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ORIGINAL ARTICLE
ORİJİNAL ARAŞTIRMA

Lomber Spinal Stenozda Ligamentum Flavum Hipertrofisinin Transforming Growth Factor Beta-1 Ile Ilişkisi

Relationship of ligamentum flavum hypertrophy in lumbar spinal stenosis with transforming growth factor β-1

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ÖZ

Amaç: Lomber spinal stenoz yaşlılardaki bel ağrısının en sık görülen sebebidir. Lomber spinal kanal stenozu faset eklemlerinin kemik proliferasyonu ve ligamentum flavum hipertrofisini içeren posterior kanaldaki dejeneratif değişiklikler sonucu gelişir. Biz bu çalışma ile lomber spinal kanal stenozunda ligamentum flavum hipertrofisinin stenoz yerindeki bu potent sitokin olan TGF-β1'in yükselen konsantrasyonu tarafından yönlendirilebileceğini göstererek literatüre katkı sağlamayı amaçladık.

Gereç ve Yöntem: Çalışmamızda lomber disk hemisi ve lomber spinal dar kanal hastalarından cerrahi girişim esnasında alınan ligamentum flavum örneklerindeki TGF-βl konsantrasyonu ölçülmüştür. Ayrıca bu hastalardaki ligamentum flavum kalınlığı lomber manyetik rezonans görüntüleme-doku kalınlığı ortalaması alınarak saptanmış ve hastalarda tüm bu sonuçlar istatistiksel olarak karşılaştırılmıştır.

Bulgular: Her iki gruptan elde edilen ligamentum flavum kalınlıkları lomber disk hernisi grubunda 3.46±1 mm, ve lomber spinal stenozda 5.63±1.35 mm olarak bulunmuştur. İstatistiksel olarak farklar anlamlı bulunmuş (p=0.000),. Transforming Growth Factor Beta-1 grup ortalamaları standart sapmaları ile HNP'de 1676.47±642 pg/g, ve lomber spinal stenozda 6816.68±5147.57 pg/g olarak bulunmuştur. İki grup arasındaki fark istatistiksel olarak anlamlı bulunmuştur (p=0.000).

Sonuç: Çalışmamızda, lomber spinal stenozda ligamentum flavum hipertrofisine TGF-β1'in etkisinin olabileceği gösterilmiştir.

Anahtar Kelimeler: Ligamentum Flavum Hipertrofisi, lomber spinal stenoz, TGF-β1.

ABSTRACT

Aim: Lumbar spinal stenosis is the most common cause of low back pain in the elderly. Lumbar spinal canal stenosis develops as a result of degenerative changes in the posterior canal including bone proliferation of the facet joints and ligamentum flavum hypertrophy. With this study, We aimed to contribute to the literature by demonstrating that ligamentum flavum hypertrophy in lumbar spinal stenosis may be directed by increased concentrations of TGF-β1, at the stenosis site.

Materials and Methods: In our study, TGF-βl concentrations in the ligamentum flavum samples taken from patients with lumbar disk hernia and lumbar spinal stenosis during surgical intervention. In addition, thickness of ligamentum flavum in these patients was calculated by averaging the lumbar MRI-tissue thickness, and all these results were statistically compared among the patients.

Results: Ligamentum flavum thickness values in two groups were 3.46±1 mm in lumbar disk hernia and 5.63±1.35 mm in lumbar spinal stenosis and the differences were statistically significant (p<0.001). Group averages of Transforming Growth Factor β -1 with standard deviations were 1676.47±642 pg/g in lumbar disk hernia and 6816.68±5147.57 pg/g in lumbar spinal stenosis. The average difference in these results was considered statistically significant (p<0.001).

Conclusion: In conclusion, we demonstrated in our study that TGF-β1 has an effect on ligamentum flavum hypertrophy in lumbar spinal stenosis.

