

Matrix Metalloproteinase-3

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Role of Matrix Metalloproteinase-3 in Degenerative Lumbar Scoliosis

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– Abstract –

Purpose: This study was performed to investigate the differences in the expression of matrix metalloproteinase-3 in degenerative scoliosis compared with other degenerative disc disease of the spine.

Materials and Methods: The intervertebral disc materials were obtained during discectomies. Six, 13 and 12 cases of herniated nucleus pulposus, spinal stenosis and degenerative lumbar scoliosis, respectively, were included in the experimental group. The expression of MMP-3 was evaluated three times that of the means, in the immunohistochemical staining, western blotting using anti human MMP-3 antibody and RT-PCR with MMP-3 primer, respectively

Results: On the immunohistochemical stains, extensive and strong staining was noted in the discs of degenerative lumbar scoliosis compared to those with spinal stenosis and HNP. In the western blotting, greater expression of MMP-3 was noted in the discs of degenerative lumbar scoliosis (mean optical density: 20.68) than in other degenerative disc diseases (SS: 6.24, HNP: 2.0). In the RT-PCR, a similar result was shown (DLS: 62.1, SS: 27.4 & HNP: 10.4). There were statistically significant differences between degenerative lumbar scoliosis and degenerative disc disease (p<0.05).

Conclusion: Rapid degeneration of the intervertebral disc might be an important factor in the pathogenesis of degenerative lumbar scoliosis. MMP-3 could be a key enzyme for the rapid degeneration of the intervertebral discs, especially in degenerative lumbar scoliosis.

Key Words: Intervertebral disc, Degenerative lumbar scoliosis, MMP-3

¹⁷⁾
(proteoglycan)

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1.

2002 6 2003 8 6 , 12 (Table 1).

Prichett Bortell⁵⁾ 4

가 , Robin¹⁸⁾ 가 (cage or strut bone)

3-4, 4-5 . Grubb⁵⁾ 가 , 10¹⁸⁾ (: 65.8) (: 63.3) 50~70 30 unit) (functional spinal 50 Thompson

가 matrix metalloproteinase-^{2,6,7,10,15)} 2. MMP-3

3(MMP-3)가 1) 10% formalin paraffin 60 °C

가 MMP-3 MMP-3 warmer 3 μm 1 xylene 3 PH 6.0 cit- 10

hyaluronic acid 2) 10 buffer 0.3% H₂O₂-

MMP-3 western blotting, 10 methanol 10

(RT-PCR ; Reverse Transcription- Polymerase Chain Reaction) 15 1:60 antihuman MMP-3 monoclonal antibody (IM70, Oncogene Research Products, San Diego, CA, USA) 90

avidin biotin ABC

biotin gel transfer
 30 avidin 가 buffer(25 mM Tris, 192 mM glycine, 20% methanol)
 . avidin 10~15 가 nitrocellulose filter elec-
 lectin avidin transfer kit 250~300 mA 1
 streptavidin . peroxidase transfer . nitrocellulose filter Ponceau S (Ponceau
 AEC(3-amino-9-ethylcarbazole) . 2 S 2 g, trichloroacetic acid 30 g, sulfosalicylic acid 30 g)
 hematoxylin-eosin . blocking buffer filter transfer band

2) Western blotting MMP-3 Anti human MMP-3 antibody 가 1
 homogenizer peroxidase labeled anti-mouse anti-
 Ready body (Amersham Pharmacia Biotech, Buckinghamshire, UK)
 PrepTM(163-2103, Bio-Rad, CA, USA) pro- ECL (enhanced
 teinase inhibitor , 4 °C chemiluminescence) Kit (Amersham Pharmacia Biotech)
 15,000 rpm Image Master VDS (Amersham Pharmacia Biotech) software
 SDS-PAGE (sodium dodecyl sulfate-poly- optical density (OD)
 acrylamide gel electrophoresis)

Table 1. Demographics of the samples

Age/Sex	Diagnosis	MRI Grade
35/M	HNP	III
30/F	HNP	III
42/F	HNP	IV
37/M	HNP	III
44/F	HNP	III
40/M	HNP	III
67/F	SS	IV
78/F	SS	IV
69/F	SS	IV
70/M	SS	V
67/M	SS	IV
63/F	SS	IV
68/M	SS	IV
63/F	SS	IV
61/F	SS	IV
62/M	SS	IV
72/M	SS	V
71/F	SS	IV
66/F	SS	IV
65/F	DLS	V
70/F	DLS	V
63/M	DLS	V
67/M	DLS	V
69/F	DLS	V
63/M	DLS	V
66/F	DLS	V
69/F	DLS	V
66/M	DLS	V

