Changes in Basic Metabolic Elements Associated With the Degeneration and Ossification of Ligamenta Flava

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Abstract

Objective: To determine the association between levels of basic metabolic elements and degeneration and ossification of the ligamentum flavum (LF).

Subjects: Fourteen consecutive patients with degenerative lumbar stenosis, 11 with ossification of the thoracic ligamenta flava, and 11 control subjects.

Methods: The basic elements of calcium (Ca), phosphorus (P), magnesium (Mg), zinc (Zn), copper (Cu), manganese (Mn), molybdenum (Mo), and fluoride (F) in the specimens were measured using atomic absorption spectrometry, the phosphomolybdic blue method, and a fluoride-selected electrode.

Results: Ca content and the ratio of Ca/Mg in the LF specimens increased significantly in the sequence of control, degeneration, and ossification groups. Compared with values for the control group, the Zn, Mn, and Mo contents in the ossification and degeneration groups were significantly lower ($P < 0.01$); in contrast, Cu content was significantly higher ($P < 0.01$). As to F, its content in the specimens of the ossification group was much higher than those in the degeneration and control groups ($P < 0.01$); the F content in the ligamenta flava and sera from patients with fluorosis was also significantly higher than in those from patients without fluorosis ($P < 0.01$). Compared with the control group, there were no differences in the F content in serum from patients without fluorosis; however, the F content in ligamenta flava specimens from patients without fluorosis was significantly higher ($P < 0.01$).

Conclusions: There are trends in the contents of basic metabolic elements in the degeneration and ossification of ligamenta flava. These basic metabolic elements may play an important role in this process.

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Key Words: Lumbar stenosis; Ossified ligamentum flavum; Spinal cord injuries; Fluorosis; Hyperphosphatemia; Spinal degeneration; Metabolic elements; Fluoride

The posterior spinal structures, including hypertrophied and ossified ligamentum flavum (LF), play a major role in the pathogenesis of spinal stenosis, which could be responsible for the compression of the spinal cord and nerve roots (1,2). The etiology of hyperplastic and ossific changes in LF remains obscure, although the release of growth factors during the repair process following ligament injury seems to be involved (3). According to Yoshida et al (2), yellow ligament (LF) hypertrophy can be ascribed to 3 mechanisms: proliferation of type II collagen, ossification, and calcium crystal deposition. The ossifica-

tion of ligamentum flavum (OLF), a definite clinical entity affecting predominantly the lower thoracic spine in middle age, is viewed as a form of ectopic ossification (4–6) and has been observed in a variety of disorders of mineral metabolism, such as vitamin D-resistant hypophosphatemic rickets and fluorosis. It has been suggested that basic metabolic elements might play some roles in the degeneration and ossification of the LF. Until now, few reports have discussed the association between basic metabolic elements and the degeneration and ossification of LF. The purpose of this study is to examine the contents of calcium (Ca), phosphorus (P), magnesium (Mg), zinc (Zn), copper (Cu), manganese (Mn), molybdenum (Mo), and fluoride (F) in the LF and sera specimens from patients with thoracic OLF and degenerated lumbar stenosis to determine whether basic metabolic elements contribute to the degeneration and ossification of the LF.

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Table 1. Ca, P, and Mg Levels and Ca/Mg Ratio in LF Specimens (means $\pm$ SD, $\mu\text{mol/g}$)

Group	n	Ca	P	Mg	Ca/Mg Ratio
Ossification	11	664.7 $\pm$ 34.8***	302.75 $\pm$ 76.19***	15.79 $\pm$ 2.32**	45.5**
Degeneration	14	319.9 $\pm$ 79.6**	104.04 $\pm$ 65.36	11.98 $\pm$ 6.07	34.2*
Control	11	180.8 $\pm$ 25.4	138.06 $\pm$ 63.43	11.29 $\pm$ 2.33	16.5

Ca, calcium; P, phosphorus; LF, ligamentum flavum; Mg, magnesium; Ca/Mg ratio, calcium to magnesium ratio.

* $P < 0.05$, ** $P < 0.01$ vs control; # $P < 0.05$, ## $P < 0.01$ vs degeneration group.