Keywords: Ligamentum flavum hypertrophy, lumbar spinal stenosis, TGF-β1

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INTRODUCTION

Today low back pain is a major and common community problem requiring admission to clinics (1). Lumbar spinal stenosis a condition very commonly seen in the elderly, and ranks first among the causes of lumbar spinal surgeries (2,3). It is classified into two groups based on its etiology: congenital and acquired (4-8). Narrowing of spinal canal is generally a result of age-related degeneration; however, it can be seen in congenital disorders of spinal canal such as achondroplasia, scoliosis and congenital narrow canal (3,5,9). Acquired spinal canal stenosis can be secondary to facet joint hypertrophy, increased thickness of ligamentum flavum, protrusion of intervertebral disk, or spondylolisthesis (5-7,9-14). Lumbar spinal stenosis is mostly seen at the L4-L5 level followed by L3-4 and L2-3 levels (1,15,16). Its diagnosis is based on the canal diameter (10). Lumbar spinal canal stenosis develops as a result of degenerative changes in the posterior canal including bone proliferation of the facet joints and ligamentum flavum hypertrophy (17).

Ligamentum flavum hypertrophy is known to be associated with aging process, or with degenerative changes secondary to mechanical instability (18-21). Ligamentum flavum hypertrophy is a characteristic feature of lumbar spinal stenosis which results in cauda equina or nerve root compression (17,20-22). As a factor mimicking chondrocytes and fibroblasts causing extracellular matrix proteins, Transforming Growth Factor-beta (1 TGF- βl) has recently drawn attention (17). TGF βl is the multifunctional growth factor involved in synthesis, differentiation and proliferation of extracellular matrix proteins (20). Increased collagen content was reported to cause ligamentum flavum hypertrophy. During recent years, many investigators shown that TGF-β1 also increases collagen synthesis in soft tissues. The mechanical stress causing collagen synthesis was reported to work in vitro via TGF-β1 in mesenchymal cells and soft muscle cells (17).

While some authors reported that hypertrophic ligamentum flavum plays an important role in lumbar spinal canal stenosis pathogenesis, whereas others mention the contrary (17). Studies have long been focused on histological or morphological changes. Biological mechanisms of ligamentum flavum hypertrophy in lumbar spinal stenosis are still unknown (20). With this study, we aimed to contribute to the literature by demonstrating that ligamentum flavum hypertrophy in lumbar spinal stenosis may be directed by increased concentrations of TGF-β1, at the stenosis site.

MATERIAL and METHOD

In our study, patients admitted to Adnan Menderes University Faculty of Medicine Hospital Neurosurgery Clinic between 2001 and 2005, diagnosed with lumbar disk hernia and lumbar spinal stenosis with surgical treatment indication were included and 39 ligamentum flavum biopsies were used. 18 of these were of HNP cases and 21 were of lumbar spinal stenosis cases. Of the HNP cases, 11 were males and 7 were females and of the lumbar spinal stenosis 13 were males and 8 were females.

Radiculopathy findings were predominant in patients with lumbar disk hernia, neurogenic claudication and radiculopathy were predominant in patients diagnosed with lumbar spinal stenosis. During the surgical intervention ligamentum flavum was removed carefully with all folds, and placed at -85°C without delay.

From patients ligamentum flavum samples for measurement of TGF-β1 concentrations were taken during the surgical intervention, and analyzed using Enzyme Linked-Immuno-Sorbent Assay (ELISA) method. TGF-β1 in ligamentum flavum was determined using immunohistochemical method.

Measurement of Ligamentum Flavum Thickness

Ligamentum flavum thickness measurements were made at the section in which the thickness of ligamentum flavum is the highest in the axial T1-weighed magnetic resonance imaging, using calipers and the scale on the film.

Ligamentum flavum samples obtained during the surgical intervention could be

measured with an accuracy to 0.01 mm on its thickest point using calipers. In our study, the average of both values was calculated. Biochemical and radiological results were compared for two cases.

Taking and Storing Tissue Samples

During the operations of patients, ligamentum flavum was removed with all folds, and placed in a deep freezer at -85°C without delay. Immediately after tissue homogenization, tissue supernatants were frozen in eppendorf tubes at -85°C. All works were completed in 2 weeks.

Tissue homogenization was performed using tissue homogenization buffer so as to be 1:10 (w/v). Tissue homogenization buffer (1mM, pH:7.4) was prepared using phenylmethylsulfonylfluoride (C7H7F025, Sigma, Cat. No. P-7626), di-natriumhydrogenphosphate-dihydrate (Na2HP04.2H20, MERCK, Cat.No.K25979680), potassiumdihydrogenphosphate (H2KP04. MERCK, Cat. No. A986373), ethylenediaminetetraacetic asid-disodium salt (Na2EDTA) (C10H14N2O8Na2.2H20, Sigma, Cat. No. E-1644).

