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RESEARCH ARTICLE

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Analysis of intervertebral disc CCR6 and IL-6 gene levels with short-term postoperative low back pain after spinal fusion in lumbar degenerative disease

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Abstract

Background: Previous studies have reported that specific pro-inflammatory cytokines or chemokines are more highly expressed in painful than in nonpainful intervertebral discs (IVDs). However, few studies have investigated their correlation with postsurgical outcomes or the relationship between postoperative pain and inflammatory cytokines in IVDs. Thus, the present study examined the correlation among the gene expression levels of pro-inflammatory cytokines and chemokines in IVD tissues removed during surgery and low back pain (LBP), leg pain (LP), and leg numbness (LN) at one year after spinal fusion surgery in patients with a lumbar degenerative disease (LDD).

Methods: Chemokine and cytokine gene expression levels were measured in IVD samples from 48 patients with LDD. The associations between chemokine and cytokine gene expression levels and pain intensity (numeric rating scale [NRS]) were also analyzed. A correlation analysis was performed between gene expression in each IVD and preoperative and postoperative pain intensity.

Results: In the preoperative analysis, CCR6 was associated with NRS_{LBP} ($r = -0.291$, $P = 0.045$). Postoperative pain analysis revealed correlations between postoperative NRS_{LBP} and CCR6 ($r = -0.328$, $P = 0.023$) and between postoperative NRS_{LBP} and IL-6 ($r = -0.382$, $P = 0.007$). Furthermore, patients with high postoperative LBP intensity ($NRS_{LBP} \geq 7$) also had high LBP intensity ($NRS_{LBP} \geq 6$) before surgery, and a correlation was observed ($r = 0.418$, $P = 0.003$). None of the gene mRNAs correlated with NRS_{LP} or NRS_{LN}, respectively.

Conclusions: CCR6 and IL-6 gene expression in the IVD was associated with postoperative LBP intensity and may indicate a need for postoperative pain management.

KEYWORDS
degeneration, pain

1 | INTRODUCTION

The causes and risk factors of low back pain (LBP) remain unclear. Because it affects work performance and social responsibilities and is a major factor in escalating healthcare costs, LBP has become a significant health concern worldwide.[1] The data for LBP in the general adult population shows a prevalence of about 12%, with a 1-month prevalence of 23%, a 1-year prevalence of 38%, and a lifetime prevalence of 40%.[2] These data suggest spine research studies are still needed to address the etiology of LBP and associated risk factors for better clinical management.

Progressive intervertebral discs (IVD) degeneration impairs spinal stability and is ultimately the leading cause of LBP from various spinal diseases. The role and mechanism of specific molecules, including proinflammatory cytokines and enzymes that degrade the IVD extracellular matrix, have gradually become clear. Multiple studies have observed significant differences in proinflammatory cytokines such as interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and interleukin-1 beta (IL-1β) compared with normal controls depending on pain intensity and the development of IVD degeneration.[3–5] In human specimens, we also found that IL-6 and CCR6 gene expression levels correlated strongly in IVD tissues and white blood (WB) cell samples. Their expression levels in WB cell samples correlated with the intensity of present pain.[6] Previous studies also showed that IL-6 and TNF-α cytokine expression were both significantly higher in patients who experienced worse levels of LBP.[7–9] Moreover, specific cytokines or chemokines are more highly expressed in painful compared with nonpainful IVD tissues.[10] Based on this background, among various inflammatory cytokines and chemokines, we have been focusing on the relationship between IL-6 and CCN20–CCR6 system and LBP. A previous study on the direct association between IL-6 expression and clinical outcomes reported that patients with elevated IL-6 expression in IVD tissues have worse early outcomes than those with lower expression.[11] However, few other studies have been conducted on the correlation between IL-6 and postoperative clinical outcomes or the relationship between postoperative pain and inflammatory cytokines in IVD tissues. Given this background, we questioned whether the expression of inflammatory cytokines and chemokines in the IVD before surgery might affect the postoperative course of LBP after spinal fusion surgery. Thus, the purpose of this study was to investigate the correlations among the gene expression of pro-inflammatory cytokines and chemokines in IVD tissues removed during surgery and LBP, leg pain (LP), and leg numbness (LN) at 1 year after spinal fusion surgery in patients with a lumbar degenerative disease (LDD).