HNP: Herniated Nucleus Pulposus, SS: Spinal Stenosis, DLS: Degenerative Lumbar Scoliosis

3) MMP-3 (RT-PCR) 30 PCR DNA 2%

(1) Total RNA agarose gel in Tris-acetate-EDTA buffer ethidium bromide (EtBr)

100 mg/ml 가 trizol 가 1.5 ml eppendorf tube 0.2 ml (3)

chloroform 가 5

12,000 g 4 °C 15 NIH (National Institute of Health) Macin-

RNA ice-cold isopropanol toshTM (image analysis)

10 4 °C, 12,000 g 10 image 1.48 gray scale

g 5 pellet 75% ethanol 7,500 densitometry gel

genomic DNA RNA preparation RQ1

RNase-free DNase(1 U/5 µg RNA; Promega, Madison, WI) 3. Western blotting MMP-3

37 °C 45 , phenol-chloroform optical density independent sample

ethanol t-Test (SPSS for windows 11.0)

(2) RT-PCR (Reverse Transcription- Polymerase Chain Reaction) RT-PCR 가 ststistical analysis system program (PC-SASTM) student t-Test (non-paired, p<0.05)

total RNA , cDNA reac- tion mixture containing 1 µg of total RNA, 2 U RNase inhibitor, 1 mM each dNTP, 1.6 µg oligo p (dT), 30 U M-MLV reverse transcriptase, and 1X reaction buffer (Promega) 20 µl

15 , 42 °C 1 cDNA 2 µl one microliter of the first strand cDNA reaction mixture (Promega) , MMP-3 primers 10 pmole/µl, 1X PCR buffer without Mgcl₂, 1.5 mM Mgcl₂, 0.2 mM each dNTP and 1U of *Thermus aquaticus* (Taq) DNA polymerase (Promega) 20 µl

1. PCR . MMP-3 PCR MMP-3

94 °C 1 , 58 °C 2 , 72 °C ,

2 35 , 5 µl MMP-3 (Fig. 1A, B).

primers . Primer MMP-3 GAPDH (Fig. 2A, B)

(Table 2). (Fig. 3A, B).

GADPH (glyceraldehyde-3-phosphate dehydrogenase)

94 °C 30 , 55 °C 30 , 72 °C 30

Table 2. Sequence of primers

Gene		sequence	Size (bp)
MMP-3	Sense	5 -AGATGCTGTTGATTCTGCTGTTGAG-3	515
	Anti-sense	5 -ACAGCATCAAAGGACAAGCAGGAT-3	
GAPDH	Sense	5 -GAGTCAACGGATTTGGTCGT-3	156
	Anti-sense	5 -GGTGCCATGGAATTTGCCAT-3	

2. Western blotting

MMP-3 western blotting
band(Fig. 4A, B) optical density (OD)

OD 2.0 ± 1.4 ,
OD 6.24 ± 3.6 ,

OD 20.68 ± 8.4
OD 가 (p<0.05)(Fig. 5).

3. RT-PCR

PCR , PCR MMP-3
35 GAPDH 30 DNA

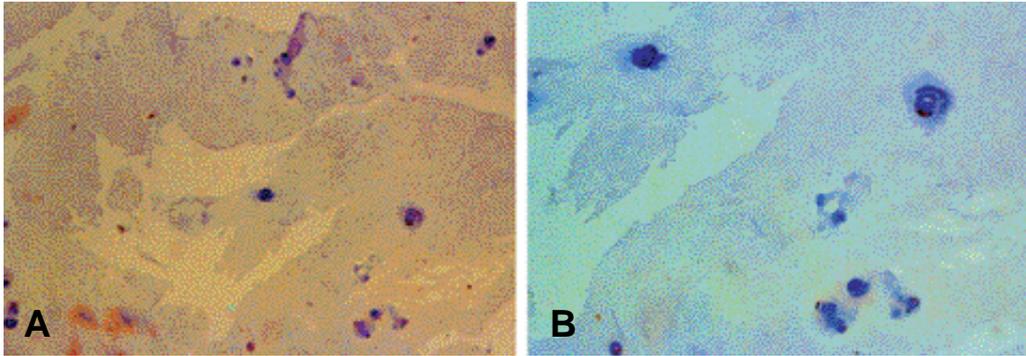


Fig. 1. (A, B) Photomicrographs of immunohistochemical staining for MMP-3(counter-stain with H-E, × 100, × 400). Slight expression of MMP-3 in HNP.

