

## 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

<sup>17)</sup>  
(proteoglycan)

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1.

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2002 6

2003 8

13

12

(Table 1).

<sup>5)</sup>.

Prichett

Bortell<sup>17)</sup>

4

, Robin

<sup>18)</sup>

가

(cage or strut bone)

가

3-4, 4-5

. Grubb

<sup>5)</sup>

가

10

<sup>18)</sup>,

가

: 65.8 )

( : 63.3 )

50~70

(functional spinal

unit)

30

50

가

<sup>6,7)</sup>.

Thompson

가

matrix metalloproteinase-

<sup>2,6,7,10,15)</sup>.

3(MMP-3)가

2. MMP-3

1)

가

10% formalin

MMP-3

paraffin

. MMP-3

60 °C

warmer

3 µm

1

hyaluronic acid

. xylene 3

PH 6.0

cit-

<sup>21)</sup>.

rate buffer

5

2

10

,

10

buffer

peroxidase

0.3% H<sub>2</sub>O<sub>2</sub>-

MMP-3

western blotting,

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

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

- 14 -

3)	MMP-3	(RT-PCR)	30	PCR	DNA	2%
(1) Total RNA			agarose gel in Tris-acetate-EDTA buffer			
	trizol		ethidium bromide (EtBr)			
100 mg/ml	1.5 ml eppendorf tube	0.2 ml	(3)			
chloroform	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	
	pellet	75% ethanol	7,500	densitometry	gel	
g	5					
genomic DNA	RNA preparation	RQ1				
RNase-free DNase(1 U/5	µg RNA; Promega, Madison, WI)		3.			
37 °C	45	, phenol-chloroform				
ethanol			Western blotting	MMP-3		
			optical density			
(2) RT-PCR (Reverse Transcription- Polymerase Chain Reaction)					independent sample	
total RNA	, cDNA	reac-	t-Test (SPSS for windows 11.0)			
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)			RT-PCR	가		
20 µl			ststistical analysis system program (PC-SASTM )		student t-Test (non-paired,	
15	, 42 °C	1	p<0.05)			
cDNA 2 µl	one microliter of the first strand					
cDNA reaction mixture (Promega)						
primers 10 pmole/µl, 1X PCR buffer without Mgcl <sub>2</sub> , 1.5 mM Mgcl <sub>2</sub> , 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 ,				
2	35					
primers		5 µl			(Fig. 1A, B).	
		20				
Primer	MMP-3	GAPDH				
		(Table 2).	2A, 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

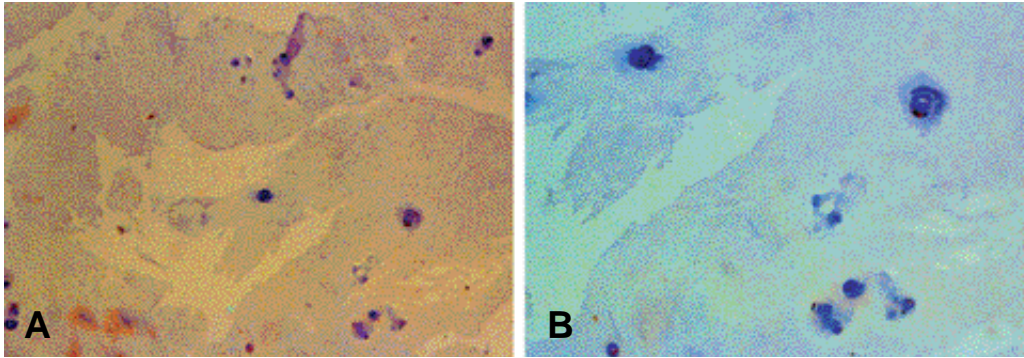
MMP-3  
band(Fig. 4A, B)      western blotting  
optical density (OD)