MATERIALS AND METHODS

Patient Samples

From 1995 through 1996, a total of 36 specimens of LF tissue and serum were harvested from patients who underwent decompressive laminectomy at the authors' institute, including 11 cases of thoracic ligamentum flavum ossification, 14 cases of lumbar LF degeneration, and 11 cases of fresh thoracic and/or thoracolumbar spinal fracture with spinal cord injury. Written consent from each patient for the use of surgical specimens for research purposes was obtained before surgery. The study design was approved by the institutional review board. The diagnosis of ossification of the thoracic ligamentum flavum was made based on clinical findings, radiographs, computerized tomography (CT), and magnetic resonance imaging (MRI) scans. Ossified LF specimens were harvested from 6 patients with ossification of thoracic LF and 5 patients with fluorosis, including 8 men and 3 women with a mean age of 50.4 years, ranging from 42 to 60 years. The diagnosis of degeneration of the lumbar LF was based on CT and MRI scans.

Specimens of degenerated LF were harvested from 14 patients with lumbar stenosis and/or lumbar disk herniation. There were 10 men and 4 women, ranging in age from 42 to 61 years (mean 50.2 y). For the control group, LF specimens were harvested from 11 patients with fresh thoracic and/or thoracolumbar spinal fracture with spinal cord injury, including 8 men and 3 women with a mean age of 36.6 years, ranging from 21 to 44 years. In all control group samples, no ossification and hypertrophy were observed by CT, MRI, and/or radiography before surgery. These findings were confirmed by observation during surgery and by histopathology after operation. In our research, we found that the degree of ossification in LF was highest on the side close to the capsular ligaments, especially around the superior articular process. So in every patient, we used the LF closest to the capsular ligaments around the superior articular process in order to maintain consistency.

Methods

Specimens of LF (0.3–2.0 g) were digested with 5 to 10 mL HNO_3 – HClO_4 mixture (ratio 4:1) in beakers, and then placed on an electric heating plate for decarbonization. After decarbonization, the specimens were brought to

2.0 mL with 1% HNO_3 and measured with flame atomic absorption spectrometry (FAAS) (7–9) (Hitachi 180–80, Tokyo, Japan) for Cu, Mn, and Mo, then diluted 50-fold with 1% HNO_3 for detecting Zn; and finally, diluted 500- to 2500-fold with 1% HNO_3 including 0.1% La, for measuring Ca and Mg.

Another batch of ligament specimens (0.2–0.5 g) was incinerated for 8 hours at 500°C and placed into 10 mL 0.25N HCl. Then, 0.2 mL of the mixture was diluted to 10 mL with deionized water, after which 1.0 mL $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$ was added. After 15 seconds, the luminosity of P in the samples was measured at 420 nm and the P content was calculated. To measure F, 5 mL of the mixture was added to 0.05 mL bromphenol blue, counteracted with 0.5 M NaOH to just blue (pH = 4), and then added to 12.5 mL TISAB solution and diluted with deionized water to the expected scale. The electro-potential was measured, and the F content was calculated. Venous blood samples (4 mL) were collected for analysis of Ca, P, Mg, Zn, Cu, Mn, Mo, and F content. After centrifugation, serum was stored at –20°C until analysis. The samples were thawed at 37°C. Serum Zn, Mg, Cu, and Ca were directly detected by flame atomic absorption spectrometry (AAS) (10). Serum Mo and Mn were detected with graphite furnace AAS (9,11) after dilution with 0.1% Triton X-100. Serum F and P were measured by the fluoride-selected electrode and phosphomolybdic blue methods, respectively.

Statistical Analysis

We performed the statistical analysis using analysis of variance (ANOVA). The data were presented as the mean $\pm$ standard deviation (SD).

