Comparison of Histological Changes in the Ligamentum Flavum from Spinal Canal Stenosis Patients with and without Diabetes Mellitus

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Abstract
Objective: Lumbar spinal canal stenosis (LSCS) has prominent position in spinal disorders of the elderly. Ligamentum flavum hypertrophy plays a dominant role in narrowing of the LSCS. There are some clues that Ligamentum flavum hypertrophy is associated with diabetes mellitus. Our objective was comparison of histological properties of ligamentum flavum in Lumbar spinal canal stenosis patients with and without diabetes mellitus.

Materials and Methods: In this case control study, during 9 months, twenty-nine patients, who were candidate for decompressive laminectomy because of ligamentum flavum hypertrophy, were studied. They were labeled as diabetic if had history of diabetes mellitus or fasting blood sugar ≥126mg/dl for two times or blood sugar >200mg/dl anytime with symptom of hyperglycemia. Ligamentum flavum was removed through surgery. Samples were evaluated by pathologist using Hematoxylin &Eosin, Masson’s trichrome and Verhoeff van Gieson staining. Evaluation includes grading of fibrosis, loss of elastin fibers, calcification and number of cellularity of samples. Data analyzed with Fisher’s test and Mann-Whitney test.

Results: In this study 41% of patients (12 persons) were suffering from diabetes mellitus. There was no significant difference between diabetic and nondiabetic patients in histological properties. (P>0.05); but the weight of diabetic patients was higher than nondiabetics (P-value =0.038)

Conclusion: These results show that diabetes mellitus has no effect on histological change in ligamentum flavum of spinal canal stenosis and mechanical stress (due to overweight) has a more important role in pathogenesis of spinal canal stenosis.

Keywords: Diabetes mellitus, Spinal canal stenosis, Ligamentum flavum, Histological change, Overweight, Tumor growth factor-β

Introduction

Lumbar spinal canal stenosis (LSCS) is one of the most common spinal disorders in the elderly. The causes of LSCS include ligamentum flavum (LF) hypertrophy, hypertrophy of the facet joints, bulging of the intervertebral discs, and vertebral endplate osteophytosis. LF hypertrophy plays a dominant role in the
narrowing of the lumbar spinal canal (1). Fibrosis is considered as the main cause of LF hypertrophy. Transforming growth factor \(\text{TGF-}\beta\) released by endothelial cells may stimulate the fibrosis, especially during the early phase of hypertrophy (2). The structure of LF is unique, because of a predominance of elastic fibers, and its intrinsic innervations at each level of the spine. The innervations decrease with increasing degeneration (3). Histological changes in the hypertrophied LF from LSCS patients include fibrosis, degradation of elastic fibers with an increase in collagen fibers, granulation tissue proliferation, chondroid metaplasia, and calcification (2,4,5) increasing of \(\text{TGF-}\beta\) (2,6,7,8). Although the serum concentration of \(\text{TGF-}\beta\) was normal (9). In the other hand \(\text{TGF-}\beta\) has an important role in complications of diabetes mellitus. \(\text{TGF-}\beta\) is increased in diabetic nephropathy and stimulates basement membrane production of collagen and fibronectin by mesangial cells (10). In some articles, mechanical stress introduced as the increasing of \(\text{TGF-}\beta\) cause (2,11,6). In the other study, it was seen that expression of MMP13, which is involved in inflammation and fibrosis, in the LF from lumbar canal stenosis patients with diabetes mellitus was higher than non-diabetic patient (12). It has mentioned that diabetes mellitus is a risk factor for the development of lumbar spinal stenosis (13).

Our purpose was to compare the histological changes (fibrosis, loss of elastin, calcification and cellularity) in LF samples from the patients with spinal canal stenosis between the diabetic and non-diabetic groups.

**Materials and Methods**

In this study, from April 2013 up to 9 months we studied all the patients that get under decompressive laminectomy because of hypertrophy of LF in Shahid Rahnemoun Hospital. The inclusion criteria was, LF hypertrophy that was diagnosed by neurosurgeon in the lumbar MRI. Patients, who had spinal canal stenosis because of tuberculosis, ossification of posterior longitudinal ligament (OPLL), vertebral fracture and spondylolisthesis were excluded. Twenty-nine patients were studied. They were diabetic if had history of diabetes mellitus or fasting blood sugar \(\geq 126\text{mg/dl}\) for two times or random blood sugar>200mg/dl anytime with symptom of hyperglycemia. According to this definition 12 people have diabetes mellitus. First time, FBS was checked in the hospital and for second time after discharging. LF was removed during surgery. All surgeries were done in Shahid Rahnemoun hospital in Yazd, Iran. In the operation, LF was removed and immediately was placed in formalin4% solution. Then paraffin blocks was prepared from samples and got sectioned for hematoxylin & eosin, Masson’s trichrome and Verhoeff van Gieson staining.

In next step, prepared sections get evaluated about grading of fibrosis, elastin fiber decrease, calcification and number of cellularity. According to these point 5 fields was selected randomly from central (not dorsal or dural) with the ×40 magnification and this four factors determined by a pathologist. Gradings were selected according to table1 and figure 1. In order to determine calcification, the minimum amount of calcification was assumed as + (figure 1-A) and the maximum amount of calcification was assumed as +++ (figure 1-B) and the amount of calcification which was among them as ++ (figure 1-C). Data input in SPSS17 and analyzed with Fisher’s exact test and Mann-Whitney test.