2 | MATERIALS AND METHODS

This study was approved by the Institutional Review Board of Tokai University School of Medicine and the Profit Reciprocity Committee (approval No.: 20R-263) and conducted following the principles outlined in the Helsinki declaration.[12]

Written informed consent was obtained from all participants. The informed consent form explained that at the time of surgery, the degenerated IVD tissue or hernia would be removed, and the participants would be asked to donate a portion of that tissue to investigate the relationship between IVD gene expression and pain intensity.

2.1 | Participants

The study participants were all 20 years or older, had received conservative treatment-resistant therapy for at least 3 months, and had undergone lumbar spine (L1–L2 to L5–S1) surgery to treat LDD. All patients were diagnosed based on their detailed medical history, neurological and radiographic examinations, myelogram results, computed tomography (CT) scans after myelography, and/or magnetic resonance imaging (MRI). Before surgery, the authors informed the patients about the use of IVD samples obtained during surgery, and the participants read the consent forms and provided their consent for sample donation. The exclusion criteria were severe mental illness, difficulty standing or moving because of severe impairment or paralysis, prior (within 5 years) or current cancer diagnosis, suspicion of a current infection, or the presence of a condition considered by the principal investigator to be a contraindication for inclusion in this study. Demographic and clinical data were obtained after the study began.

Between August 2019 and May 2021, IVD tissues were collected from 58 patients (35 males, 23 females; mean age 70.3 ± 9.7 years) who had been diagnosed with LDD accompanied by LBP, LP, and/or LN and hospitalized for surgical purposes. IVD tissues from multilevel discs were collected in a single tube and used in genetic testing. Genes in IVD tissues were analyzed along with patient backgrounds, clinical data, and surgical data. We also investigated the correlation between proinflammatory cytokine and chemokine gene expression in IVD tissues and pain intensity before surgery and 1 year later. We analyzed the 48 patients (31 males, 17 females, mean age 70.0 ± 9.4 years) who underwent fusion surgery and were available for pain assessment at the final observation 1 year after surgery. Our criteria for fusion surgery were >3 mm of sagittal translation of, or >10° segmental angulation angle on dynamic radiographic evaluation, or posterior widening of the disc space of >5° on a flexion radiograph.

2.2 | Lumbar MRI

All patients underwent a routine lumbar MRI scan before surgery. MRI of the lumbar spine (L1–L2 to L5–S1) was performed using T2-weighted sequences with a 1.5- or 3.0-T imaging system (Ingenia or Achieva; Philips Medical Systems, Best, The Netherlands). The grade of IVD degeneration was evaluated using midsagittal T2-weighted images according to the Pfirrmann classification system[13] and modified Pfirrmann classification.[14] The modified Pfirrmann grade is a widely used grading system based on MRI features used to evaluate IVD degeneration degree noninvasively.

2.3 | RNA isolation and quantitative reverse transcription-polymerase chain reaction

Total cellular RNA was isolated using the Trizol method. The isolated IVD tissues were soaked in RNALater (Ambion, Foster City, CA) and stored at −80°C until extraction. On the day of RNA extraction, RNALater was removed, and the tissues were snap-frozen with liquid nitrogen and transferred into Trizol (Invitrogen, Carlsbad, CA). The tissues were homogenized with Omni Tissue Homogenizer (Omni International, Kennesaw, GA) for solid tissues. Total RNA was precipitated with 70% ethanol and further purified using the RNeasy Mini Kit (Qiagen, Valencia, CA) in accordance with the manufacturer's protocols. Reverse transcription was performed using a High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, Waltham, MA) with 100 ng total RNA template and 20 μL reaction volume. The synthesized cDNA solution was diluted with nuclease-free water with 10-fold dilution. Quantitative real-time PCR (qPCR) was performed using an SYBR Green Real-Time PCR kit (Thermo Fisher Scientific) in order to quantify the transcripts of target genes on the LightCycler® 480 System (Roche Applied Science, Mannheim). qPCR reaction was carried out in triplicate on a 96-well plate with 10 μL per well using ABI 2x SYBR Green Master Mix. PCR conditions were as follows: 20 s initial denaturation at 95°C and the reactions continue for 40 cycles at 95°C for 3 s and 60°C for 30 s. All samples were normalized for GAPDH expression. All reactions were run on a real-time polymerase chain reaction (PCR) system, and ΔC_t was calculated by subtracting the C_t value of GAPDH from that of the target gene. The primer sequences for the CCL20, CCR6, IL-6, IL-1β, TNF-α, Agg, Col2, and GADPH genes were synthesized by Sangon Biotech (Shanghai, China) and are listed in Table S1.

