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# Effect of Nd:YAG Laser on Experimental Disc Degeneration Part I. Biochemical and Radiographical Analysis*

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## Summary

Intervertebral disc herniation is an important health problem from both the social and economic aspect. It is often accompanied by a decrease in the content of water and proteoglycan (PG). The present study was undertaken to elucidate the effect of neodymium-yttrium-aluminum-garnet (Nd:YAG) laser on degenerated disc tissue.

In this study a ventral disc herniation model in guinea pigs was performed to study the effects of Nd:YAG laser on degenerated disc with low water and PG content. Effect of Nd:YAG laser on degenerated cervical disc tissue is examined from the aspects of biochemistry and radiology. In the acute period water, PG, and collagen content decreased due to the ablative effect of Nd:YAG laser on disc tissue. When changes in the degenerated disc in the post-laser chronic period were compared with degenerated disc findings, statistically significant changes could not be be found.

From the results of this study, there is no question that the Nd:YAG laser offers many potential benefits, it remains to be proven whether or not this is of real benefit in the treatment of patients with degenerated disc disease.

*Keywords:* Intervertebral disc; neodymium:YAG laser; experimental model; proteoglycan; degeneration.

## Introduction

Anatomically, the intervertebral disc which has an important role in the continuation of biomechanical functions of the spinal column is composed of three regions: annulus fibrosus, nucleus pulposus, and cartilage end plate. Movement ability and shock absorption capabilities of the vertebral column are provided by proteoglycan (PG), collagen, and water found in the structure of the intervertebral disc [1, 2, 4, 10, 19, 21, 22, 40, 43, 49, 55]. As a result of disc degeneration related to ageing or disc herniation, water and PG content of the disc decreases with loss of its hydrostatic properties [1, 4, 8–10, 26, 27, 29, 34, 37, 40, 55]. Disc herniation associated with degenerative change is one of the most common problems encountered in neurosurgical practice.

As is known, disc surgery is the most important part of vertebral column surgery and the standard surgical procedure for disc herniation is partial laminectomy and discectomy. Recently, due to advances in spinal surgery, percutaneous disc surgery (PDS) as an alternative procedure to standard discectomy has evolved for disc herniations. In 1986, Choy and Ascher applied laser surgery for the first time, to the intervertebral disc resulting in coagulation and shrinkage in the size of the disc [12, 13]. It is postulated that due to vaporization of water, internal disc pressure decreases which in turn results in disc decompression as well as decompression of neighbouring neural elements [13, 24, 32, 36, 44, 45]. This type of disc surgery showed some technical difficulties, such as causing damage to the surrounding tissues by the laser energy [13, 15–17, 25].

In neurosurgery, it is well known that the vaporative effect of neodymium-yttrium-aluminium-garnet (Nd:YAG) laser increases in proportion to the increase in water content of the tissue [9, 12]. Although much effort has been made to ascertain the possible effect of laser energy on disc tissue, the results of laser surgery on degenerated disc with low water and PG content is not yet clearly defined. To show acute and chronic changes in degenerated cervical disc after laser irradiation, an experimental disc degeneration model described in 1951 by Smith and Walmsley [49] was used in this study. The aim of this

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study is to demonstrate biochemical and radiological influences of Nd:YAG laser on degenerated disc tissue. In the second part, the influence will be further studied together with the histological and magnetic resonance (MR) imaging findings [52].

## Materials and Methods

*Animals and Surgical Procedure*

Twenty adult, male guinea pigs (each weighing between 250 g to 350 g, 2 months old) were used in this experiment. They were obtained from litters bred at the animal resource unit of the School of Medicine, Hacettepe University. The care of the animals complied with that stipulated by the Principles of Laboratory Animal Care and the Guide for the Care and Use of Laboratory Animals produced by the American National Society for Medical Research and the National Academy of Sciences, respectively. Prior to surgery, each animal was anaesthetized with a combination of 10 mg/kg xylasine (BAYER Birleşik Alman Ilaç Fabrikaları T.A.Ş, Istanbul) and 60 mg/kg ketamine hydrochloride (Parke-Davis, Istanbul), administered intramuscularly.