Transforming Growth Factor βl (TGF βl)

TGF β1 determination was performed using BIOSOURCE Immunoassay commercial kit (Cat. No: KAC1688/KAC1689). Measurement principle of the kit is based on ELISA method. Wells in the microplate contained in the kit are pre-coated with TGF-β1-specific monoclonal antibodies. After the samples and the standards which come with the kit are prepared, they are placed into the wells, and the second biotinylated antibody is added. After the first incubation step, it will bind to immobilized antibody with the TGF-β1 portion, and to the biotinylated antibody with the other portion. After the excess antibodies are removed via washing step, Streptavidin-Peroxidase enzyme is added. After the second incubation step, unbound enzyme is removed, the substrate solution is added so that the enzyme bound to the antibody develops color. The intensity of this color is directly proportional to the amount of TGF-β1 present. TGF-β1 amounts were determined directly from the microplate reader and then tissue amounts were found by calculation.

Wholes (lumbar disk hernia and lumbar spinal stenosis) were considered dependent variables, and age, TGF-β1 amount and thickness were considered independent variables. Average values of the two groups were evaluated from non-parametric tests using Kruskal-Wallis Variance Analysis. A p value of 0.05 was considered significant. When a significant difference is found between groups, Mann-Whitney U Test was applied using Bonferroni correction to determine the group from which the difference originates. The tests were performed using SPSS 11.0 software.

RESULTS

Average ages in the groups with standard deviations were 49± 13 in lumbar disk hernia and 62±9 in lumbar spinal stenosis. The average age difference were found statistically significant (p<0.001)

Ligamentum flavum thicknesses were 3.46±1 mm in lumbar disk hernia, and 5.63±1.35 mm in lumbar spinal stenosis (Table 1). The differences were statistically significant (p<0.001).

Table 1: Average of ligamentum flavum thicknesses the groups with standard deviations.

Diagnosis	Average of ligamentum flavum thicknesses	N	Standart Deviations
HNP	3,4611	18	1,01236
Lumbar Stenosis	5,6381	21	1,34925

Group averages of TGF-β1with standard deviations were 1676.47±642 pg/g and 6816.68±5147.57 pg/g in lumbar spinal stenosis (Table 2). The mean difference for these results was considered statistically significant (p<0.001).

DISCUSSION

The ligamentum flavum hypertrophy is a pathological process, which has an important role in the etiology of the lumbar spinal stenosis (23,24). Despite the ligamentum flavum hypertrophy was frequently

investigated previously via histological and morphological approaches, the responsible biochemical changes are still not clarified. TGF-β1 is an important growth factor responsible for the synthesis of extracellular matrix proteins and also for the cell differentiation and proliferation. It is emphasized that this growth factor, which involves in the physiological and pathological processes of many tissues and organs, may also associate with the hypertrophy of the ligamentum flavum (24).

Table 2: Averages of Transforming Growth Factor B-1 (pg/g) with standard deviations.

Diagnosis	Averages of Transforming Growth Factor B-1	N	Standart deviations
HNP	1676,4656	18	642,02202
Lumbar Stenosis	6816,6848	21	5147,57652

In our study, the thickness of the ligamentum flavum is measured among two groups of patients with lumbar spinal stenosis and lumbar disc hernia. The thickness of the ligamentum flavum was found significantly higher in patients with lumber spinal stenosis.

In recent years, the likely association of the ligamentum flavum hypertrophy with TGF-β1 levels was asserted. In a study conducted by Park et al., which compared the lumbar spinal stenosis and lumbar disc hernias, the levels of the TGF-β1 were found significantly higher in the patients with lumbar spinal stenosis (24). In another experimental study, it was revealed that the ligamentum flavum hypertrophy is associated with TGF-β1 expression (25). In our study too, the TGF-β1 concentrations were found significantly higher among the patients with lumbar spinal stenosis in comparison to other two groups, which is consistent with the existing literature. These findings indicate that the TGF-β1 levels - which substantially induce the synthesis of the extracellular collagen – may play a role in the ligamentum flavum hypertrophy-pathogenesis.

In conclusion, increased concentrations of TGF-β in the ligamentum flavum might be a possible pathogenesis for ligamentum flavum hypertrophy in spinal stenosis. This result may guide for further studies regarding the prophylaxis and novel therapeutic approaches of lumbar spinal stenosis, which can be currently treated only with decompressive surgery.