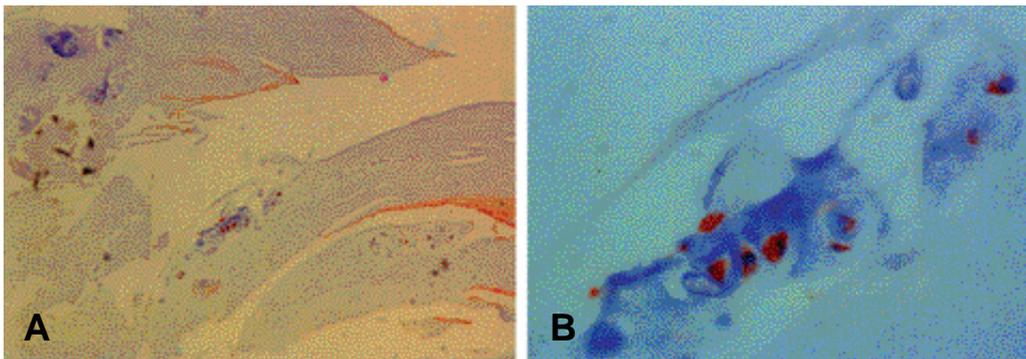


Fig. 2. (A, B) Photomicrographs of immunohistochemical staining for MMP-3(counter-stain with H-E, × 100, × 400). Degenerations of the disc and expression of MMP-3 are noted in the sample, obtained in SS. Profuse expression of MMP-3 with reddish area and more cellular proliferation than HNP are noted.

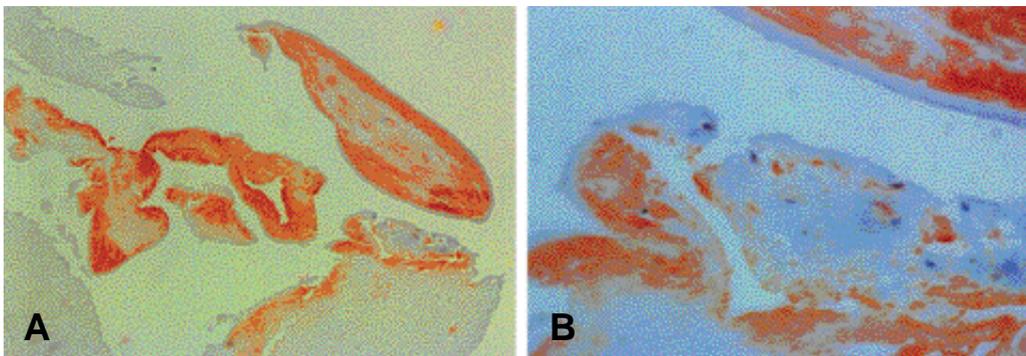


Fig. 3. (A, B) Immunohistochemical staining for MMP-3(counter-stain with H-E, × 100, × 400). Severe disc degeneration with many cleft of the disc material and cellular proliferation are noted in DLS. Also marked expressions of MMP-3 is noted with reddish stains.

RNA
MMP-3
GAPDH
agarose gel
band
MMP-3
cDNA
MMP-3
MMP-3
MMP-3
GAPDH mRNA

가
6.1
10.4 ± 2.0,
27.4 ±
62.1 ± 12.3
(P<0.05)(Fig. 6).