2.4 | Pain intensity

Pain intensity was assessed using a three-level numeric rating scale (NRS).[15] NRS scores were obtained on a scale from 0 to 10 for LBP (NRS_{LBP}), LP (NRS_{LP}), and LN (NRS_{LN}), where 0 is no pain and 10 is the worst pain imaginable. Each pain condition was investigated before and at 1 year after surgery, and Δ indicated the change in pain from postoperative to preoperative.

2.5 | Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics (version 23.0; IBM Corp., Armonk, NY). All values are expressed as mean ± standard deviation. The Shapiro–Wilk test was used to confirm the normality of the data distribution. For the primary analysis, Student's *t*-test or the Mann–Whitney *U* test was used to compare the two groups. Student's *t*-test was used to analyze normally distributed data, whereas the Mann–Whitney *U* test was used to analyze nonnormally distributed data. Pearson's product–moment correlation analysis or Spearman's product–moment correlational analysis was used to identify significant associations. The type 1 error was set at 5% for all statistical analyses, and *P* values <0.05 were considered significant.

TABLE 1 Characteristics of the subjects in the present study.

Characteristic	Data
No. of patients	48
Age (years)	70.0 (9.4)
Sex (male/female)	31/17
Height (cm)	160.1 (7.9)
Body weight (kg)	64.3 (12.0)
BMI (kg/m^2)	25.0 (4.2)
Indications	
LCS + (LDS)	44 (91.7)
LDH	2 (4.2)
FS	2 (4.2)
MRI grading (P)	4.3 (0.5)
MRI grading (M–P)	6.1 (1.6)
Levels treated, *n* (%)	
L1–L2	0 (0)
L2–L3	4 (6.0)
L3–L4	23 (34.3)
L4–L5	39 (58.2)
L5–S1	1 (1.5)
Overall	67
Number of treated segments	
1 level	31 (64.6)
2 level	15 (31.3)
3 level	2 (4.2)
4 level	0 (0)
Mean	1.4 (0.6)
Average OR time (min)	108.8 (39.4)
Average EBL (mL)	95.9 (122.1)
CRP on POD1 (mg/dL)	2.7 (1.4)
Average length of stay (days)	15.7 (4.3)

Note: Data presented as mean (SD) or number of patients (%).
Abbreviations: ASD, adult spinal deformity; BMI, body mass index; CRP, C-reactive protein; EBL, estimated blood loss; FS, foraminal stenosis; LCS, lumbar canal stenosis; LDS, lumbar degenerative spondylolisthesis; MRI grading (M-P), modified Pfirrmann classification; MRI grading (P), Pfirrmann classification; OR, operation; POD, postoperative day.

3 | RESULTS

3.1 | Patient backgrounds and grade of IVD degeneration

Table 1 shows the participants' basic demographic information (e.g., age, sex, height, weight, body mass index) and clinical data such as the underlying diagnosis and surgical information (e.g., surgical procedure, surgical level, operation time, bleeding). The 48 patients

included 44 with spondylolisthesis with lumbar spinal stenosis, two with lumbar disc herniation, and two with foraminal stenosis. The most common segment was L4–5 (39 segments, 58.2%), followed by L3–4 (23 segments, 34.3%), L2–3 (4 segments, 6.0%), and L5–S1 (1 segment, 1.5%). The average Pfirrmann grade was 4.3 ± 0.5, and the modified Pfirrmann grade was 6.1 ± 1.6.