Guinea pigs were then divided randomly into four treatment groups (five animals in each) according to the experimental procedures. In the first control gorup (Group 1) five animals were killed, two intervertebral discs were dissected from $C_3$–$C_4$ and $C_4$–$C_5$ levels and immediately placed into a freezer at –60° C until the time of biochemical analysis. In the remaining three groups (Groups 2, 3 and 4), each animal's neck was shaved and the skin was scrubbed with providone iodine (Betadine). Using standard aseptic microsurgical techniques, the ventral surface of the cervical vertebral bodies and disc spaces in between were exposed through an anterior cervical approach under a binocular operating microscope (Opmi 99, Zeis, Germany). Then, a 2–3 mm long transverse incision through the annulus fibrosus was made in order to obtain a degenerated disc at $C_3$–$C_4$ and $C_4$–$C_5$, using a number 11 blade knife. The $C_5$–$C_6$ level was marked with metallic clips and silk sutures. At the end of surgery, all wounds were closed in a standard manner with absorbable sutures. In the second group (Group 2, n = 5) with degenerated disc guinea pigs were killed 60 days after the surgical procedure, all discs incised previously were dissected, and samples were taken for biochemical analysis. In the other two groups with degenerated discs, all animals were re-anaesthetized and the original cervical incision was re-opened and Nd:YAG laser device (wavelength 1,064 nm, MBB medilas Fa. Germany) attached to the operating microscope was used to vaporize the degenerated discs. Laser irradiation was done under the following conditions: duration of 0.4 seconds at a power of 20 watts and an interval of 1.5 to 2.0 seconds. Repetition time was 15 to 20 times (total energy = 90–160 joules). All animals were killed on the 1st day (Group 3, n = 5) and 60 (Group 4, n = 5) days after laser irradiation, respectively. As previously described, samples were then harvested from each of these groups for biochemical analysis.

*Reagents and Biochemical Analysis*

In our present experimental study, p-Dimethylaminobenzaldehyde, D-glucuronic acid, papain, l-cysteine, HCl, carbazole, and cethylpyridinum chloride were obtained form the Sigma Chemical Co., St. Louis, Mo. PG content was determined by the modified uronic acid carbazole reaction of Bitter and Muir [7], with D-glucuronic acid as the standard. The hydroxyproline content as a measure of collagen content was estimated colourimetrically using a method based on the methods of Woessner [47] and Bergman and Loxley [5]. The wet weight of each disc material group was obtained and the dry weights were measured after freeze-drying the weighed samples. Water content was calculated as % water/wet weight.

*Roentgenography*

Lateral roentgenograms of the cervical region of each animal were taken just prior to their deaths, in order to monitor disc height. The disc height at $C_3$–$C_4$ and $C_4$–$C_5$ was measured from the roentgenograms for statistical evaluation of the data to determine the effects of the disc degeneration and Nd:YAG laser on degenerated disc height as a function of time. The height of the disc space was measured at the centre of the interspace and divided by the vertical midbody vertebral dimension. This ratio, which was named the disc index, was then used for comparison [3].

*Statistical Analysis*

One-way analysis of variance (ANOVA) was employed to compare variations between the disc index values at all time periods. Because of significant difference between the values, the analysis was followed by Tukey's test. The difference among the biochemical values at all time periods were evaluated with Kruskal-Wallis one-way analysis of variance tests for comparisons followed by Tukey's test. Statistically significant differences were defined as $p<0.05$ for all tests.

## Results

*Biochemical Findings*

*a. Water content of disc tissue:* The water content of the disc tissue was determined by obtaining the dry weight of the tissue (Table 1). The water content of the normal intervertebral disc in Group 1 was 81% of the tissue wet weight. After experimental disc incision, the water content of the tissue in Group 2 decreased to 57% of its original weight. In Groups 3 and 4, the water content decreased to 53% and 55% of the tissue wet weight on the 1st day and 60 days after laser irradiation of the degenerated disc, respectively. However, the ratio of dry weight to wet weight increased with time; in Group 2, the dry weight at 60 days after experimental disc incision was 43% of the weight of tissue and in Group 4, 60 days after laser irradiation on the degenerated disc, 45% of the original value, while it was 19% of the wet weight of tissue in Group 1. This means that the water content of the disc had significantly recovered and that other components such as protein had increased with time.