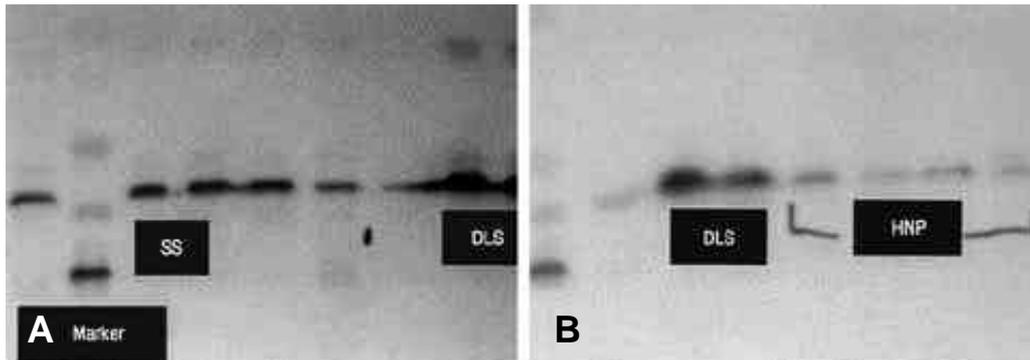


Fig. 4. (A, B) Expression of MMP-3 in intervertebral disc of various spinal disease. More thick band expressed in DLS than SS. Sparse expression shows in HNP

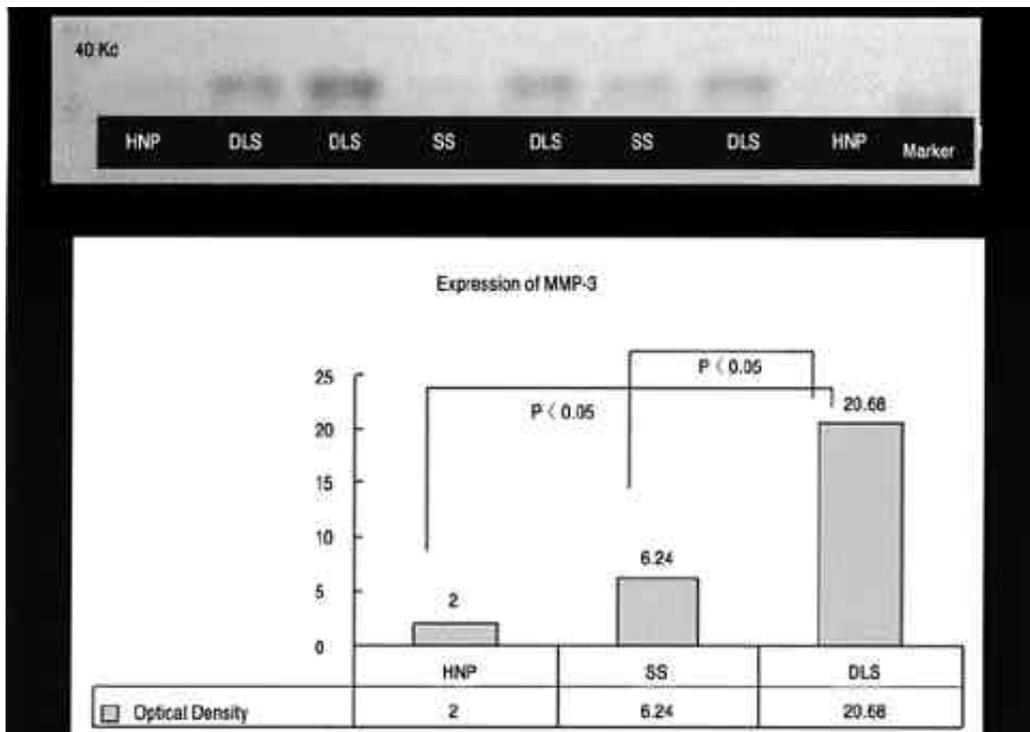


Fig. 5. Expression of MMP-3 in intervertebral disc of various spinal disease. Intensity of the bands is evaluated using Image Master VDS software and standardized for β -actin expression. HNP: Herniated Nucleus Pulposus, SS: Spinal Stenosis, DLS: Degenerative Lumbar Scoliosis *: P<0.05.

(macrophage) promoter 10). MMP-3 chemoattractant 가 9), MMP-3 가 가 23). 가 MMP-3 가 MMP-3가 가 MMP-3가 가 MMP-3가 가 MMP-3 (protrusion) (extrusion or sequestrum) MMP-3 14). MMP-3

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matrix metalloproteinase-3

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matrix metallopro-

teinase-3 (MMP-3; stromelysin)

6 , 13

12 , western blotting, RT-PCR (Reverse Transcription-Polymerase
Chain Reaction)

MMP-3 ,
western blotting (

optical density: 20.68 ± 8.4) (optical density: 6.24 ± 3.6) (2.0 ± 1.4)
RT-PCR MMP-3 (10.4 ± 2.0)

(27.4 ± 6.1) (62.1 ± 12.3) 가 MMP-3
MMP-3가

, MMP-3

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