The average operation time was 108.8 ± 39.4 min (range 61–293 min), the average estimated blood loss was 95.9 ± 122.1 mL (range 2–695 mL), and the average length of hospital stay was 15.7 ± 4.3 days (range 5–24 days). The mean postoperative C-reactive protein concentration was 2.7 ± 1.4 mg/L (range 0.38–6.05 mg/L).

3.2 | Expression of chemokines and inflammatory cytokines in IVD tissues

As shown in Table 2, CCL20, CCR6, IL-6, IL-1β, TNF-α, Agg, and Col2 mRNAs were expressed in the degenerated IVD tissues. GAPDH was selected as a reference sample to evaluate the ΔCt value. In comparison with CCL20 using $2^{-(\Delta\Delta Ct)}$, gene expression levels were higher in CCR6 (78.8 fold) and IL-6 (13.0 fold) compared with CCL20 (data not shown).

TABLE 2 Gene expression of chemokine and inflammatory cytokine in IVD tissues by qPCR.

IVD tissues	Target gene, Avg C_t	ΔC_t Target-normalizer
CCL20	36.4 (3.9)	13.3 (3.3)
CCR6	30.4 (1.0)	7.0 (3.4)
IL-6	32.9 (2.3)	9.6 (3.9)
IL-1β	33.7 (2.8)	10.3 (2.7)
TNF-α	33.8 (2.4)	10.5 (2.5)
Agg	23.4 (3.7)	0.2 (1.5)
Col2	22.6 (3.6)	−0.8 (1.6)
GAPDH	23.3 (3.6)	0

Note: Data are expressed as mean ± standard deviation (SD).
Abbreviations: ΔC_t, the difference in threshold cycles for the target gene ($C_t^{target} - C_t^{GAPDH}$); IVD, intervertebral disc; SD; standard deviation; WB, whole blood.

3.3 | Correlation analysis between chemokines and inflammatory cytokines in IVD tissues

Table 3 shows the correlations between chemokine and inflammatory cytokine expression levels for IVD tissues. Some IVD tissues had low gene expression, and some data were missing. The missing data for the IVD tissues were for CCL20 in six patients, IL-1β in two, and TNF-α in two. They were excluded from the analysis because they were considered zero values or very low

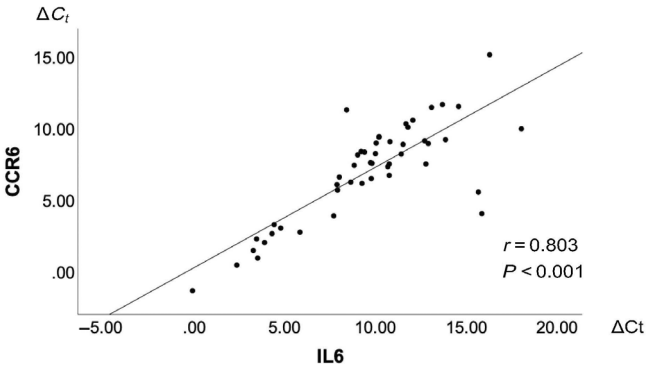

FIGURE 1 Correlational analysis (ΔC_t: Target − GAPDH) for IL-6 and CCR6 expression in IVD tissues. Each point on the scatter plot represents one patient, and the line represents the correlation fit ($n = 48$).

TABLE 4 Pain intensity for each group.

	Preope	Postope (12M)	Change	P[a]
NRS_{LBP}	7.2 (2.1)	3.6 (3.1)	−3.6 (2.9)	<0.001*
NRS_{LP}	7.1 (2.2)	2.4 (2.6)	−4.7 (2.6)	<0.001*
NRS_{LN}	6.9 (2.5)	2.8 (3.1)	−4.1 (3.5)	<0.001*

Abbreviations: NRS_{LBP}, numeric rating scale for low back pain; NRS_{LN}, numeric rating scale for leg numbness; NRS_{LP}, numeric rating scale for leg pain.
[a]Comparison with pre op.
*Statistically significant.

TABLE 3 Correlation matrix showing the Pearson's product moment correlation (*r*) between each chemokine/cytokine pair (ΔC_t) in IVD tissues.