OD     $2.0 \pm 1.4$       ,  
OD     $6.24 \pm 3.6$       ,

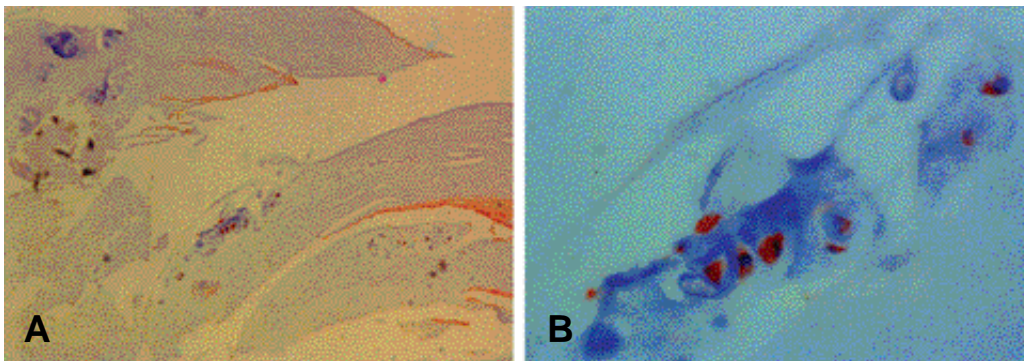
OD     $20.68 \pm 8.4$       .  
OD    가      (p<0.05)(Fig. 5).

## 3. RT-PCR

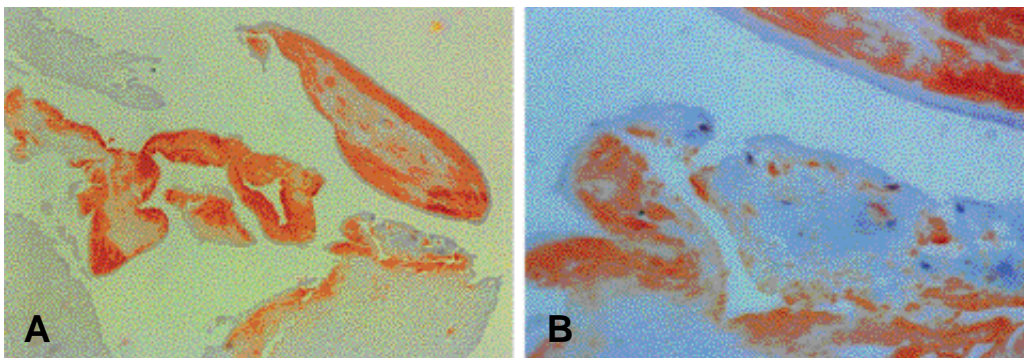
PCR      , PCR      MMP-3  
35      GAPDH    30      DNA



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

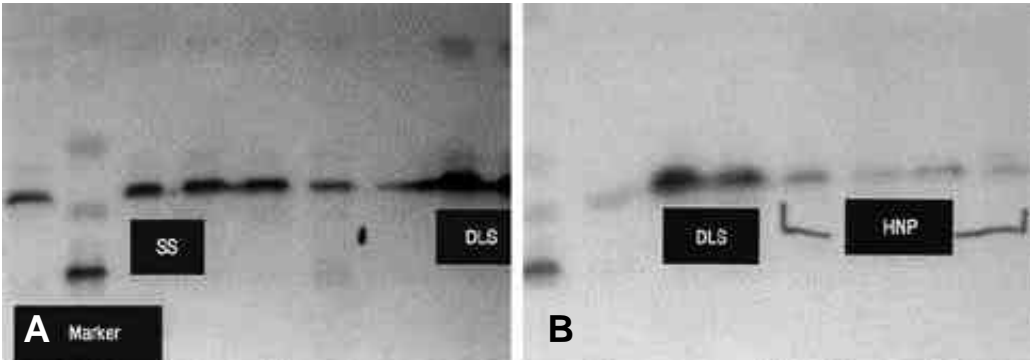


**Fig. 2.** (A, B) Photomicrographs of immunohistochemical staining for MMP-3(counter-stain with H-E,  $\times 100$ ,  $\times 400$ ). Degenerations of the disc and expression of MMP-3 are noted in the sample, obtained in SS. Profuse expression of MMP-3 with reddish area and more cellular proliferation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