RESULTS

Content of Ca, P, and Mg in LF

The results in Table 1 show that the content of Ca and the ratio of Ca to Mg increased significantly in the sequence of control, degeneration, and ossification groups ($P < 0.01$). The levels of Ca in the control, degeneration, and ossification groups were 180.8 ± 25.4 , 319.9 ± 79.6 , and $664.7 \pm 34.8 \mu\text{mol/g}$, respectively. The Ca to Mg ratios were 16.5, 34.2, and 45.5, respectively. The contents of P and Mg in the ossification group were 302.75 ± 76.19 and $15.79 \pm 2.32 \mu\text{mol/g}$, significantly

Table 2. Zn, Cu, Mn, Mo, and F levels in LF Specimens (means $\pm$ SD, $\mu\text{mol/g}$)

Group	n	Zn	Cu	Mn	Mo	F
Ossification	11	568.11 $\pm$ 138.3**	34.36 $\pm$ 8.95**	8.11 $\pm$ 2.37**	4.17 $\pm$ 1.08**	17,543.6 $\pm$ 7680.9**
Degeneration	14	406.10 $\pm$ 74.9**	36.72 $\pm$ 10.18**	6.92 $\pm$ 1.23**	3.37 $\pm$ 0.61**	2960.1 $\pm$ 1512.3
Control	11	704.11 $\pm$ 138.3	15.33 $\pm$ 2.06	14.66 $\pm$ 2.19	6.87 $\pm$ 1.14	2902.7 $\pm$ 1024.6

Cu, copper; F, fluoride; LF, ligamentum flavum; Mn, manganese; Mo, molybdenum; Zn, zinc.

** $P < 0.01$ vs control.

higher than those in the degeneration and control groups ($P < 0.05$).

Content of Zn, Cu, Mn, Mo, and F in LF

As Table 2 shows, the contents of Zn, Mn, and Mo in the ossification group were 568.11 ± 138.3 , 8.11 ± 2.37 , and $4.17 \pm 1.08 \mu\text{mol/g}$, respectively, and levels in the degeneration group were 406.10 ± 74.9 , 6.92 ± 1.23 , and $3.37 \pm 0.61 \mu\text{mol/g}$, respectively; both were measurably lower than those in the control group ($P < 0.01$). In contrast, the contents of Cu in the ossification and degeneration groups were 34.36 ± 8.95 and $36.72 \pm 10.18 \mu\text{mol/g}$, significantly higher than that in the control group ($P < 0.01$). Compared with the degeneration and control groups, F content in the ossification group was much higher ($17,543.6 \pm 7680.9 \mu\text{mol/g}$) ($P < 0.01$).

Content of Ca, P, and Mg in Sera

As that data in Table 3 show, the P content in the ossification group was $1.08 \pm 0.26 \mu\text{mol/L}$, which was lower than the level in the control group ($P < 0.05$). Although the Mg content in both the ossification and degeneration groups was higher (1.24 ± 0.04 and $1.25 \pm 0.06 \mu\text{mol/L}$, respectively), than that in the control group ($P < 0.05$), there were no differences in the Ca content or the ratio of Ca to Mg among the 3 groups.

Content of Zn, Cu, Mn, Mo, and F in Sera

As the data in Table 4 show, the contents of Mn and F in the ossification group were 59.52 ± 18.09 and $10.29 \pm 2.14 \mu\text{mol/L}$, respectively, which were significantly higher than those in the control group ($P < 0.05$). However, the Mo content in the ossification group was 81.02 ± 17.21

$\mu\text{mol/L}$, significantly lower than that in control group ($P < 0.05$).

Content of F in LF and Sera Specimens from Patients With or Without Fluorosis

As Table 5 shows, the F content in LF and sera from patients with fluorosis was $24.40 \pm 4.00 \text{ mmol/g}$ and $12.10 \pm 0.76 \mu\text{mol/L}$, respectively, significantly higher than in patients without fluorosis ($P < 0.01$). Although there were no differences in the F content in sera from patients without fluorosis and controls, the F content of LF from patients without fluorosis was significantly higher ($11.82 \pm 4.35 \text{ mmol/g}$, $P < 0.01$) than in controls.