IVD tissues	CCL20	CCR6	IL-6	IL-1β	TNF-α	Agg	Col2
CCL20	1.000						
CCR6	0.402*	1.000					
IL-6	0.426**	0.803***	1.000				
IL-1β	0.461**	0.675***	0.651***	1.000			
TNF-α	0.423**	0.758***	0.751***	0.805***	1.000		
Agg	0.001	0.022	0.012	−0.047	−0.127	1.000	
Col2	0.101	0.172	0.126	0.049	0.005	0.799***	1.000

Note: The **$P < 0.01$, ***$P < 0.001$ indicates significant differences.
Abbreviations: ΔC_t, the difference in threshold cycles for the target gene ($C_t^{target} - C_t^{GAPDH}$); IVD, intervertebral disc.

TABLE 5 Correlation coefficient (r) of NRS_{LBP}, NRS_{LP}, and NRS_{LN} in IVD tissues chemokine/cytokine (ΔC_t).

	Preoperative			Postoperative		
IVD tissues	NRS_{LBP}	NRS_{LP}	NRS_{LN}	NRS_{LBP}	NRS_{LP}	NRS_{LN}
CCL20						
r	−0.239	0.070	0.031	−0.029	−0.004	0.036
P	0.127	0.661	0.847	0.857	0.981	0.822
CCR6						
r	−0.291	−0.263	−0.178	−0.328	−0.138	−0.135
P	0.045*	0.070	0.225	0.023*	0.350	0.359
IL-6						
r	−0.209	−0.140	−0.063	−0.382	−0.163	−0.118
P	0.154	0.344	0.670	0.007**	0.269	0.425
IL-1β						
r	−0.173	−0.197	−0.170	−0.203	0.081	−0.084
P	0.251	0.190	0.259	0.176	0.593	0.581
TNF-α						
r	−0.230	−0.251	−0.249	−0.235	−0.049	−0.165
P	0.123	0.093	0.095	0.116	0.746	0.273
Agg						
r	0.193	0.065	0.050	0.024	−0.088	0.119
P	0.193	0.666	0.736	0.871	0.557	0.424
Col2						
r	0.066	−0.039	−0.053	−0.027	−0.281	−0.102
P	0.658	0.793	0.723	0.855	0.053	0.491

Abbreviations: ΔC_t, the difference in threshold cycles for the target gene ($C_t^{target} - C_t^{GAPDH}$); IVD, intervertebral disc.

*$P < 0.05$, indicates significant differences.

**$P < 0.01$ indicates significant differences.

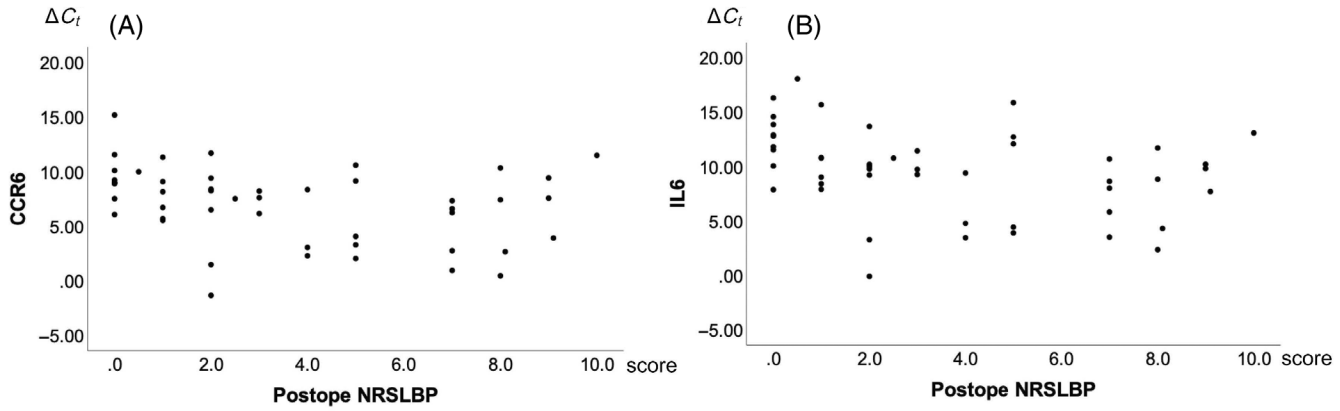

FIGURE 2 Scatter plot of postoperative NRS_{LBP} and gene expression (ΔC_t: Target − GAPDH) of CCR6 (A; $n = 48$) and IL-6 (B; $n = 48$).

expression levels. We did not consider missing values in statistical analyses. Correlations of $r > 0.8$ or higher were observed in IVD tissues for the expression levels of CCR6 and IL-6 ($r = 0.803$, $P < 0.001$) and IL-β and TNF-α ($r = 0.805$, $P < 0.001$). Figure 1 shows a correlation diagram for CCR6 and IL-6 mRNA expression levels in IVD tissues.