*b. Collagen content of disc tissue:* The collagen content of the disc tissue for all groups was measured (Table 2). The collagen content of the degenerated disc in Group 2 was significantly greater than those of

Table 1. *Water Content of Guinea Pig Discs in Wet and Dry Tissues*

| | Wet weight (mg) | Dry weight | | Water content | |
|---|---|---|---|---|---|
| | | mg | % | mg | % |
| Normal | 112 | 21 | 18.7 | 91 | 81.3 |
| Control[a] | 65 | 28 | 43.0 | 37 | 57.0 |
| 1 day[b] | 36 | 17 | 47.2 | 19 | 52.8 |
| 60 days[b] | 62 | 28 | 45.1 | 33 | 54.9 |

Wet or dry weight is expressed as the total weight of ten intervertebral discs.
[a]Degenerated disc.
[b]Disc after laser irradiation.

Table 2. *Collagen Content of Guinea Pig Discs in Wet and Dry Tissues*

| | mg collagen/g dry tissue | mg collagen/g wet tissue |
|---|---|---|
| Normal | 173.75±25.40 | 32.58±4.77 |
| Control[a] | 200.077±29.49 | 86.20±12.70 |
| 1 day[b] | 133.47±4.38 | 63.03±2.07 |
| 60 days[b] | 212.66±32.51 | 98.47±10.69 |

All values are given as means standard deviations.
[a] Degenerated disc.
[b] Disc after laser irradiation.

Table 3. *Proteoglycan Content of Guinea Pig Discs in Wet and Dry Tissues*

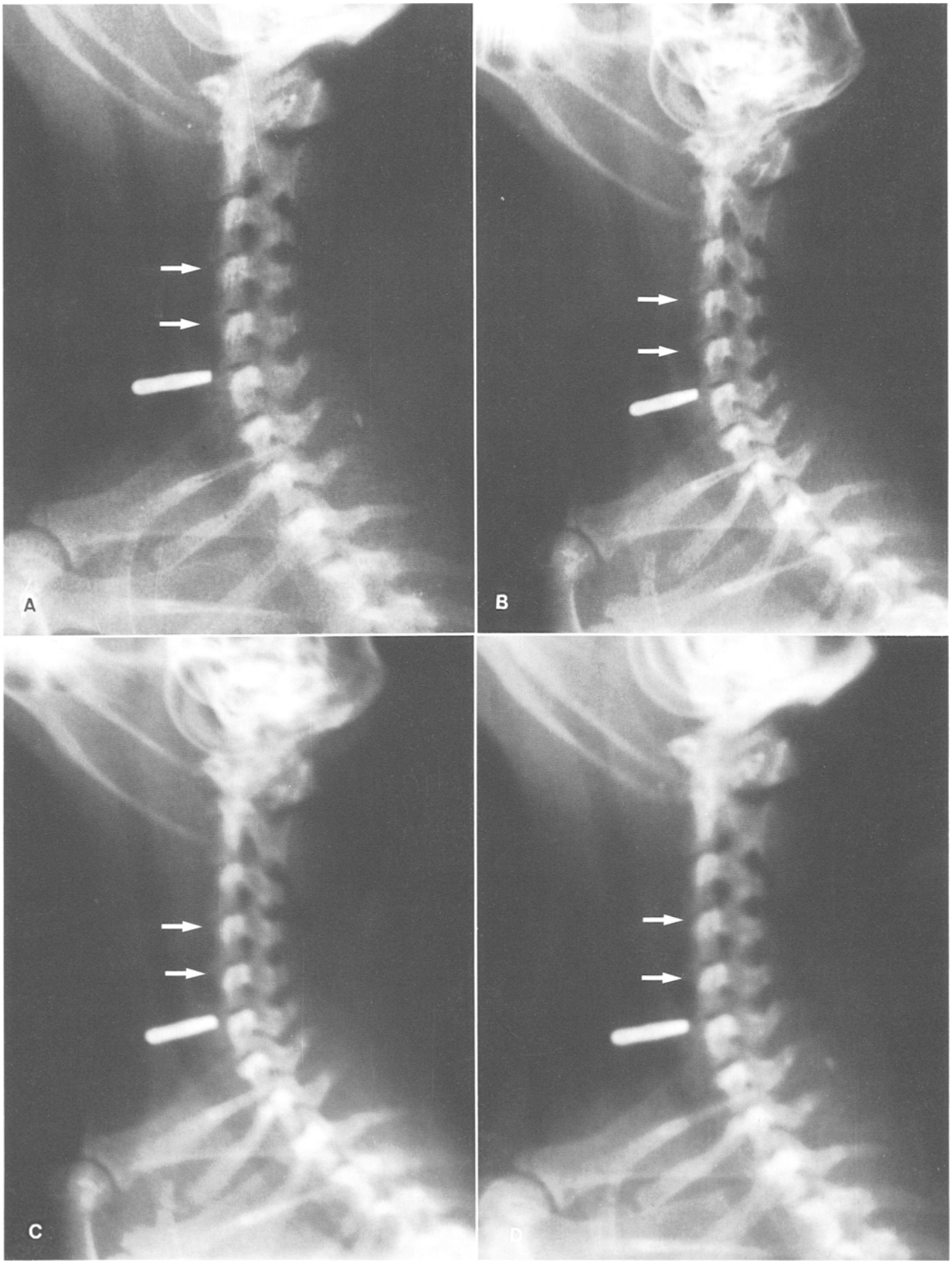
| | mg proteoglycan/g dry tissue | mg proteoglycan/g wet tissue |
|---|---|---|
| Normal | 165.03±1.39 | 30.94±0.26 |
| Control[a] | 150.99±3.48 | 65.04±1.50 |
| 1 day[b] | 112.41±2.29 | 53.08±1.08 |
| 60 days[b] | 154.28±4.69 | 69.68±2.12 |