DISCUSSION

The LF is a yellow elastic ligament extending from the second cervical vertebra to the first piece of sacrum. It is composed of a longitudinal network of elastic connective tissue and is routinely demonstrated on CT scans as an isodense, 2- to 4-mm thick structure on the posteromedial aspect of the laminae, contrasted by the adjacent fat (12). Any thickening of LF can result in a narrowing of the spinal canal, either centrally or laterally within the foramina, and can cause radiculopathy, myelopathy, or even a cauda equina syndrome, depending on its level and extent (13). This may be due to hypertrophy or ossification of the ligaments. The hyperplasia of LF is secondary to degenerative change or aging (12). In addition, mucoid swelling and hyalinization of the elastic fibrous connective tissue may occur, as can buckling secondary to facet degeneration and spondylosis (14).

OLF, first reported by Polgar in 1920 (15), has been reported to occur most commonly in the thoracic, then lumbar, and rarely in the cervical spine (16). Within the

Table 3. Ca, P, and Mg Levels and Ca/Mg ratio in Sera Specimens (means $\pm$ SD, $\mu\text{mol/L}$)

Group	n	Ca	P	Mg	Ca/Mg ratio
Ossification	11	1.73 $\pm$ 0.20	1.08 $\pm$ 0.26*	1.24 $\pm$ 0.04*	1.38
Degeneration	14	1.78 $\pm$ 0.13	1.21 $\pm$ 0.26	1.25 $\pm$ 0.06*	1.41
Control	11	1.71 $\pm$ 0.17	1.45 $\pm$ 0.45	1.19 $\pm$ 0.05	1.45

Ca, calcium; P, phosphorus; Mg, magnesium; Ca/Mg ratio, calcium/magnesium ratio.

* $P < 0.05$ vs control.

Table 4. Zn, Cu, Mn, Mo, and F Levels in Sera Tissue Specimens (means $\pm$ SD, μ mol/L)

Group	n	Zn	Cu	F	Mn	Mo
Ossification	11	16.54 $\pm$ 1.92	20.02 $\pm$ 2.77	10.29 $\pm$ 2.14*	59.52 $\pm$ 18.09*	81.02 $\pm$ 17.2*
Degeneration	14	15.66 $\pm$ 1.48	19.14 $\pm$ 1.95	9.10 $\pm$ 1.23	56.25 $\pm$ 14.97	97.40 $\pm$ 30.76
Control	11	16.39 $\pm$ 2.49	20.96 $\pm$ 3.03	8.58 $\pm$ 1.19	46.66 $\pm$ 8.17	97.40 $\pm$ 15.18

Cu, copper; F, fluoride; Mn, manganese; Mo, molybdenum; Zn, zinc.

* $P < 0.05$ vs control.

thoracic spine itself, the majority of OLF cases involve the lower third (4).

Histological examination reveals ossification along the superficial layers of the yellow ligaments, calcified areas into an abundant cartilaginous matrix, a decreased number of elastic fibers, and an increased number and size of collagen fibers and fibrocartilaginous cells between these collagen fibers, as well as premature osteons and osteoblasts (17,18).

The etiology of OLF remains unclear, and it is unlikely that it is unique. Both systemic and local mechanisms are hypothesized. The local mechanisms considered responsible of the OLF include repeated mechanical stress that initiates the degenerative processes of the ligament (18,19). However, this hypothesis does not fit in well with the selective involvement of the thoracic spine: the cervical and lumbar segments, although subjected to greater mechanical stress, are less frequently involved than the thoracic segment is (18,20).

Significantly increased levels of fibronectin, a glycoprotein in growth plates and an essential factor in endochondral ossification, have been shown in patients with OLF (18). Glycosamine metabolism alteration within LF has also been shown to be a contributory factor (21,22). Other systemic factors such as hormones, growth factors, and vitamins may also predispose to the development of ligamentous ossification since such ossification is prevalent in diseases such as obesity, hyperinsulinism, and calcium and phosphorous metabolism abnormalities (12,18).

Matsui et al (23) suggested that the mechanical stress on the spine and the increased retention of Ca in LF may be the factors influencing the OLF. In the current study, the authors found retention of Ca in LF in the ossification

and degeneration groups and retention of P in the ossification group. The Ca content in the ossification and degeneration groups was significantly higher than that in the control group, and the P content in the ossification group was also significantly higher than in the degeneration and control groups.

