness of disc ablation was directly a function of the laser energy's water absorption characteristics. Lasers with the highest water absorption had the highest amount of ablation and the lowest amount of surrounding thermal necrosis from heat transmission to the end plates or posterior longitudinal ligament.

The disc itself is avascular but contains chondrocytes, which are mesenchymal cells which produce and maintain the collagen and proteoglycans of the disc. Only a few studies in the literature have described the histopathologic features of disc degeneration. According to Coventry's study annular changes are characterized by loss of fibrous connective tissue and its replacement with hyalinized collagen fibres. In the nucleus pulposus of infants, residues of notochordal cell aggregates are replaced by proliferating chondrocytes, beginning in the second decade of life. Weidner and Rice reported that neovascularity, granular changes, chondrocyte cloning and fibrillation of fibrocartilage indicate disc degeneration(2).

Most of the studies on the effects of laser irradiation have showed a significant decrease in the water content following the application. Our finding regarding the water content of the disc tissue was similar.

The effect of laser radiation on the intervertebral discs of rabbits was studied and the results demonstrated that laser radiation of spinal discs induced the metaplasia of fibrous cartilage into hylaine-type cartilage(10). Two months later, signs of regeneration were seen in the inner layers of the annulus fibrosus and nucleus pulposus. Another study on cadaveric sheep discs showed that laser
irradiation caused scattering, thinning and homogenization of the connective tissue, a decrease in the number and volume of the nucleus and microcyte formation\(^5\). In this study, we found similar histopathologic changes to those in previous studies in the hematoxylin-eosin stained laser irradiated disc tissue. However, there is no information about the staining characteristics of laser irradiated and/or conventional discectomy tissue with MMP staining (MMP-2, MMP-3, MMP-9, TIMP and fibronectin). Although there was no significant staining difference between the laser irradiated and conventional discectomy tissue with the immunohistochemical dyes, there were no stained zones at the MMP-11 component in the degeneration territory and intense staining of fibronectin showed that the extracellular matrix in particular was affected in the degenerated intervertebral disc tissue and the metalloproteinase activity and protein compound of the extracellular matrix had changed.

During the embryogenic stage of life, the chondrogenic cells differentiate into chondroblasts, which will then synthesize the extracellular matrix of the cartilage. After trapping themselves in a small space the chondroblasts change into chondrocytes, which are usually inactive but can still secrete and degrade the matrix, depending on the conditions. Beside chondrocytes, chondrocyte-like cells in the inner annulus mainly produce type II collagen. A recent study has showed that cells derived from the human annulus were able to differentiate into the chondrogenic and adipogenic lineages. It is well known that chondrocytes are important cells for the regeneration of cartilage. In the literature, there is little information about the effects of laser irradiation on the chondroid component\(^9\). In this study, chondrocyte depletion is the most significant effect of the laser irradiation applied to the disc tissue and it is obvious that the regeneration capability of the disc tissue decreases after laser discectomy.

Therefore, more disc tissue irradiation means more chondrocyte degeneration and less regeneration capability. We think that when the laser probe is used in various directions and angles, the volume of irradiated disc tissue increases, and the success rate of laser discectomy surgery increases.

**CONCLUSION**

The findings for the patients on whom the laser discectomy procedure had previously been performed using the hematoxylin-eosin stained disc materials were similar to those in the literature. The most significant finding was depletion of chondrocytes. In order to increase the efficacy of PLDD, greater amount of disc tissue should be irradiated.

However, there has not been any study of the changes in fibronectin, TIMP and MMPs such as MMP-2, MMP-3, MMP-9 in the disc tissue with immunostaining.

There was no significant difference between the laser irradiated and open discectomy materials with immunohistochemical dyes but a depletion of MMP-11 and massive staining of fibronectin in the degeneration fields indicated that the extracellular matrix was effected and the metalloproteinase activity and protein compound of the extracellular matrix had changed. Further immunostaining studies may show new methodologies for percutaneous disc surgery.

**Correspondence to:**
Arif Ösün
E-mail: arifosun@yahoo.com

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