3.4 | Pain intensity

All patients reported LBP (mean NRS_{LBP} 7.2 ± 2.1), LP (mean NRS_{LP} 7.1 ± 2.2), and LN (mean NRS_{LN} 6.9 ± 2.5), but at 1 year postoperatively, these values significantly improved to 3.6 ± 3.1, 2.4 ± 2.6, and 2.8 ± 3.1, respectively ($P < 0.001$; Table 4).

3.5 | Correlation analysis between preoperative and postoperative pain intensity and gene expression levels in IVD tissue

A correlation analysis was performed between gene expression in each IVD and preoperative and postoperative pain intensity (Table 5). In the preoperative analysis, no pro-inflammatory cytokines or chemokines were associated with NRS_{LBP}, NRS_{LP}, or NRS_{LN}. Postoperative pain analysis showed a correlation between postoperative NRS_{LBP} and CCR6 expression ($r = -0.328$, $P = 0.023$) and postoperative NRS_{LBP} and IL-6 ($r = -0.382$, $P = 0.007$). None of the gene mRNAs correlated with NRS_{LP} or NRS_{LN}. Figure 2 shows a graph of postoperative NRS_{LBP} and CCR6 and IL-6 gene expression. There was no correlation between IVD matrix Agg and Col2 genes and preoperative and postoperative pain. Furthermore, there was no statistically significant difference when investigating the change (Δ) in each pain before and after surgery and gene expression in each IVD (Table 6).

TABLE 6 Correlation coefficient (r) of ΔNRS_{LBP}, ΔNRS_{LP}, and ΔNRS_{LN} in IVD tissues chemokine/cytokine (ΔC_t).

	Δ(Postoperative − Preoperative)		
IVD tissues	**NRS_{LBP}**	**NRS_{LP}**	**NRS_{LN}**
CCL20			
r	0.135	−0.140	−0.017
P	0.393	0.376	0.914
CCR6			
r	−0.085	0.037	−0.084
P	0.564	0.803	0.572
IL-6			
r	−0.188	−0.079	−0.088
P	0.201	0.595	0.553
IL-1β			
r	−0.068	0.164	0.005
P	0.655	0.276	0.974
TNF-α			
r	−0.044	0.093	−0.011
P	0.771	0.539	0.944
Agg			
r	0.102	−0.083	0.026
P	0.494	0.579	0.860
Col2			
r	−0.059	−0.217	−0.096
P	0.692	0.138	0.515

Abbreviations: ΔC_t, the difference in threshold cycles for the target gene ($C_t^{target} - C_t^{GAPDH}$); IVD, intervertebral disc.

3.6 | Correlation analysis between preoperative and postoperative pain intensity

Postoperative NRS_{LBP} correlated with preoperative NRS_{LBP} ($r = 0.418$, $P = 0.003$), NRS_{LP} ($r = 0.462$, $P < 0.001$), and NRS_{LN} ($r = 0.451$, $P < 0.001$). Similarly, postoperative NRS_{LP} and NRS_{LN} correlated with NRS_{LBP} and NRS_{LP}, respectively (Table 7). Figure 3 shows the

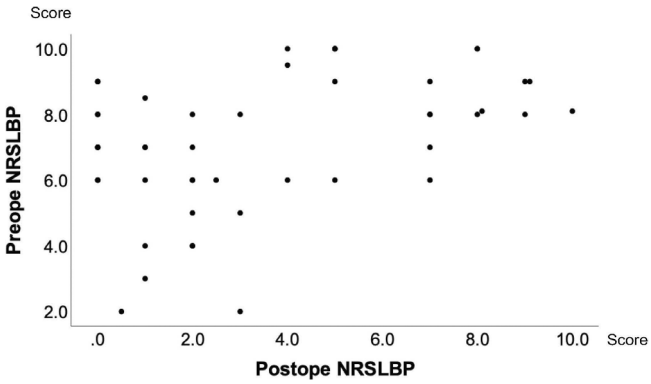

FIGURE 3 Scatter plot of postoperative NRS_{LBP} and preoperative NRS_{LBP}. Some of them look like one point because the plots match, but it is a scatterplot of 48 patient.

