PATHOMECHANISM OF HYPERTROPHY AND LOSS OF ELASTICITY IN LUMBAR LIGAMENTUM FLAVUM AMONG AGED PATIENTS WITH LUMBAR SPINAL CANAL STENOSIS.

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INTRODUCTION:
The most common spinal disorder in elderly patients is lumbar spinal canal stenosis (LSCS). Canal narrowing (stenosis) in part results from hypertrophy of the ligamentum flavum (LF). Loss of elasticity of the LF may cause its folding into the neural canal, which could further induce canal narrowing. The purpose of this study was to investigate the pathomechanism of hypertrophy and loss of elasticity of LF in the aged patients with LSCS.

METHODS:
Histological study:
LF was collected during surgery from 5 young (20-40 y.o) and 5 aged (60-80 y.o.) patients. Each LF was sagittally cut and evaluated histologically. Three staining techniques were used for evaluation; Elastica Van Gieson (EV), Alucian blue, and Trichrome (T) stain. One fetus (9 months gestational age) LF sample was also collected for the complete control.

Immunohistochemistry:
Cellular location of Type II collagen in LF was investigated using immunohistochemical technique. A routine 3-stage immunoperoxidase staining technique using avidin- biotin- immunoperoxidase complex was performed.

Biological study:
Quantitative analysis of mRNA expression using real time reverse transcriptase- coupled polymerase chain reaction (RT-PCR) was undertaken to investigate the ligament expression type I collagen and elastin. The relationship between the expressions and age was analyzed.

RESULTS AND DISCUSSION:
Qualitative analysis for LF elasticity:

Figure 1. EV stain of LF sample (right: aged; left: fetus)

Fetus sample was stained mostly in black color (EV stain), indicating the majority of the LF was elastic fibers (Fig. 1-left). The dorsal aspect of LF in the aged group was not stained in color black, indicating that the elastic fiber was sparse; whereas, at the dural aspect it consisted of many elastic fibers like the fetus samples (Fig. 1-right). The LF in the young group showed minor degree of loss of elastic fibers at the dorsal aspect.

Figure 2. Elastic fiber occupying ratio in the LF

For these 11 LF samples, elastic fiber occupying ratio was calculated using software Winroof. In results, the LF of fetus had elastic fiber in about 75% of the entire area (Fig. 2). Dural aspect of the LF in both young and aged group showed similar ratio around 75%; however, at the dorsal aspect the ratio was becoming smaller (losing elastic fibers) as the age increased.

Figure 3. Alucian blue and type II collagen immunohistochemistry

At the almost half of the area showing loss of elastic fibers, alucian blue and type II collagen immunohistochemistry indicated that the dorsal aspect converted to cartilaginous tissue, which produces type II collagen and proteoglycan (Fig.3). The other half was positively stained by T stain, indicating scarring formation at the site. The results indicated that the LF from the aged patients, elastic fiber contents decreased; while scarring and chondrometaplasia occurred instead, leading to the loss of elasticity in the LF.

Quantitative analysis for LF hypertrophy:
Using the software WinRoof, we measured the thickness of normal dural layer (consisting of many elastic fibers) and abnormal dorsal layer (showing loss of elastic fibers) for both young and aged group. In results, the thickness was 2.0 and 2.3 (mm), for young and aged group in the normal layer, respectively. Accordingly, it was 1.1 and 1.8 (mm), for the dorsal abnormal layer. Thus, it is revealed that both elastic and collagenous layers became thicker in the aged group as compared to the young group.

Figure 4. Results of real time RT-PCR

The expressions of elastin and type I collagen showed positive linear correlations with age. Thus, in the aged patients, cells in the LF may produce more elastic and type I collagen fibers when compared to the young LF, which may induce hypertrophy (Fig. 4).

CONCLUSION:
In the LF in the aged patients, elastic fiber contents decreased due to occurrence of scarring and chondrometaplasia, leading to the loss of elasticity in the LF. Also LF produced more elastic and type I collagen fibers, which could induce its thickening (hypertrophy).

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