**Results**

Totally 29 patients were studied. Demographic characteristics of patients is presented in table 2.

The cellularity average in five fields and the amount of calcification in LF samples were determined; but there were no significant differences between diabetic and non-diabetics. (Figures 2-3) Branching and variability of sizes in elastin fibers which had been placed in margin of
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Figure 1. section without calcification stained by H&E (a) section with +++ calcification stained by H&E (b) section with + calcification stained by H&E (c) section demonstrate fibrocartilage tissue (d) section demonstrate grade0 in loss of elastin fibers stained by Verhoeff van Gieson (e) section demonstrate grade1 in loss of elastin fibers stained by Verhoeff van Gieson (f) section demonstrate grade0 in fibrosis stained by Masson’s trichrome (g) section demonstrate grade1 in fibrosis stained by Masson’s trichrome(h)

fibrotic region, was increased. (Figure 4).there wasn’t significant difference in fibrosis grading between diabetics and non-diabetics. (Figure 5)

Conclusion
Lumbar spinal canal stenosis (LSCS) is one of the most common spinal disorders in the elderly (12). Since 1913 that Elsberg first has reported the case showing sciatica caused by the LF hypertrophy, there are several attempts to clarify the pathomechanism of LF hypertrophy but it is still unclear (2). It was reported that in lumbar canal stenosis unique histological changes are seen including, increase of collagen fibers (fibrosis) (2,4,12,14,15,16), loss of elastic fibers (2,12,15,16), calcification (4,16) and increase of cellularity (11). Decreased elastin fibers and increased collagen fibers have several reasons: aging (2), inflammation (14) and mechanical stress (6,11,17). Fibrosis is the main cause of LF hypertrophy, and TGF-β released by endothelial cells, may stimulate the fibrosis, especially during the early phase of hypertrophy (2). Nakatani et al (6) confirmed the effects of TGF-β application on collagen synthesis of cultured LF fibroblasts. It was reported that TGF-β mediated by mechanical stress induces collagen synthesis on a variety of cells in vitro (6,18,19,20). There are documents that can interpret high expression of TGF-β in LF: 1-Mechanical stress 2-diabetes mellitus.

In some studies, mechanical stress introduced as the cause of TGF-β increasing (2,6,11); so the dorsal side of LF was subjected to more stress when compared to the dural side so dorsal side of LF has more fibrotic region than dural side (2).

TGF-β has an important role in complications

<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls</th>
<th>Diabetic patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>17 (59%)</td>
<td>12 (41%)</td>
</tr>
<tr>
<td>Age</td>
<td>54.8±12.3</td>
<td>61.2±9.5</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>6 (35%)</td>
<td>8 (65%)</td>
</tr>
<tr>
<td>Female</td>
<td>11 (65%)</td>
<td>4 (35%)</td>
</tr>
<tr>
<td>Metabolic syndrome</td>
<td>8 (47%)</td>
<td>10 (83%)</td>
</tr>
<tr>
<td>BMI</td>
<td>25.7±4.8</td>
<td>26.2±8.0</td>
</tr>
<tr>
<td>Weight</td>
<td>67.0±10.0</td>
<td>72.0±18.1</td>
</tr>
</tbody>
</table>
of diabetes mellitus. TGF-β is increased in diabetic nephropathy and stimulates basement membrane production of collagen and fibronectin by mesangial cells (10). High blood glucose could promote collagen synthesis in the posterior longitudinal ligament mainly via endogenous TGF-β1, resulting in hypertrophy of ligament (21). This fact supports this hypothesis that TGF-β in LF can be attributed to diabetes mellitus. Diabetes mellitus introduced as a risk factor of several soft tissue disorders such as ossification of posterior longitudinal ligament (OPLL) (21), Achilles tendon thickness (22,23), plantar fasciitis (22), plantar aponeurosis thickness (24), flexor hallucis longus tendon thickness (24), supraspinatus tendon thickness (25), biceps tendon thickness (25), adhesive capsulitis (26) and diffused idiopathic skeletal hyperostosis (DISH) (27). But there are a few numbers of studies about the effect of DM on LF. Fibrosis is the major change in LF of spinal canal stenosis patients (2), so if diabetes mellitus become the cause of spinal canal stenosis, different histological difference of histological change between diabetic and non-diabetic patients. In order to this point, we compare the histological change, include fibrosis, loss of elastin fibers, calcification and cellularity between the
The study compared histological changes in the ligamentum flavum (LF) in diabetic and non-diabetic patients, but found no significant difference between them. It was concluded that diabetes mellitus cannot be assumed as a risk factor for spinal canal stenosis.

Diabetes patients were heavier than non-diabetics, with a BMI of $\geq 25$ kg/m$^2$ being a risk factor for type 2 diabetes (T2DM). The mean weight in diabetic patients was 72.0±18.1 kg vs. 67.0±10.0 kg in non-diabetics ($P=0.024$). It was noted that patients with a BMI $\geq 25$ kg/m$^2$ had the thickest LF at the L3–L4 level.

High weight can result in high mechanical stress, which can explain why spinal canal stenosis is more prevalent in diabetic patients, as reported by Yoram Anekstein et al. (13). This fact was seen in the study as well. As diabetes mellitus has a prevalence of 16.3% in the general Yazd population, and 41% of patients were suffering from diabetes mellitus, the study concluded that diabetes mellitus is not a direct cause of spinal canal stenosis.

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References


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