TABLE 7 Correlation coefficient (r) of preoperative and postoperative NRS_{LBP}, NRS_{LP}, and NRS_{LN}.

		Preoperative			Postoperative		
		NRS_{LBP}	**NRS_{LP}**	**NRS_{LN}**	**NRS_{LBP}**	**NRS_{LP}**	**NRS_{LN}**
Postoperative	NRS_{LBP}						
	r	0.418	0.462	0.451	1.000	0.606	0.567
	P	0.003**	0.001**	0.001**		0.000***	0.000***
	NRS_{LP}						
	r	0.267	0.457	0.233	0.606	1.000	0.678
	P	0.067	0.001**	0.111	0.000***		0.000***
	NRS_{LN}						
	r	0.314	0.435	0.226	0.567	0.678	1.000
	P	0.030*	0.002**	0.122	0.000***	0.000***	

Note: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ indicates significant differences.

distribution of postoperative and preoperative NRS_{LBP}. Thirteen patients with a postoperative NRS_{LBP} score ≥7 also had a preoperative NRS_{LBP} score ≥6.

4 | DISCUSSION

Various reports have been published on postoperative pain.[16–18] At present, factors affecting patients' postoperative pain can be broadly grouped into categories of sex,[19] obesity,[20] preoperative opioid abuse,[21] preoperative chronic pain,[22] and many other factors. Molecular analysis of the factors involved in post-spine surgery pain may lead to new treatment methods. In other words, if we can intervene in patients with such factors before surgery, it may be possible to solve the problem of postoperative pain. We speculated that a high expression of proinflammatory cytokines and chemokines within preoperative degenerative IVD might influence postoperative pain intensity. Therefore, we investigated the correlation between gene expression of pro-inflammatory cytokines and chemokines within the IVD and pain intensity before and at 1 year after spinal fusion surgery in this study.

To our knowledge, this is the first study to investigate preoperative inflammatory cytokines and chemokines in the IVD and pain at one year after spinal fusion surgery in patients with LDD. We found no correlation between preoperative pain, inflammatory cytokines, and chemokines in the IVD. However, a correlation was observed between postoperative LBP and preoperative IL-6 and CCR-6 gene expression in IVD by correlation analysis.

Moreover, patients with severe postoperative LBP (NRS_{LBP} ≥7) had preoperative LBP (NRS_{LBP} ≥6), a similar result to previous observations. These findings indicate that high levels of CCR6 and IL-6 mRNA in degenerated IVDs may be risk factors for postoperative LBP in patients with LDD.

Pro-inflammatory cytokines rapidly and directly activate nociceptors that generate action potentials and induce pain hypersensitivity. That is, proinflammatory cytokines may be involved in promoting pain. These data support our notion that CCR6 and IL-6 in degenerated IVDs might influence the improvement of postoperative LBP. However, there was no correlation when analyzed by changes in pain. Other factors may be involved in pain relief. Further investigation is needed to determine how gene expression in unstable IVDs tissue resected during surgery is associated with postoperative pain. However, the collected IVD tissue is only a part, and the remaining IVDs tissue may be involved in inflammation and residual pain.