REFERENCES

1. Chung SS, Lee CS, Kim SH et al. Effect of low back posture on the morphology of the spinal canal. Skeletal Radiology 2000;29:217-223.
2. Benoist M. Natural history of the aging spine. Eur. Spine J 2003;12:86-89.
3. Jayakumar PN, Devi BI, Bhat DI et al. Thoracic cord compression due to ossified hypertrophied ligamentum flavum. Neurology India 2002;50:286-289.
4. Aydın Y, Ziyal İM, Duman H et al. Clinical and radiological results of lumbar microdiskectomy technique with preserving of ligamentum flavum comparing to standard microdiskectomy technique. Surg Neurol 2002;57:5-14.
5. Epstein NE. Symptomatic lumbar spinal stenosis. Surg Neurol 1998;50:3-10.
6. Honsraj KK, O'Leary PF, Commisa FP et al. Decompression, fusion and instrumentation surgery for complex lumbar spinal stenosis. Clin Orthop Relat Res 2001;(384):18-25.
7. Kalbarezyk A, Lukes A, Seiler R W. Surgical treatment of lumbar spinal stenosis in elderly. Acta Neurochirurgica 1998;140:637-641.
8. Kucich U, Rosenbloom JC, Herrick DJ et al. Signaling events required for transforming growth factor-p stimulation of connective tissue growth factor expression by cultured human lung fibroblasts. Arch Biochem Biophys 2001;395:103-112.
9. Carano RA, Filvaroff EH Angiogenesis and bone repair. Drug Discov Today 2003;8(21):980-9.
10. Brock M, Ramsbacher J. Lumbar spinal stenosis. Crit Rev Neurosurg 1998;8:333- 337.
11. Goh KJ, Khalifa W, Anslow P et al. The clinical syndrome associated with lumbar spinal stenosis. Eur Neurol 2004;52:242-249.
12. Grossman RG, Loftus CM. Spinal instability. Second Edition. Principles of Neurosurgery. Philadelphia: 1999.
13. Hering S, Jost C, Schulz H et al. Circulating transforming growth factor beta1 (TGFbeta1) is elevated by extensive exercise. Eur J Appl Physiol. 2002;86(5):406-10.
14. Kim S L, Lim RD. Spinal stenosis. Pis Mon 2004;51:6-17.
15. Bridwell K H, DeWold R L, Lubicky J P et al. Spinal stenosis. The Textbook of Spinal Surgery, Philadelphia: 1991.
16. Inoue T, Okada H, Kobayashi T et al. TGF-31 and HGF coordinately facilitate collagen turnover in subepithelial mesenchyme, and Biophysical Research Communications 2002;297: 255-260.
17. Goldman R. Growth factors and chronic wound healing: Past, present, and future. ADV Skin Wound Care 2004;17:24-35.
18. Daniels JT, Schultz GS, Blalock TD et al. Mediation of transforming growth factor pi stimulated matrix contraction by fibroblasts: A role for connective tissue growth factor in contractile scarring. Am J Pathol 2003;163(5):2043-52.
19. Franklin JT. Therapeutic approaches to organ fibrosis. Int J Biochem Cell Biol. 1997;29(1):79-89.
20. Greenberg MS. Spinal vertebra ve spinal kord. Birinci Baskı. Nörosirürii Elkitabı. İstanbul, 1996;42:488-492.
21. Kaye AH, Black PM, Popovic EA. Decompressive lumbar laminectomy. Operative Neurosurgery: London, 2000.
22. Giles LGF. Mechanisms of neurovascular compression within the spinal and intervertébral canals. J Manipulative Physiol Ther 2000;23(2):107-11.
23. Nakatani T, Marui T, Hitora T et al. Mechanical stretching force promotes collagen synthesis by cultured cells from human ligamentum flavum via transforming growth factor [beta] 1. J Orthop Res 2002;20(6):1380-6.
24. Park J B, Chang H, Lee J K. Quantitative analysis of transforming growth factor- beta 1 in ligamentum flavum of lumbar spinal stenosis and disc herniation. Spine 2001;26:492-495.
25. Löhr M, Hampl JA, Lee JY et al. Hypertrophy of the lumbar ligamentum flavum is associated with inflammation-related TGF-β expression. Acta Neurochirurgica 2011;153(1):134-4.