All values are given as means standard deviations.
[a] Degenerated disc.
[b] Disc after laser irradiation.

the normal disc in Group 1; 150% of the dry weight of the tissue and 250% of the wet weight of the tissue. At one day after laser irradiation in Group 3, this ratio had decreased to about 70% of the value of the degenerated disc in Group 2. Statistical analysis of this results demonstrated that there was a significant difference between Groups 2 and 3 ($p = 0.014$). At 60 days after laser irradiation, this ratio had increased significantly in Group 4 and there was no significant difference between Groups 2 and 4 ($p = 0.895$).

*c. PG content of disc tissue:* To determine the alterations in the PG content of the disc tissue with time, the PG amount of both the dry and wet weights of each disc was measured (Table 3). The percentage

Fig. 1. Radiographic changes in the intervertebral discs in one guine pig following experimental disc degeneration and laser irradiation. The arrows show the two disc spaces which were incised and irradiated. (A) Normal intervertebral discs in Group 1. (B) Sixty days after experimental disc incision, the same two spaces showed narrowing and loss of disc space in Group 2. (C) One day after laser irradiation, heights of irradiated discs had decreased to less than two-thirds of the original height in Group 3. (D) Sixty days after laser irradiation, the disc spaces at the irradiated levels are restored in Group 4 (see arrows)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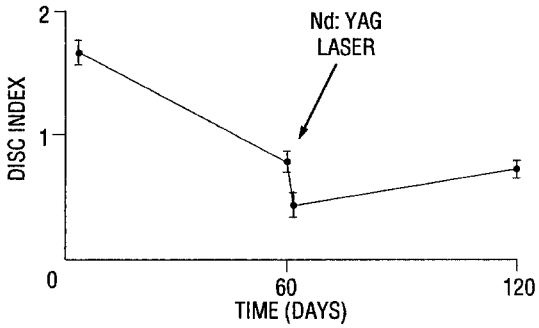


Fig. 2. Changes in the disc space height after the experimental disc degeneration and laser irradiation of the degenerated disc

ratio of PG content of the degenerated disc to dry weight in Group 2 decreased to about 90% of the normal disc in Group 1. At one day after laser irradiation, the percentage ratio of PG content in Group 3 decreased to 65% of that in the degenerated disc in Group 2. However, the percentage ratio of PG content to dry weight increased to 90% of the normal disc, but there was no significant difference between Groups 2 and 4 ($p = 0.315$). Statistical analysis of the results of PG content demonstrated that there was a significant difference between the other groups ($p = 0.00$).

*Radiological Findings*

Significant narrowing of the disc height was shown in all roentgenograms at 60 days after experimental incision of the intervertebral disc in Group 2 (Fig. 1A and B). When the changes were measured on the first day after laser irradiation on the degenerated disc, the height in Group 3 had decreased to about 60% of its original value (Fig. 1C). At 60 days after laser irradiation on the disc, the disc height in Group 4 had returned to its original value (Fig. 1D). The disc index value for each period is shown in Fig. 2. The difference in the disc height was statistically significant ($p>0.05$).