A previous article detected CCL20 expression in NP cells and has reported a potential link between CCL20 and CCR6 expression levels in degenerate human IVDs.[23] However, the mechanism of CCL20–CCL6 system in IVD degeneration remains to be elucidated yet. CCR6 is a chemokine receptor, but IL-6, a proinflammatory cytokine, is said to function as follows. Proinflammatory cytokines are important neuromodulators in central sensitization by activating glial cells within the spinal cord and inducing hyperalgesia and allodynia.[24,25] In the nervous system, IL-6, the classical proinflammatory cytokine, not only plays an essential role in the development, differentiation, regeneration, and degeneration of neurons but also acts as a molecule with both beneficial and destructive potentials.[26] Previous reports on IL-6 involvement speculated that IL-6 levels in blood samples are higher in patients with LBP than in healthy controls.[6] On the other hand, a systematic review reported that no firm conclusions could be established.[27] The present study noted that only one[28] of the seven studies[28–34] reported a statistically significant difference in IL-6 levels. Inflammatory biomarkers in the blood normally change with pain exposure.[35] However, the results of the present study suggest that the preoperative IVD expression of proinflammatory cytokines and chemokines is associated with LBP intensity at 1 year after surgery.

Intradiscal injections of tocilizumab, an IL-6 antibody, have been used in trials to treat LBP. In the tocilizumab group, NRS and Oswestry disability index scores improved significantly at 2 and 4 weeks after treatment, respectively, thereby offering promising short-term results.[36] Furthermore, an earlier study comparing IVD tissues from patients undergoing fusion or discectomy surgery showed significantly higher levels of IL-6 in the fusion group, suggesting that IL-6 plays a role in painful disc degeneration.[37] These data suggest that modulating intradiscal levels of IL-6 in patients with LBP may lead to improved functional outcomes after surgery. Moreover, a clearer understanding of this potential relationship may help predict which patients will have better or worse outcomes in the postoperative period. Based on a genetic analysis of IVD tissue collected during surgery, patients with high CCR6 and IL-6 gene expression may have higher postoperative LBP intensity. Therefore, if the postoperative course could be predicted, pain intervention could be implemented from the early postoperative period.

This study did have some limitations. First, the sample size was small, and no control sample was used. Further analyses are needed to examine the chemokine and inflammatory cytokine expression in IVD tissue samples from healthy controls without LBP. However, it remains an ethical and moral challenge to obtain IVD tissues from pain-free people preoperatively because even patients with trauma experience pain. In addition, this study did not consider various patient biases such as age, gender, and surgical method. In particular, the surgical method may affect the outcome. The observation period is 1 year, so the effects of spinal fusion surgery can be denied as much as possible. In the future, it will be necessary to conduct an evaluation that rigorously considers disease, age, and gender. Furthermore, the duration of patient-identified pain could not be accurately assessed; therefore, this study did not analyze the duration of pain. However, many patients resistant to conservative treatment for at least 3 months underwent surgery. Moreover, we have yet to investigate the role of CCR6 and IL-6 in preoperative degenerative IVD over the long term. Finally, many factors contribute to LBP, but this study did not analyze these factors. Psychological factors should be assessed using patient-reported outcomes, but we have yet to be able to investigate how psychological factors influence outcomes. Moreover, we did not assess the impact of bone fusion or postoperative complications on pain factors. Despite these limitations, to our knowledge, this is the first study to analyze the correlation between the gene

expression of pro-inflammatory cytokines and chemokines in IVD tissues and postoperative pain. In the future, we intend to increase the number of samples to ameliorate these limitations.

In summary, we found that CCR6 and IL6 gene expression within degenerative IVD tissues were found to be correlated with postoperative LBP intensity, suggesting that postoperative pain management may be required. No correlation with preoperative pain was found, and the clinical relevance of why there was a correlation with postoperative LBP remains unclear. Further analyses with additional cases are required to determine whether the expression of these genes is the cause, process, or consequence associated with IVD degeneration.

AUTHOR CONTRIBUTIONS

Akihiko Hiyama participated in the design of the study, performed experiments, analyzed data, and wrote the article. Daisuke Sakai participated in the design, and performed experiments, and performed the statistical analysis. Masato Sato participated in the design. Masahiko Watanabe participated in the design and coordination. All authors read and approved the final article.

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CONFLICT OF INTEREST STATEMENT

Akihiko Hiyama is an Editorial Board member and Daisuke Sakai is an Editor of JOR Spine and are co-authors of this article. To minimize bias, they were exclude from all editorial decision-making related to the acceptance of this article for publication. [Correction added on 22 June 2023, after first online publication: Conflict of Interest statement was revised]

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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