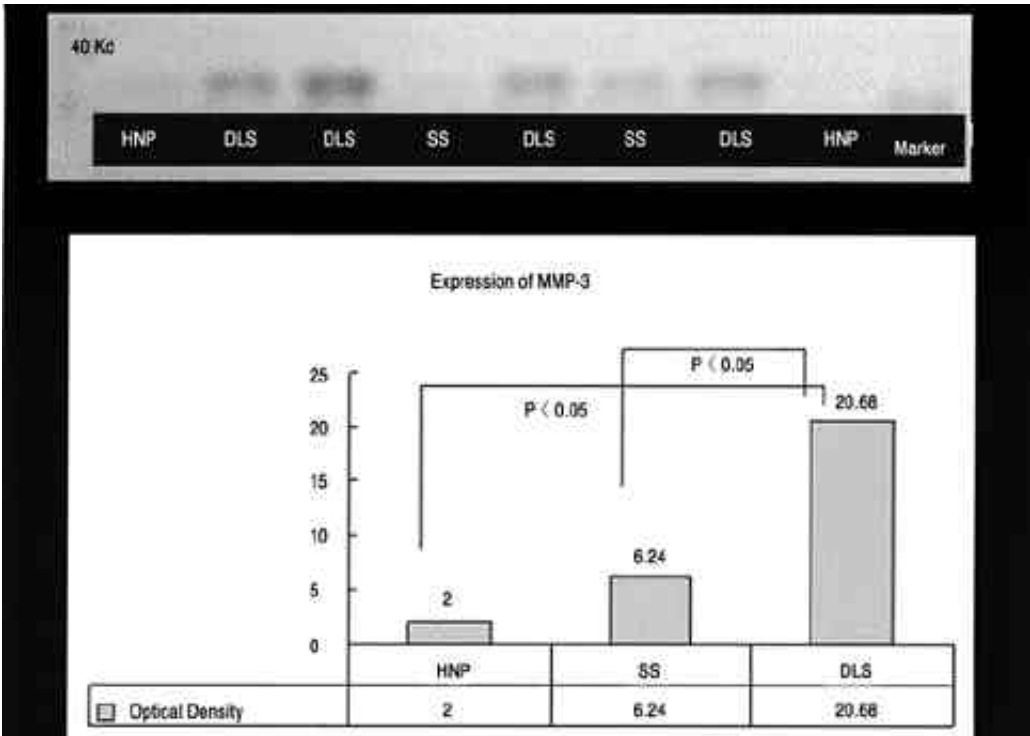


**Fig. 3.** (A, B) Immunohistochemical staining for MMP-3(counter-stain with H-E,  $\times 100$ ,  $\times 400$ ). Severe disc degeneration with many cleft of the disc material and cellular proliferation are noted in DLS. Also marked expressions of MMP-3 is noted with reddish stains.

RNA 가 10.4 ± 2.0, 27.4 ±  
MMP-3 GAPDH 6.1 62.1 ± 12.3  
agarose gel , (P<0.05)(Fig. 6).  
MMP-3 band  
cDNA MMP-3  
MMP-3  
MMP-3 PCR GAPDH  
MMP-3  
GAPDH mRNA