Magnesium, the antagonist to Ca, has a close relationship with elastin. The constant ratio of Ca to Mg plays a fundamental role in maintaining the elasticity of LF (24). The elasticity of LF decreases and the fragility increases in OLF (12,13). In this study, the ratio of Ca to Mg also increased significantly in the sequence of control, degeneration, and ossification groups. The changes in the ratio of Ca to Mg might reflect changes of the elasticity of LF (ie, the higher the ratio of Ca to Mg, the greater the possibility of degeneration).

In this study, similar changes were also observed in the other essential metabolic elements—Zn, Cu, Mn, and Mo. The Zn, Mn, and Mo contents in the ossification and degeneration groups were measurably lower than those in the control group; in contrast, Cu content was significantly higher in the degeneration and ossification groups. The increase of Cu content and decrease of Zn, Mn, and Mo contents and the antagonism to Cu suggested the vigorous anabolism of collagen in degenerated or ossified LF. Copper is an important cofactor of lysyl oxidase, which is the key enzyme in anabolism of collagen (25).

It has been reported that there is a close relationship between F and ligament ossification, and that F might play an important role in chemically induced ligament ossification (26,28). In this study, 5 patients with fluorosis were found in the ossification group. The contents of Ca and F in the ossification group were measurably higher than those in the degeneration and control groups.

Table 5. F Levels in LF Tissue Specimens and Sera Tissue Specimens (means $\pm$ SD)

Group	n	LF (mmol/g)	Serum (μ mol/L)
Fluorosis	5	24.40 $\pm$ 4.00** ##	12.10 $\pm$ 0.76** ##
Nonfluorosis	6	11.82 $\pm$ 4.35**	8.79 $\pm$ 1.62
Control	11	2.90 $\pm$ 1.02	8.58 $\pm$ 1.19

LF, ligamentum flavum.

** $P < 0.01$ vs control; ## $P < 0.01$ vs nonfluorosis group.

Although the Ca content in the degeneration group was also higher than that in the control group, there was no statistical difference in F content between the degeneration and control groups. The F content in LF from nonfluorosis patients with OLF was also significantly higher than that in the control group, even though there was no difference between their serum F levels. A reasonable explanation was that patients without fluorosis may have endured a period of excessive F intake, which promoted the development of OLF. After that period, although the intake of F decreased to within normal limits, OLF still developed. So when compared, the F content of the LF of nonfluorosis patients was much higher than that in the controls; however, the serum F remained normal. The exact sources of F in nonfluorosis patients are still not known. It is reported that F can activate intracellular c-AMP, causing a significant increase of intracellular Ca (29) and could stimulate the activation and proliferation of osteoblast-like cells with enhanced expression of messenger ribonucleic acid and the proteins c-fos and c-jun (27).

Although it has not been confirmed that the retention of Ca in chondrocytes caused by F leads to the degeneration and necrosis of chondrocytes followed by chondral matrix calcification and endochondral ossification, these findings suggest that F and Ca might play important roles during the development of OLF.

CONCLUSIONS

The Ca content and the ratio of Ca to Mg in LF increased significantly in the sequence of control, degeneration, and ossification groups. Compared with the control group, the contents of Zn, Mn, and Mo in the ossification and degeneration groups were measurably lower; in contrast, the Cu content was significantly higher. The F content in the LF of the ossification group was extremely high compared to that in the degeneration and control groups. The F content in the LF and sera from patients with fluorosis was also significantly higher than in specimens from patients without fluorosis. Compared with the control group, although there were no differences in the F content in sera from patients with and without fluorosis, the F content in LF from the nonfluorosis subjects was significantly higher. This pattern suggests that there is a trend in changes in the contents of basic metabolic elements in LF during the process of degeneration to ossification and that basic metabolic elements may play an important role in this process. Whether OLF results from degeneration or some unknown activity of F, the exact mechanism from degeneration to ossification remains unclear. Further investigations, such as elucidating the sources of F and the detailed mechanism of its activity, need to be performed to clarify the etiology of OLF.

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