## Discussion

The intervertebral disc is one of the structures placed between the two adjacent vertebral bodies and plays an important role in the maintanance of the functions of the spinal column. The first anatomicopathological studies related to the disc were performed by von Luschka in 1958 [28]. The exact relationship between the disc lesions and sciatica was first described by Mixter and Barr in 1934 [38]. Nowadays, intervertebral disc herniations are still one of the most important health problems from both the public and economic aspects [3, 4, 9, 14, 16, 20, 29–35, 37, 39, 41, 42, 46, 47, 51]. It is estimated that about 60 to 80% of the people had experienced an acute attack of back pain at sometime in their life [4, 20, 39]. Prevention of morbidity and mortality, complete relief of the pain, and having a rapid healing process are the main principles of solving this problem. Surgical decompression of the presenting disc lesion by a posterior approach to the spinal column is the commonly used conventional treatment modality.

Normal disc tissue is quite a rigid material which is closed to the surrounding structures. Therefore, it is assumed that a small volume change due to the vaporization effect of laser irradiation on the disc material would achieve decompression and a significant decrease in the pressure within the disc [13, 36]. It is accepted that vaporization created by Nd:YAG laser is primarily due to the heat effect of the laser [15, 18]. As a result, shrinkage occurs in the target tissue, leading to the formation of end products like vapour, carbon, $CO_2$ and carbonized tissue fragments [18, 23, 32, 48]. Some of the studies suggested that laser application not only lengthened the surgical intervention period but also increased the surgical risks. In an experimental study, Trauner *et al.* [53] showed that the amount of laser energy necessary for vaporization of water for a material completely composed of water is 2.5 kJ/cm$^3$, for the articular cartilage containing 70 to 80% water is 10.5 kJ/cm$^3$ and for the fibrous cartilage with a lower content of water is 12 kJ/cm$^3$. From the results of this study, it can be concluded that as the water content of the target tissue decreases, the amount of laser energy necessary to provide vaporization increases. On the other hand, the depth of vaporization created by Nd:YAG laser can not be controlled completely. Therefore, the increased heat produced by laser energy can cause damage to the surrounding tissue [11, 15, 33, 53]. Recently, the introduction of new laser types has been started with the purpose of decreasing thermal damage and avoiding the prolongation of wound healing [9, 20, 44, 45].

Previous studies on normal disc material showed that laser was more effective on the disc with a high water content [13, 23, 32]. This effect is probably a result of the better absorption of laser by these tissues. In this study, acute and chronic biochemical and radiological changes after Nd:YAG laser irradiation on degenerated disc tissue were evaluated. As used in the present study, there is no experimental study showing

the effects of laser irradiation on degenerated disc material which contains a lesser amount of water. Radiologically, decrease in the height of disc after disc herniation is a well-known process [3, 6, 23, 25, 32, 42, 50, 54, 56, 57]. When normal disc tissue in Group 1 was degenerated, more than 50% decrease in the height of the disc was observed in our study. On the other hand, after laser irradiation on degenerated disc, there was only a 40% decrease in disc height in Group 3. In Group 4, disc height was increased again, but when the height of disc in Group 4 was compared with the height of disc in Group 2, there was no statistically significant difference ($p>0.05$).

It is accepted that decreasing content of water in a disc is the result of the decrease in the amount of PG [4, 10, 34, 55]. Therefore, a decrease will occur in the hydration of a disc with decreased PG content. On the other hand, it is suggested that collagen has an effect on PGs by forming a fibrous network and controls the swelling pressure of the disc [19, 22, 26, 48]. After laser irradiation to the degenerated disc, decrease in water, PG, and collagen content in Group 3 is due to the ablative and vaporizative effects of laser. There was a statistically significant difference in the biochemical values of the discs between Groups 2 and 3, whereas the biochemical findings in Group 4 were not significantly different from Group 2. In addition, there was statistically significant difference in biochemical findings of degenerated disc between Groups 1 and 3. This result is also in accordance with the radiological findings.

Although results of the current study cannot be directly related to clinical efficacy, they allow us to draw the following conclusions. First, an experimental degeneration model can easily be obtained by making a ventral disc incision in guinea pigs. Second, by ventral disc herniation in normal disc tissue, water and PG content decreases while collagen content increases. Third, biochemical and radiological changes between Groups 2 and 4 were found to be insignificant statistically. Fourth, it is quite difficult to see Nd:YAG laser disc surgery as an effective treatment modality. Although it seems attractive to use laser technology in disc surgery, the results of this experimental study do not support the effectiveness of laser in disc surgery. However, Nd:YAG laser may have different effects on various collagen types and PG monomers. Therefore, from a clinical standpoint, further studies are necessary to show these effects.

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