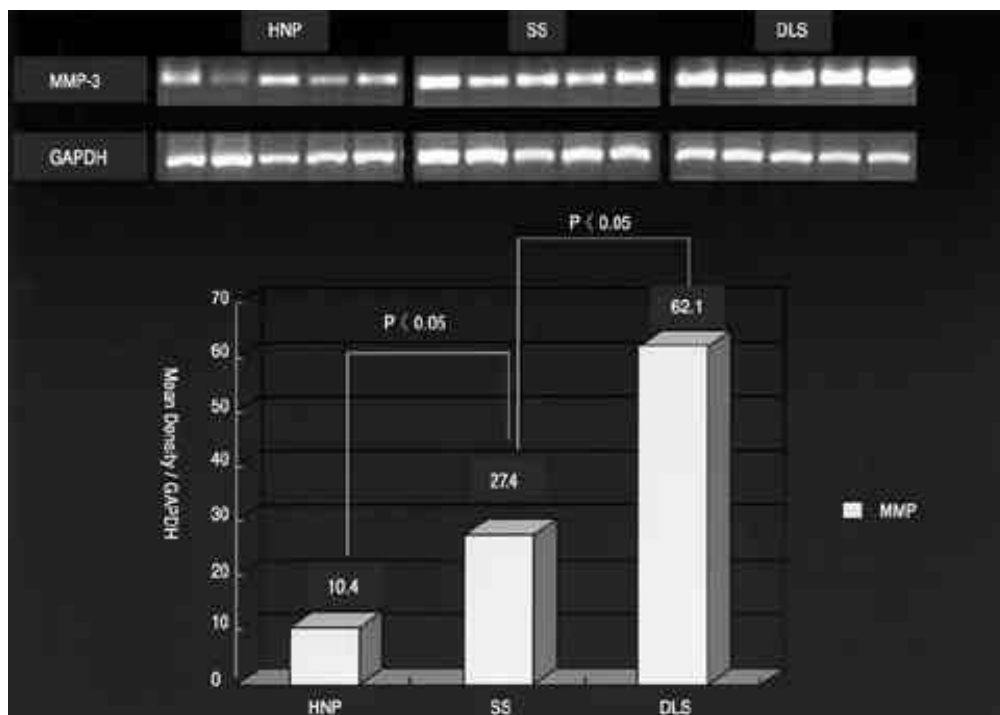


**Fig. 4.** (A, B) Expression of MMP-3 in intervertebral disc of various spinal disease. More thick band expressed in DLS than SS. Sparse expression shows in HNP



**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  $\alpha$ -actin expression. HNP: Herniated Nucleus Pulposus, SS: Spinal Stenosis, DLS: Degenerative Lumbar Scoliosis \*: P<0.05.

가 tor 가 vitamin D recep-  
polymorphism  
MMP-3  
Frymoyer Cars-Baril<sup>3)</sup>  
hyaluronic acid  
proteinase cathepsin D thiol proteinase  
Greis<sup>4)</sup>  
Norcross<sup>20)</sup>  
MMP-3  
MMP-3 (matrix)  
MMP-3  
tissue inhibitor  
MMP-3  
positive cell ratio  
MMP-3



**Fig. 6.** RT-PCR amplification of MMP-3 mRNA in the human intervertebral disc which are excised from DLS, SS and HNP. The photograph of 1% agarose gel electrophoresis shows the gene expression of MMP-3 and GAPDH as single bands of expected sizes in each specimen. and Paragraphs show comparison of gene expression levels of MMP-3 between HNP, SS and DLS. (HNP; Herniated nucleus pulposus, SS: Spinal Stenosis, DLS: Degenerative Lumbar Scoliosis)

(macrophage) <sup>10)</sup> MMP-3 chemoattractant  
가  
promoter 5A allele <sup>9)</sup> MMP-3 가  
<sup>23)</sup> 가  
가  
MMP-3  
가  
MMP-3가  
가  
가  
가  
가  
MMP-3  
(protrusion)  
(extrusion or sequestrum)  
MMP-3  
<sup>14)</sup>  
MMP-3

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matrix metallopro-  
teinase-3 (MMP-3; stromelysin)  
:  
12 , 6 , 13  
Chain Reaction) , western blotting, RT-PCR (Reverse Transcription-Polymerase  
:  
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)  
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MMP-3가  
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: , MMP-3

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